Efficacy of Egyptian Parasitic Nematodes, *Heterorhabditis bacteriophora* (BA1) and *Steinernema carpocapsae* (BA2) in Bio-control of Economically Important Pests

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Abstract

Background: Entomopathogenic nematodes (EPNs) are obligate lethal parasites of insects and are globally used as safe biocontrol agents against a wide range of insect pests. They occur in most agricultural soils all around the world. **Method:** The insecticidal activity of two native EPNs *Heterorhabditis bacteriophora* (BA1) and *Steinernema carpocapsae* (BA2), isolated from the Egyptian soil was examined against eight different economic insect pests under laboratory conditions. These pests are the Greater wax moth larvae, *Galleria mellonella;* the cotton leafworm, *Spodoptera littorallis;* the cutworm, *Agrotis ipsilon;* the european corn borer, *Osterinia nubilalis;* the greater sugarcane borer, *Sesamia critica;* the apple tree borer, *Zeuzera pyrina;* the sugar beet fly, *Pegomyia mixta and* the tortoise beetle *Cassida vittatta*. Three nematode concentrations (100, 50, and 25 /ml water) of infective juveniles (IJs) were applied per insect. The test was conducted at 25± 2 °C and about 70± 2 % RH.

Results: The median lethal concentration (LC_{50}) of *H. bacteriophora* BA1– was almost– higher than that of *S. capocapsae* BA2 against the different tested insect pest larvae. The percentage of the cumulative mortality ranged between 46 and 100% in general, according to the tested concentration and/or the nematode species. The comparison between the mortality percentages caused by BA1 and BA2 nematodes to differently treated insect larvae revealed that BA1 was almost more virulent than BA2. Results showed that the lowest LC_{50} value was found to be 2.15 IJs, for *H. bacteriophora* BA1 isolate. **Conclusion:** The results showed that both indigenous EPNs isolates had a good impact in the management of the eight economic insect pests tested in this study had insecticidal properties and could be positively enrolled in integrated pest management programs against different insect pests.

Significance | Bio-control of economically important pests

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Abbreviations: EPNs, Entomopathogenic nematodes; IJs, Infective juveniles.

Introduction

The Entomopathogenic nematodes (EPNs) from the genera *Heterorhabditis* and *Steinernema* are obligate lethal parasites of insects and are globally used as safe biocontrol agents against wide range of insect pests (Hussein & El-Mahdi, 2019; Hussein *et al.*, 2015; Nouh & Hussein, 2014; Saleh *et al.*, 2009; El-Wakeil & Hussein, 2009; Ehlers, 2005; Burnell and Stock, 2000;). They occur in most agricultural soils all around the world (Hominick, 2002). The only continent where EPNs have not been found is Antarctica (Griffin *et al.*, 1991). These nematodes are mutualistically associated with

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enteric bacteria in the genus *Photorhabdous* and *Xenorhabdus*. The infective juveniles (IJs) - non-feeding resistant stage - carry the bacterial symbiont in bacterial pouch. When the IJ finds a susceptible insect host, it enters the insect via natural openings (anus, spiracles or mouth), penetrates into the hemocoel, and releases the symbiotic bacteria. The bacterial cells multiply in the hemocoel and kill the host within 48 hours causing septicemia. The nematodes feed on the bacterial cells and the host tissues digested by the symbiotic bacteria. There is an intense interest to isolate EPNs from different regions of the world so that they can be used as biological control agents for insect pests in the area or region they were isolated (Iraki *et al.*, 2000; Abdel-Razek *et al.*, 2018; Hazir *et al.*, 2003). Such nematodes will be adapted climatically as well as adapted to the insect pest and will avoid the introduction of exotic nematodes to the region. Therefore, many surveys have been also conducted in many parts of the world to find well-adapted and more suitable strains and/or species for inundated biological control.

Cotton (*Gossypium barbadense* L.), corn (*Zea mays* L.) and sugar beet, *Beta vulgaris* L. are among the most important commercial crops in Egypt, Under Egyptian ecosystem, heavy losses occurred yearly (about 30-40% losses) due to numerous insect pests during plants development (Drazz, 2009; CATGO, 2009; Al-Eryan *et al.*, 2019; El-Dessouki *et al.*, 2019).

The major key cotton pests recorded in Egypt are the cotton leafworm, *Spodoptera littoralis* (Boisd.); the cotton bollworms, pink bollworm, *Pectinophora gossypiella* (Saund.) and spiny bollworm, *Earias insulana* (Boisd.). The corn plants are also attacked by numerous pests during its growth from seedlings till the full maturation. These insects are: The cut worms, *Agrotis ipsilon* (Hufn.); the greater sugarcane borer, *Sesamia cretica* Led.; the European corn borer, *Ostrinia nubilalis* (Hb.) and *Chilo Agamemnon* (Bles.) (El-Wakeel and Hussein, 2009: Al-Eryan *et al.*, 2019). During its different growth stages of sugar beet plants, they are attacked by several pests. Sugar beet fly *Pegomyia mixta* (Vill.); tortoise beetle *Cassida vittata* (Vill) and beet moth *Scrobipalpa ocellatella* (Boyd.) are considered the most serious pests attacking sugar beet (Saleh *et al.*, 2009; Saleh *et al.*, 2011; El-Dessouki *et al.*, 2019).

These pests are exclusively controlled by insecticides which caused severe problems to human health and environment. Moreover, these pests have developed resistance to most of these chemicals (Amin and Gergis 2006). Therefore, searching for safe and effective alternatives is a must.

The objective of this work was screening the role of the native isolates of both *Heterorhabditis bacteriophora* (BA1) and *Steinernema carpocapsae* (BA2) in the biological control of the greater wax moth larvae, *Galleria mellonella;* the cotton leafworm, *Spodoptera littorallis;* the cutworm, *Agrotis ipsilon;* the european corn borer, *Osterinia nubilalis;* the greater sugarcane borer, *Sesamia critica;* the apple tree borer, *Zeuzera pyrina;* the sugar beet fly, *Pegomyia mixta and* the tortoise beetle *Cassida vittatta* under laboratory conditions.

Methods

Parasitic nematodes

The entomopathogenic nematodes, *Steinernema carpocapae* (BA2) and *Heterorhabditis bacteriphora* (BA1), were isolated from the Egyptian soils and identified by Hussein and Abouel-Sooud, 2006. Third stage IJs of the Egyptian species of *S. carpocapae* (BA2) and *H. bacteriphora* (BA1) were used in this study. Nematodes were cultured *in vitro* according to Hussein & El-Mahdi (2020) on the last instar larvae of the greater wax moth, *Galleria mellonella* which were reared according to Metwally *et al.* (2012) under laboratory conditions. After the harvest, IJs were stored in horizontally placed, 500 ml vented culture flasks, containing 150 ml distilled water at 14°C. Flasks were shaken weekly to improve aeration and IJs survival. IJs were used within the first three weeks after emerging and harvested from White's traps (White, 1927). IJs were kept at 22°C for 24 hours prior to use in experiments. Before conducting experiments, IJ concentrations were quantified for all trials by using the method developed by Navon & Ascher (2000).

Tested insect pests

Laboratory studies were carried out on eight different economic pests belonging to four different families and three different orders using the aforementioned two native species of EPNs. These pests were listed in table (1). The insect larvae obtained from Pests and Plant Protection Department, National Research Centre.

Bioassay

Insect mortality bioassays were conducted with two different isolates of EPNs, *H. bacteriophora* (BA1) and *S. carpocapsae* (BA2). Water suspensions of the studied EPNs were prepared at three concentrations (25, 50 and 100 infective juveniles (IJs)/ml). As many as 5 larvae of each tested pest were placed in a Petri dish 9 cm-diameter furnished with filter paper and treated with 1 ml of assigned nematode concentration along with pieces of common larval media for their feeding. Each experiment consisted of 3 treatments (concentrations). Each treatment consisted of 6 replicates (dishes) including 5 insects/replicate. The dishes were kept under controlled conditions (25±2°C) and observed daily for larval mortality. Percentages of larval mortality were assessed 24, 48, 72 and 96 hours post treatment (HPT). Development of nematodes in infected larvae was also recorded. Infected insects were dissected for nematode development and percentages of insects with nematode development were recorded. Treatments were arranged in a complete randomized design with 6 replicates. Control experiment received 1.0 ml of distilled water was used to wet filter paper before adding the tested larvae. The lethal concentration (LC_{50}) of the nematodes *H. bacteriophora* BA1 and *S. capocapsae* BA2 to tested insect pest larvae were calculated according to Finny (1952).

Statistical analysis

Mortality percentages obtained from laboratory experiments were corrected according to Abbott formula (Abbott 1925). Data were subjected to analysis of variance (ANOVA) through the Computer Statistical Package "SPSS". Means were compared using Duncan Multiple Range Test (Duncan, 1965). The values of LC_{50} were calculated according to Finney (1952).

Results

Data represented in Tables (2 and 3) show the virulence of the two Egyptian isolates of the EPNs, *H. bacteriophora* (BA1) and *S. carpocapsae* (BA2) against 8 different insect pests. The data represented as mortality percentage. Larvae of the greater wax moth, *G. mellonella*, were highly susceptible to IJs of *H. bacteriophora*

Table 1| **List of the insect pests used to evaluate the virulence of the Egyptian isolates of entomopathogenic nematodes, *Heterorhabditis bacteriophora* BA1 and *Steinernema carpocapsae* BA2.**

Pest	Latin name	Taxonomy		Tested larval instar	Host Crops
		Order	Family		
Greater Wax moth	*Galleria mellonella*	Lepidoptera	Pyralidae	5th	Stored Wax Comb
Cotton Leafworm	*Spodoptera littorallis*		Noctuidae	5th	Cotton and Vegetables
Cutworm	*Agrotis ipsilon*		Noctuidae	4th	Cotton, Corn, Vegetables
European corn borer	*Osterinia nubilalis*		Pyralidae	4th	Corn
Greater sugarcane borer	*Sesamia critica*		Noctuidae	4th	Corn and sugar cane
Apple tree borer	*Zeuzera pyrina*		Pyralidae	5th	Apple, peach, Grapes
Sugar beet fly	*Pegomyia mixta*	Diptera	Muscidae	3rd	Sugar beet
Tortoise beetle	*Cassida vittatta*	Coleoptera	Curculionidae	3rd	Sugar beet

(BA1) than *S. carpocapsae* (BA2), especially at the low concentration level. In general, the heterorhabditid nematode (BA1) caused the highest mortality percentages, except in case of *O. nubilalis*, *Z. pyrina* and *P. mixta*. The mortality percentages caused by *H. bacteriophora* ranged between 83 to 100% at the highest concentration used (100 IJs/ml), where the corresponding figures for *S. carpocapsae* was 80 to 100% mortality.

The corresponding figures for the two other concentrations (50 and 25 IJs/ml) was nearly the same, where the *H. bacteriophora* was more virulent than *S. capocapsae*. The mortality percentage for (BA1) ranged between 65 and 100% and 45 and 93% at 50 and 25 IJs/ml concentration, respectively (Table 2). As for (BA2), nearly the same trend was observed with some exception; the mortality percentage ranged between 66 to 93% and 46 to 90% at 50 and 25 IJs/ml concentration, respectively. Statistically by applying "Student t-test", the comparison between the mortality percentages caused by BA1 and BA2 nematodes to different treated insect larvae, revealed that, BA1 was almost more virulent than BA2 (Table 3). On the other hand, statistical analysis revealed that, the percentages of mortality were increased with increasing the concentration applied, where the F-values obtained were highly significant in all cases except the treatment of *S. littoralis* where the F-value was significant only (Table 2). The Probit Analysis to calculate the median lethal concentration (LC_{50}), indicate that: as for BA1, *S. littoralis* was the most susceptible insect, followed by *G. mellonella*, *P. mixta*, *C. vittatta* then *Z. pyrina* (Table 3).

The heterorhabditid nematode also caused the highest mortality percentages in case of the cotton Leaf worm, *S. littoralis* and the greasy cutworm, *A. ipsilon*. Both insect larvae showed high sensitivity to IJs of *H. bacteriophora* (BA1) than *S. carpocapsae* (BA2). The percentage mortalities of *S. littoralis* due to exposure to the isolate BA1 recorded 100, 100 and 93.33% at concentrations of 100, 50 and 25 IJs/ml, respectively. The steinernematids caused 100, 86.66 and 83.33% mortality of cotton leaf worms at the same aforementioned concentrations (Table 2). *H. bacteriophora* (BA1) caused mortality percentages to the cut worms, *A. ipsilon*, ranged between 46.66 to 96.66 % and *S. carpocapsae* induced mortality ranged between 46.66 to 80%. The European corn borer, *O. nubilalis,* showed high sensitivity to both nematode strains.

The mortality percentages recorded due to the exposure to *H. bacteriophora* were 85, 65 and 45% for 100, 50 and 25 IJs/ml concentrations. Meanwhile, the mortality percentages were 95, 75 and 60% for *S. carpocapsae*, at the same concentrations (Table 2). Results also revealed that, the greater sugarcane borer larvae, *S. cretica* was highly susceptible to the infection with EPN. The mortality reached 100% for 100 IJs/ml and recorded 84 and 64% for 50 and 25 IJs/ml, respectively for *H. bacteriophora*. The corresponding figure for BA2 was 84, 64 and 52% for 100, 50, 25 IJs/ml, respectively.

On the other hand, the apple tree borer larvae, *Z. pyrina,* showed more susceptibility to *S. carpocapsae* than *H. bacteriophora.* The mortality percentages ranged between 100% to 90% for 100, 50 and 25 IJs/ml H_2O concentrations in case of BA2 and ranged between 90 and 60% for the same concentration in case of BA1, respectively.

Data in Table (2) showed that both sugar beet pests were susceptible to the infection with nematodes in a variable extent. The 3rd instar larvae of the sugar beet fly (Leaf miner), *P. mixta,* were highly susceptible to steinernematid nematodes more than heterorhabtids. Results confirmed the high sensitivity of sugar beet pests for both nematode strains. This sensitivity was varied according to the different concentration received. For example, the sugar beet fly, *P. mixta* was susceptible to *S. carpocapsae* BA2 more than the *H. bacteriophora* BA1 strain. Meanwhile, the opposite was true with the tortoise sugar beet beetle, *C. vitatta*, which recorded the highest mortality when treated with *H. bacteriophora* BA1. The percentage mortality ranged between 100%, 96.66% and 73.33% for BA1, and 93.33 and 66.66% for the BA2 strain at tested concentrations of 100, 50 and 25 IJs/ml H_2O, respectively.

Discussion

The overall aim of this study was to manipulate the endemic species in controlling native serious pests, to avoid the introduction of exotic species. The tested species, *H. bacteriophora* BA1 and *S. carpocapdae* BA2, were able to infect the pest larvae causing mortality rates comparable to those observed with other parasitic nematode strains on other insects. Despite the significant variability observed, our main goal was successfully achieved. The mean susceptibility to nematode infestations was rated by Glazer *et al.* (1999) as poor (mortality <35%), moderate (mortality 35-

Table 2| **Percentages of Mortality of different economically important insect pests treated with two isolates, *Heterorhabditis bacteriphora* (BA1) and *Steinernema carpocapae* (BA2) nematodes at 27°C using filter paper bioassay.**

Insect Host	Nematode Strain	Percentages of Mortality			F-value
		100 (IJs/ml)	50 (IJs/ml)	25 (IJs/ml)	
Galleria mellonella	(BA1)	100 aA	100 aA	90.00 aB	75.000**
	(BA2)	100 aA	90.00 bB	76.66 bC	21.209**
T-value		---	5.774**	3.168*	
Spodoptera littorallis	(BA1)	100 aA	100 aA	93.33 aB	8.160*
	(BA2)	100 aA	86.66 b AB	83.33 aB	5.079*
T-value		---	3.775*	1.633 NS	
Agrotis ipsilon	(BA1)	96.66 aA	86.66 aB	46.66 aC	141.993**
	(BA2)	80.00 bA	63.33 bB	46.66 aC	33.227**
T-value		4.409**	8.862**	---	
Osterinia nubilalis	(BA1)	85.00 bA	65.00 aB	45.00 aC	112.500**
	(BA2)	95.00 aA	75.00 aB	60.00 aC	17.453**
T-value		3.612*	2.013 NS	2.380 NS	
Sesamia critica	(BA1)	100 aA	84.00 aB	64.00 aC	127.304**
	(BA2)	84.00 bA	64.00 bB	52.00 aC	22.783**
T-value		13.856**	7.821**	2.189 NS	
Zeuzera pyrina	(BA1)	90.00 bA	75.00 bB	60.00 bC	19.471**
	(BA2)	100 aA	90.00 aB	90.00 aB	13.636**
T-value		2.00 NS	11.619**	7.661**	
Pegomyia mixta	(BA1)	83.33 bA	73.33 bB	60.00 bC	70.281**
	(BA2)	100 aA	93.33 aB	66.66 aC	167.419**
T-value		11.096**	9.413**	3.088*	
Cassida vittatta	(BA1)	100 aA	96.66 aA	73.33 aB	85.357**
	(BA2)	93.33 bA	66.66 bB	66.66 aB	65.631**
T-value		2.837*	13.560**	2.38b3 NS	

Table 3| **The median lethal concentration (LC_{50}) of nematode concentrations against different economic pests**

Insect Host	Nematode Strain	LC_{50} (IJs/ml)
Galleria mellonella	*Heterorhabditis bacteriphora* (BA1)	14.974
	Steinernema carpocapae (BA2)	13.876
Spodoptera littorallis	*Heterorhabditis bacteriphora* (BA1)	12.570
	Steinernema carpocapae (BA2)	8.861
Agrotis ipsilon	*Heterorhabditis bacteriphora* (BA1)	25.714
	Steinernema carpocapae (BA2)	28.868
Osterinia nubilalis	*Heterorhabditis bacteriphora* (BA1)	29.819
	Steinernema carpocapae (BA2)	20.435
Sesamia critica	*Heterorhabditis bacteriphora* (BA1)	20.033
	Steinernema carpocapae (BA2)	24.966
Zeuzera pyrina	*Heterorhabditis bacteriphora* (BA1)	18.260
	Steinernema carpocapae (BA2)	2.149
Pegomyia mixta	*Heterorhabditis bacteriphora* (BA1)	15.190
	Steinernema carpocapae (BA2)	19.33
Cassida vittatta	*Heterorhabditis bacteriphora* (BA1)	17.306
	Steinernema carpocapae (BA2)	16.407

65%), or high (mortality> 65%). According to this approach, tested larvae showed a high susceptibility only to the highest concentrations of the tested nematodes (*H. bacteriophora* BA1 and *S. carpocapdae* BA2) in screening bioassays.

The mortality variability caused by various species and strains of EPNs against various insect pests is well known. Our data are in line with previous studies suggesting that nematodes are generally highly effective against Noctuids and Pyrilds larvae (Lepidoptera: Noctuidae and Pyrilidae) at low levels of IJs (Bedding *et al.* 1983; Glazer and Navon, 1990; Glazer *et al.* 1991; West and Vrain, 1997; Feaster and Steinkrauss, 1999; El-Wakeil, and Hussein; 2009; Metwally *et al.* 2012; Nouh and Hussein, 2014; Yuksel and Canhilal, 2018; Hussein *et al.* 2018 ; Amutha *et al.* 2020). The recorded results are also in agreement with previous bioassays suggesting that EPNs are effective against Curculionidae larvae (Saleh *et al*, 2009; Williams *et al.*, 2015).

The study indicated that the local tested isolates, *H. bacteriophora* (BA1) and *S. carpocapsae* (BA2) gave promising results and induced high mortality percentage on the tested larvae. Although heterorhabditid isolates appeared to be more virulent than steinernematid ones, generally, there were insignificant differences statistically between the EPNs isolates.

Conclusions

Two native strains of EPNs species; *Heterorhabditis bacteriophora* (BA1) and *Steinernema carpocapsae* (BA2); were found to be effective against eight different destructive pests: *G. mellonella*; *S. littorallis; A. ipsilon; O. nubilalis; S. cretica; Z. pyrina; P. mixta* and *C. vittatta*. The results showed that both indigenous EPNs isolates have good potentials in the management of all tested pests. This study also proved that the native EPNs could be implemented successfully in an IPM to manage serious economic insect pests. However, more field studies are needed to be conducted.

Author Contribution

MAH designed and performed the experiments, and prepared and revised the manuscript.

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Competing financial interests

The author declares that she has no competing interest.

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