Egypt. J. Agronematol., Vol. 17, No.2 pp.157-170 (2018)

Role of Earthworm *Allolobophora caliginosa* in Enhancing Biological Control of Egyptian Cotton Leafworm *Spodoptera littoralis* by *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*

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Received:02July 2018 *Revised: 25July 2018* *Accepted:31July2018*

ABSTRACT

Entomopathogenic nematodes, *Steinernema carpocapsae* (All strain) and *Heterorhabditis bacteriophora* (HP 88) dispersal were enhanced by the presence of earthworms that may serve as phoretic hosts based on increased IJs dispersal through the soil columns. IJs dispersal capability either in sandy or clayey soil is limited in the absence of earthworm *Allolobophora caliginosa* as compared with PVC column pipes that contained the earthworms. After two weeks, dispersal was estimated by using the greater wax moth, *Galleria mellonella* as bioassay organism. Results showed vertical dispersal of nematodes was increased significantly in the presence of earthworm as compared with soil columns in absence of earthworms. With two species of nematodes, when IJs were placed on the surface of soil columns, significantly more nematodes dispersed to the lower half of the columns when *A. caliginosa* was present and vice versa in the absence of earthworm. Thus, earthworm could be used as vectors to introduce/disperse beneficial organism. The current results showed that the ability of *S. carpocapsae* (All strain) and *H. bacteriophora* (HP 88) to control Egyptian cotton leafworm *Spodoptera littoralis* was enhanced in the presence of earthworm and caused significant S. *littoralis* suppression with mortality percentages 70 and 94 % relative to the control in the absence of earthworm.

Key words*:* Earthworm, Nematode dispersal, *Steinernema carpocapsae, Heterorhabditis bacteriophora, Spodoptera littoralis.*

INTRODUCTION

Entomopathogenic nematodes (EPNs) belonging to families Heterorhabditidae and Steinernematidae are promising non-chemical alternatives for suppressing populations of insect pests in many crops worldwide (Kaya et al., 2006 and Georgis et al., 2006). They are widely distributed in natural and managed ecosystems of all continents except Antarctica (Adams et al., 2006). EPNs possess many attributes, an ideal biological control agents including safety (EPA exempt), ease of mass production, with high virulence and broad host range (Gaugler, 1981). The third stage infective juvenile (IJ) is the only stage that can reside in soil and infect a host. IJs have matualistic association with enteric bacteria i.e. *Xenorhabdus* spp. in the case of genus *Steinernema* and *Photorhabdus* spp. for the genus *Heterorhaditis*. The bacteria incorporate in the anterior intestine or in specific vesicles (Boemare, 2002). Persistence of EPNs populations after application showed rapid decline ranged

between 40 to 90 % within hours or days after application. It has been attributed to inactivation of IJs by ultraviolet light and desiccation at soil surface (Smits, 1996). On the other hand, density of IJs, dispersal capacity, IJs remaining energy reserves, tolerance against desiccation and temperature. interact with extrinsic factors such as biotic (i.e. predation, competition, phoretic relationships, synergistic and plant root) and abiotic (i.e. soil moisture, radiation, temperature, aeration, and soil characteristics). Moreover, phoretic relationships which can be considered beneficial through enhancing EPNs dispersal have been reported with earthworm (Shapiro et al., 1995), isopoda (Eng et al., 2005) and insects (Kruitbos et al., 2009).

Earthworms are a major component of soil fauna communities in most ecosystems. They improve soil fertility by modification of soil structure, aeration, drainage and by breaking down and distributing organic matter (Edwards, 1983). Early studies revealed that EPNs can develop in previously damaged earthworms providing nematodes with alternate host in absence of more suitable insect hosts (Capinera et al., 1982; Nuutinen et al., 1991 and Potter et al.,1994). On the other hand, IJs could be transported by the setae of earthworm body and even looked inside the gut. Futhermore, EPNs can be ingested by earthworms and casts can have some viable EPNs but only in rare cases (Campos – Herrera et al., 2006). More recently, Shapiro–Ilan and Brown (2013) demonstrated lives how earthworms can improve nematode dispersal throughout the soil and this enhancing biocontrol.

The present study was conducted to determine dispersal of EPNs i.e. *S.carpocapsae* (ALL strain) and *H.bacteriophora* (HP88 strain) in the presence or absence of the earthworm *Allolobophora caliginosa* in soil columns, and their effect on enhancing control of Egyptian cotton leaf worm, *Spodoptera littoralis* Boisd under laboratory conditions.

MATERIALS AND METHODS

Source, Rearing and Extraction of the tested animals

A. Entomogenous nematodes

Infective juveniles (IJs) of *Steinernema carpocapsae* (All strain) and *Heterorhabditis bacteriophora* (HP88 strain) were obtained from Department of Entomology and Nematology, University of Florida, USA. The nematodes were cultured separately in last instar larvae of the greater wax moth, *Galleria mellonella* L. according to the technique described by Kaya & Stock (1979). IJs were harvested and stored in distilled water at 12 C for two weeks before experiment (Woodring and Kaya, 1988). On the other hand, at the end of each trial, IJs were extracted from soil samples using two methods according to Sturhan & Mracek (2000). In the first method, sieving technique which is commonly applied for extraction plant–parasitic nematodes (Southey, 1986) in which 60 and 350 mesh sieves were employed, and Baermann trays were used to separate nematodes from soil particles. The obtained nematode suspension was examined with a research microscope and nematodes were counted using Hawksely slide. In the second method, soil samples were placed in small plastic containers measuring 22x 18 x12 cm and healthy *G. mellonella* larvae were added as bait for IJs. After 48 hours, dead larvae were recovered and examined under stereomicroscope for the presence of EPNs.

B.Greater wax moth, *Galleria mellonella* L.

Naturally infested combs with *G.mellonella* were obtained from honey in Zagazig University. The collected larvae were reared using an artificial medium consisted of honey, wheat bran, glycerol, soy flour, milk powder, dry yeast and honey bee was in glass jars kept under laboratory conditions at 24± 3°C. (Ekmen et al., 2010). Each jar was provided with a tissue paper as a physical surface for moths to lay their eggs. The egg masses were transferred frequently to new glass jars containing the nutrient medium. Enough quantity of *G. mellonella* larvae were collected by repeating this technique.

C. Earthworm, *Allolobophora caliginosa*

The earthworms were collected from clayey soil cultured with horse manure at Zagazig district, Sharkia governorate by digging and hand sorting using hand trowel. The collected earthworms were identified to generic level according to the key given by Edwards & Lofty (1976). Earthworms of *A. caliginosa* were cultured under laboratory conditions using clayey soil to a depth of about 40 cm- diameter. The pots were filled with clayey soil to a depth of about 25 cm. Ten adults of equal size of the same species were introduced on the top of the soil in each pot. Tap water was sprinkled on each pot content to keep the culture moistened but not was spread on the top of each pot at weekly intervals and covered with thin layer of clayey soil. The harvested earthworms were used in the experiments (Ibrahim et al.,2010).

Experiment 1. Vertical movement of *S. carpocapsae* (All strain) and *H. bacteriophora* (HP88 strain) in clayey and sandy soils with or without earthworms

Soil columns consisted of jointed six 5-cm- long sections of 5-cm diameter polyvinyl chloride (PVC), were used as described by Shapiro et al.(1993). Columns were filled with sterilized soil to bulk density of 1.9 and 1.2 g/cm3 for clay and sandy soil, respectively. Clayey soil was obtained from the upper 20 cm in the Experimental farm, Faculty of Agriculture, Zagazig University, while sandy soil was collected by the same manner from newly reclaimed sandy area located in El-Salhiya district, Sharkia Governorate. The mechanical analysis of sandy soil was as follows: (95.7% sand; 1.2% silt and 3.1% clay), while the parallel values for clayey soil were 3.64 % , 58.73 % and 37.63 %, respectively. Each soil was autoclaved and left for one week before experiment. Soil moisture was adjusted to field capacity in each column (18%). About 40 g of dried cow manure were placed on the top of each column earthworm treatments. Column of each soil type received one of the following treatments, downward and upward movement of *S.carpocapsae* and *H. bacteriophora* in the presence or absence of the earthworm, *A.caliginosa*. Each treatment was replicated three times. Therefore, 24 columns were needed for each soil type. In the treatments of earthworms, two adults of *A.caliginosa* were placed on the surface of columns and allowed to burrow for one week before the addition of nematodes (Shapiro et al.,1993).

In treatments of the downward movement, 5000 IJs of *S.carpocapsae* (All strain) and *H. bacteriophora* (HP88 strain) were placed in 0.5 ml water to one hole of about 1-cm wide to the surface of each soil column. Literature reports indicated that *S.carpocapsae* move upward from the placement site in the soil, while *H. bacteriophora* tend to migrate downward in the soil. Therefore, to stimulate downward movement of IJs , 2-6 moth larvae were put in screen bait cage measuring 2.5 x 3 cm and placed on the bottom end of each column. On the other hand, to test upward movement, the same numbers of the above-mentioned nematodes

were applied in 0.5 ml water to soil on the bottom of each column (30 cm below the soil surface). Two weeks later, soil columns were examined for larvae mortality. The soil of each 5cm long section of PVC pipe was subdivided into two equal parts each of 115 gm. The first was processed for IJs extraction as mentioned before, while the second once was placed in plastic tray (18 x 12 x 8 cm) and provided with 10 fresh *G. mellonella* larvae. The plastic trays were kept at 28 °C in the laboratory for 3 days, thereafter numbers of dead larvae were recorded to estimate percentage of mortality.

Experiment 2. Enhancing biocontrol of Egyptian cotton leafworm, *Spodoptera littoralis* by earthworm *A. caliginosa* and entomogenous nematodes

This experiment was conducted to evaluate the phoretic role of earthworm *A. caliginosa* in controlling Egyptian cotton leafworm *S. littoralis* by two entomopathogenic nematodes i.e *S. carpocapsae* (All strain) and *H.bacteriophora* (strain HP88). The experiment work was carried out in plastic pots (5 cm - diameter, 30 cm deep), filled with 1185g autoclaved clayey soil gained from Experimental Farm , Faculty of Agriculture, Zagazig University. Mechanical analysis of soil used was above –mentioned.

Nearly 309 ml of tap water were added to pots in order to achieve moisture of field capacity. One hole of 1 cm diameter and 1 cm deep was in the center of each pot. About 5000 IJs of *S. carpocapsae* or *H.bacteriophora* in 2 ml tap water were pipetted onto the hole. Four adults of *A. caliginosa* combined with 40 g of dried cow manure were placed on the top of each earthworm treatment. Ten 5th instar larvae of *S. littoralis* were put on soil surface together with 5 leaves of Egyptian clover, *Trifolium alexandrina* as natural diet for insects. Pots were sealed with perforated upper lid to prevent insects from escaping. The pots were arranged on a bench in a completely randomized design with four treatments, each with five replicates and were left for two weeks before testing insect mortality.

Larval mortality was checked at 24h interval up to 72h. Dead larvae were removed, rinsed in distilled water and placed individually in small Petri dishes of 5 cm-diameter lined with moist filter paper. After 3 days cadavers were dissected under a stereomicroscope to confirm the presence of nematodes. Average room temperature and relative humidity during experiments were 29 ± 3 °C and 78%, respectively. *S.littoralis* was reared on castor plant (*Ricinus communis*). Egg clutches were collected from infested cotton field. Eggs were reared in separate jars and fresh surface sterilized castor leaves were provided. Cleaning, changing of food and chining of culture were done at regular intervals to get healthy culture. The containers were sealed with parafilm to prevent dehydration and left in bench at 25±3 °C during rearing period.

Statistical analysis

Movements of nematodes in sandy and clayey soils were compared using a Student Paired Sample a t-test. Data on mortality of *S.littoralis* were selected to analysis of variance using F test. Means were compared by Duncan's multiple range test at 5% level of possibility according to Snedecor (1966).

RESULTS AND DISCUSSION

Samples were collected at different column depth to recover infective juveniles of *S.carpocapsae* in the presence and absence of earthworm, *A. caliginosa*. Data in

Table (1) showed the effect of *A. caliginosa* on downward and upward movement of *S.carpocapsae* IJs. In upward movement percentage of IJs recovered from different column depth decreased significantly from 83.38 (0-5cm deep) to 4.35 at 10-15 cm deep and reach 0.00 at 15-30 cm deep in the absence of earthworm *A. caliginosa* in sandy soil. Whereas, the percentage of IJs recovered in the presence of *A. caliginosa* was increased significantly from 1.39 (10-15 cm deep) to 7.90 (15-20cm deep) and significantly farther downward (90.88) at the depth of 20-25 cm.

On the other hand, downward dispersal of *S. carpocapsae* was significantly increased farther in the presence of *A. caliginosa*. Percentage of IJs recovered were 4.77 and 5.81 at column depth 10-15 and 5-10 cm, respectively in the presence *A. caliginosa* earthworms , whereas in the absence of earthworm , percentage of IJs decreased from 6.13 to 2.32 and 0.67 at the column depth 15-20, 10-15 and 5-10 cm, respectively. At column depth 0-5cm, upward dispersal of *S. carpocapsae* was increased significantly to reach 78.26 in the presence of earthworm, whereas no IJs was found in the absence of *A. caliginosa*.

Data in Table (1) clearly showed the higher dispersal percentage (91.84%) of *S.carpocapsae* at column depth 20-25cm in the presence of earthworm *A. caliginosa* in clayey soil. Whereas in the absence of *A. caliginosa*, percentages of IJs dispersal were 6.87 and 1.45 at column depths 5-10 and 10-15 cm only. So, presence of earthworm, *A. caliginosa* caused higher percentages of IJs dispersal at deeper column than other columns. With downward movement the percentages of IJs recovered in the presence of earthworm were 5.13, 5.69 and 73.18 at column depth 15-20, 20-25 and 25-30 cm, respectively. At 5-10 cm depth, IJs recovered was 0.27 in the presence of *A. caliginosa* and 0.00 in the absence of *A. caliginosa* . When no earthworm found, dispersal of IJs of *S.carpocapsae* decreased significantly 14 days post- treatment.

The percentage of IJs of *H. bacteriophora* dispersal in absence or presence of the earthworm, *A. caliginosa* in sandy soil is shown in Table (2). In upward movement, data revealed that *A. caliginosa* increased the percentage of IJs dispersal through the top of column depth 0-5, 5-10 and 10-15 cm whereas in the absence of earthworm, IJs of *H. bacteriophora* tend to move towards the bottom of column depth. The percentages of IJs recovered were 2.81, 4.94 and 89.77 at column depth 15-20, 20-25 and 25-30 cm in the absence of earthworm.

In the presence of *A. caliginosa*, recovered infective juveniles of *H. bacteriophora* were 31.23, 33.30 and 33.66 at column depth 0.5, 5-10 and 10-15 cm, respectively. In downward movement, recovered IJs samples show that *H. bacteriophora* moved further upward in the presence of earthworm *A. caliginosa* in sandy soil. The percentage of IJs of *H. bacteriophora* was 79.18, 6.16 and 4.70 at column depth 0-5, 5-10 and 10-15, respectively. In the absence of earthworm *A. caliginosa* IJs of *H. bacteriophora* moved to bottom of column depth at 30-25, 20- 25 and 15-20 cm with percentages of 1.60, 3.43 and 92.11, respectively. With upward movement the highest percentage of *H. bacteriophora* IJs (93.31) was recovered from the top surface of clayey soil columns (0-5 deep) in the presence of the earthworm, *A. caliginosa.* However, no IJs of *H. bacteriophora* were recovered at depth 0-5, 5-10 and 10-15 cm in the absence of *A.caliginosa*.

Table 1. Percentage of nematodes recovered from sandy and clayey soil columns testing downward or upward movement of *Steinernema carpocapsae* (All strain) in the presence and absence of the earthworm, *Allolobophora caliginosa.*

Column depth/ cm	*S. carpocapsae*							
	Sandy soil				Clayey soil			
	Upward movement		Downward movement		Upward movement		Downward movement	
	No Earthworm	With Earthworm.	No Earthworm	With Earthworm.	No Earthworm	With Earthworm.	No Earthworm	With Earthworm.
0- 5	83.38 a	0.00 c	0.00 c	78.26 a	92.01 a	0.00 c	0.00 c	0.00 c
5-10	34.13 b	0.00 c	0.67 c	5.81 b	6.87 b	0.00 c	0.00 c	0.27 c
10-15	4.35 bc	1.39 c	2.33 bc	4.77 b	1.46 c	0.00 c	0.13 c	1.50 bc
15-20	0.00 c	7.90 b	6.13 bc	1.29 c	0.00 c	1.39 c	1.57 b	5.13 b
20-25	0.00 c	90.88 a	7.41 b	0.12 c	0.00 c	6.41 b	3.61 b	5.69 b
25-30	0.00 c	0.00 c	64.23 a	0.00 c	0.00 c	91.84 a	84.56a	73.18 a

Numbers represent means of three replications. Means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 2. Percentage of nematodes recovered from sandy and clayey soil columns testing downward or upward movement of *Heterorhabditis bacteriophora* (HP88) in the presence and absence of the earthworm, *Allolobophora caliginosa*.

Column depth/ cm	*H. bacteriophora*							
	Sandy soil				Clayey soil			
	Upward movement		Downward movement		Upward movement		Downward movement	
	No Earthworm	With Earthworm.	No Earthworm	With Earthworm.	No Earthworm	With Earthworm.	No Earthworm	With Earthworm.
0- 5	0.00 d	31.23 a	0.00 c	79.18 a	0.00 b	93.31 a	0.00 c	76.54 a
5-10	0.00 d	33.30 a	0.00 c	6.16 b	0.00 b	3.09 b	0.00 c	6.53 b
10-15	0.00 d	33.66 a	0.25 c	4.70 b	0.00 b	2.00 b	0.09 c	7.42 b
15-20	2.81 c	0.00 b	1.60 bc	2.55 c	2.64 b	0.00 c	0.49 c	0.94 c
20-25	4.94 b	0.00 b	3.43 b	0.81 cd	3.13 b	0.00 c	3.04 b	0.41 c
25-30	89.77 a	0.00 b	92.11 a	0.00 d	62.27 a	0.00 c	92.76 a	0.00 c

Numbers represent means of three replications. Means followed by the same letter are not significantly different at $P \leq 0.05$.

At more depths, percentage of IJs recovered were increased in absence of earthworm to reach 62.27 at depth 25-30 cm whereas, at the same depth no IJs was extracted in the presence of *A. caliginosa* (Table 2). With downward movement, earthworms have increased percentage of IJs recovered from depth 10-15, 5-10 and 0-5 cm to reach 7.42, 6.53 and 76.54, respectively. In the absence of earthworm, *A. caliginosa* IJs of *H. bacteriophora* tend to move downward of column depth and sharply reached its maximum (92.76%) at 25-30 deep. No infective juveniles were extracted from depths 0-5 and 5-10 cm.

Data in Table (3) show the mortality percentage in *G. mellonella* larvae exposed to *S.carpocapsae* isolated from each column section. Generally, number of dead larvae was higher in sandy soil than clayey soil in presence or absence of earthworms. In sandy soil, where no earthworm was found number of dead larvae in downward movement was greater in the top of soil columns sections, 0-5cm, 5-10 and 10-15 cm to reach 14.33, 16.33 and 17.00, respectively. Whereas, in the presence of earthworm was 12.00, 15.00 and 16.33 with the above-mentioned soil column sections. Earthworm increased numbers of dead *G. mellonella* larvae in the bottom of soil columns sections 15-20 and 20-25 cm to reach 9.67 and 1.67 while in the absence of earthworm , no dead *G. mellonella* larvae was observed at 20-30cm deep. In upward movement, earthworm encourage *S. carpocapsae* to move up to the top of soil columns sections 5-10 and 15-15cm whereas at the same sections , no IJs of *S. carpocapsae* were found and no dead *G. mellonella* larvae was observed.

In clayey soil, although the nematodes dispersed farther downward with earthworms and increased number of dead *G. mellonella* larvae, numbers of dead larvae were lower than in sandy soil in all soil columns sections. Numbers of dead *G. mellonella* larvae were 10.00, 12.00 , 14.00 7.33 and 0.67 at soil column depths 0-5, 5-10, 10-15 , 15-20 and 20-25 cm. whereas at 25-30cm soil column depth no *G. mellonella* larvae was observed either in presence or absence of earthworms. In upward dispersal, earthworms encourage IJs of *S. carpocapsae* to move up to near the top of soil columns and reach to 10-15 and 5-10 cm sections. The dead *G. mellonella* larvae were 1.33 and 0.67 at soil columns depth 10-15 and 5-10cm whereas, in the absence of earthworms, at above-mentioned depth the dead *G. mellonella* larvae were 0.0 for each.

Data in table (4) indicated that when earthworms were found, the nematode significantly dispersed to top column sections and more dead *G. mellonella* larvae were observed. Dismantled sandy and clayey soil columns sections and observed mortality in *G. mellonella* larvae were assessed after 14 days. In sandy soil, numbers of dead larvae caused by *H. bacteriophora* (HP88) and earthworm's treatments were increased as compared to treatments without earthworms. In downward movement, number of dead larvae was greater in treatments with earthworms than in their absence. Numbers of recovered dead larvae of *G.mellonella* were 2.67, 10.67 and 14.00 at soil columns sections, 5-10, 10-15 and 15-20 cm, respectively. Whereas, in absence of earthworm was 0.00, 3.33 and 11.33 with the same soil column sections. In upward dispersal treatments with *H. bacteriophora* (HP88) earthworm increased numbers of dead *G. mellonella* larvae in the soil columns sections 15-20 and 20-25cm to reach 8.33 and 4.00, respectively while at the same depth sections in absence of earthworm numbers of dead *G. mellonella* larvae were 6.33 and 0.0, respectively.

Table 3. Number of dead *Galleria mellonella* larvae recovered from soil column sections infected with *Steinernema carpocapsae* (All strain) in the presence and absence of earthworm (*Allolobophora caliginosa*).

Column depth/ cm	4000 IJs of *S. carpocapsae*							
	Sandy soil				Clayey soil			
	Downward movement		Upward movement		Downward movement		Upward movement	
	No Earthworm	With Earthworm	No Earthworm	With Earthworm	No Earthworm	With Earthworm	No Earthworm	With Earthworm
0 – 5	14.33 b	12.00 c	0.00 c	0.00 d	9.67 b	10.00 c	0.00 d	0.00 c
5-10	16.33ab	15.00 b	0.00 c	1.00 d	13.00a	12.00 b	0.00 d	0.67 c
10-15	17.00 a	16.33 a	0.00 c	3.33 c	13.00a	14.00 a	0.00 d	1.33 c
15-20	5.33 c	9.67 d	4.33 b	7.00 b	4.00 c	7.33 d	2.33 c	6.00 b
20-25	0.00 d	1.67 e	14.00 a	13.00 a	0.00 d	0.67 e	11.00 a	12.00 a
25-30	0.00 d	0.00 f	13.00 a	12.33 a	0.00 d	0.00 e	9.33 b	10.67 a
Average Mortality	8.83 A	9.11 A	5.22 A	6.11 B	6.61 A	7.33 A	3.77 B	5.11 A

Numbers represent means of three replications. The same lowercase letter in columns or uppercase letters in rows indicate no significant differences at $P \leq 0.05$ according to Duncan's multiple range test. *Steinernema carpocapsae* was introduced on top of soil columns in the upward and downward dispersal experiments. Twenty *G. mellonella* larvae were exposed to soil removed from column sections 2 weeks after nematodes introduction. *G. mellonella* mortality indicates relative nematode densities.

In clayey soil although, the earthworm improved the nematodes dispersal up to the half top of soil columns sections and increased numbers of dead *G. mellonella* larvae, numbers of dead larvae in downward movement still lower than in upward movement. Numbers of dead *G. mellonella* larvae in treatments with earthworms were 0.00, 7.00; 1.33, 11.00 and 6.67, 12.67 at sections depth 0-5, 5-10 and 10-15 cm with downward and upward, respectively.

Entomopathogenic nematodes are considered as good bio-agents for controlling cotton leafworm, *S.littoralis* (Saleh & Ragab, 1999 and Hussein, 2004). In the present investigation a significant ($P \leq 0.05$) percentage of mortality of *S. littoralis* was recorded by *S.carpocapsae* (70%) and *H. bacteriophora* (94%) in the presence of the earthworm, *A. calginosa* compared to control (without earthworm) (Fig.1). Its effect could be related to the interaction between entomogenous nematodes and earthworm during upward and downward movement (Poinar, 1978; Timper et al., 1988 and Shapiro et al., 1993). Soil texture and moisture (Molyneux and Bedding, 1984 and Wang et al., 1995) also play a vital role in this aspect.

EPNs disperse horizontally and vertically throughout the soil. Active dispersal of IJs by their own energy after inundative application is limited and differs from one species to another. It is usually a few centimeters per day and limited to scale of meters overall (Downes & Griffin, 1996 and Poinar & Hom (1986). Light- textured (sand) soil favor nematode movement (Georgis &Poinar, 1983 and Koppenhofer &Fuzy, 2006). *S. carpocapsea* IJs move upward in soil column under laboratory conditions, whereas *S. glaseri* and *H.bacteriophora* move downwards (Georgis & Poinar, 1983 and Schroeder & Beavers, 1987). *Heterorhabditis* spp. tended to migrate farther than did *Steinernema* spp. in soil arenas (Westerman, 1995; Downes & Griffin, 1996 and Koppenhofer & kaya, 1996). Natural populations of *S.carpocapsae* were found in upper 1-2 cm of the soil, whereas *H.bacteriophora* was detected through the upper 8 cm of soil (Campbell et al., 1995). When Ferguson et al. (1995) compared vertical distribution of certain EPN species, it was found that *S.carpocapsae* and undescribed *Steinernema* sp. remained near the soil surface , while *H.bacteriophora* move to greater depths.

The present study gave evidence that earthworm *A.caliginosa* may serve as phoretic host of *S. carpocapsae* (All strain) and *H.bacteriophora* (HP88). Nematodes may be dispersed on the surface of earthworms, or may be passed through the earthworm digestive system and remain viable (Shapero et al., 1995). Mortality percentages of *S.littoralis* were more pronounced with *H.bacteriophora* than did *S.carpocapsae* in the presence of *A.caliginosa*.

Entomopathogenic nematodes genera i.e. *Steinernema* and *Heterorhabditis* are used as biological control agents. They have a wide host range, are safety way for nontarget organisms, vectoring a bacterium, *Xenorhabdus* sp. and *Photorhabdus* sp., which precipitately kills the insect hosts (kaya and Gaugler 1993). In spite of their good prospective as biological control agents, results applications of nematode have been changefully. Biotic and abiotic factors that impact the efficacy of applications nematode must be scrutinized (Molyneux and Bedding, 1984).

Table 4. Number of dead *Galleria mellonella* larvae recovered from soil column sections infected with *Heterorhabditis bacteriophora* (HP88) in the presence and absence of earthworm (*Allolobophora caliginosa*).

Column depth/cm	4000 IJs of *H. bacteriophora*							
	Sandy soil				Clayey soil			
	Downward movement		Upward movement		Downward movement		Upward movement	
	No Earthworm	With Earthworm	No Earthworm	With Earthworm	No Earthworm	With Earthworm	No Earthworm	With Earthworm
0- 5	0.00 d	0.00 d	11.67 b	9.00 c	0.00 c	0.00 d	7.00 c	7.00 c
5-10	0.00 d	2.67 d	17.00 a	15.33 b	0.00 c	1.33 d	10.00 b	11.00 b
10-15	3.33 c	10.67 c	18.00 a	18.00 a	0.67 c	6.67 c	13.00 a	12.67 a
15-20	11.33 b	14.00 b	6.33 c	8.33 c	4.67 b	10.33 b	5.00 c	3.00 d
20-25	17.33 a	17.67 a	0.00 d	4.00 d	10.67 a	13.67 a	2.33 d	0.00 e
25-30	16.00 a	13.00 bC	0.00 d	0.00 E	11.67 a	11.67 b	0.00 e	0.00 e
Average Mortality	7.99 B	9.66 A	8.83 A	9.11 A	4.61 B	7.27 A	5.05 B	7.26 A

*Numbers represent means of three replications. *Heterorhabditis bacteriophora* was introduced 30 cm below the soil surface in the upward dispersal experiment and on the soil surface in the downward dispersal experiment. Twenty *G. mellonella* larvae were exposed to soil removed from column sections 2 weeks after nematodes introduction. *G. mellonella* mortality indicates relative nematode densities. * The same lowercase letter in columns or uppercase letter in rows indicates no significant differences at $P \leq 0.05$ according to Duncan's multiple range tests.

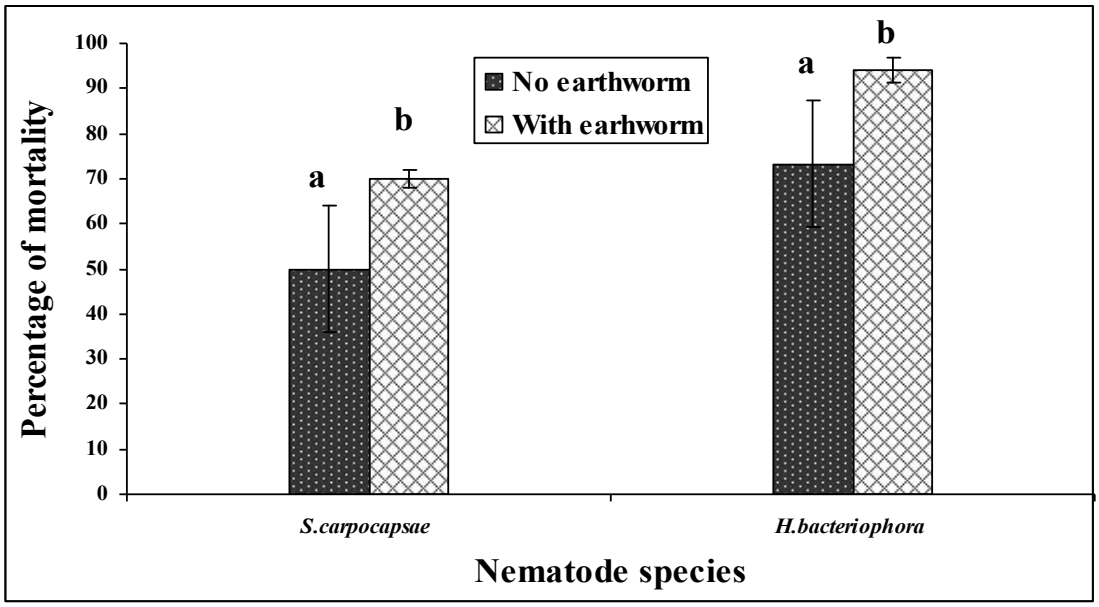

Fig. 1. Percentage of mortality caused by entomopathogenic nematodes infection of *Spodoptera littoralis* without (control) or with *Allolobophora caliginosa* 14 days post-treatment. The control contained only nematodes, *S.carpocapsae* (All strain) or *H. bacteriophora* (HP88) while treated contained nematodes and earthworm, *Allolobophora caliginosa*. Different letters above bars indicate statistically significant ($P \leq 0.05$).

Enhancing both *S. carpocapsae* and *H.bacteriophora* dispersal in the presence of earthworm *A.caliginosa* is a promising option to suppress economic damage resulting from the Egyptian cotton leafworm, *S. littoralis*. Further studies on entomogenous nematode dispersal in the presence of earthworm, are needed to confirm current findings.

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الملخص العربى

دور دودة الأرض *Allolobophora caliginosa* في تحسين المكافحة الحيوية لدودة ورق القطن الكبيرة *Spodoptera littoralis* Boisd بواسطة نيماتودا الحشرات *Steinernema carpocapsae* ونيماتودا *Heterorhabditis bacteriophora*

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حسنت ديدان الأرض من إنتشار النيماتودا الممرضة للحشرات *Steinernema carpocapsae* السلالة (All strain) كما أنها تستخدم كوسيلة لانتقال العوائل حيث ساعدت على إنتشار يرقات الطور المعدى خلال أعمدة التربة. وكانت قابلية إنتشار يرقات الطور المعدى فى التربة الرملية اوالطينية محدودة فى غياب دودة الأرض *Allolobophora caliginosa* بالمقارنة مع أنابيب البلاستيك التي احتوت على ديدان الأرض.

وبعد أسبوعين،تم تقييم الانتشار للنيماتودا عن طريق استخدام دودة الشمع الكبيرة *Galleria mellonella* ككائن تقييم حيوى وأوضحت النتائج زيادة الانتشار العمودي للنيماتودا بصورة معنوية فى وجود ديدان الأرض مقارنة بأعمدة التربة الخالية من ديدان الأرض. ومع استخدام نوعين من النيماتودا وبصورة أكبر مع النوع (السلالة HP88) *Heterorhabditis bacteriophora* ، عندما وضعت يرقات الطور المعدى على سطح أعمدة التربة ،زاد إنتشار أعداد النيماتودا بدرجة أكبر حتى عمق ٢٠- ٢٥ سم وخاصة في التربة الرملية من تلك الأعداد التي وضعت فى النصف السفلى لأعمدة عندما تواجدت ديدان الأرض عن تلك التى غابت بها ديدان الأرض والعكس بالعكس في غيابِ دودةِ الأرض . ولهذا ،يمكن استخدام ديدان الأرض كناقل لإدخال أو لنشر الكائنات الحية المفيدة.

وتوضح نتائج التجربة الحالية أن النيماتودا *S. carpocapsae* (السلالة All) والنيماتودا (السلالة HP88) *H. bacteriophora* قد حسنت من مكافحة دودة ورق القطن الكبيرة *S.littoralis* Boisd في وجود دودة الأرض وأحدثت خفض معنوى فى دودة ورق القطن الكبيرة *S. littoralis* بنسبة ٧٠ و ٩٤ % على التوالى عن تجربة المقارنة التى غابت فيها دودةِ الأرض