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Efficacy of entomopathogenic nematodes against *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (H.) (Lepidoptera: Noctuidae)

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Abstract

The study aimed to evaluate the efficacy of the EPNs against the larvae of Egyptian cotton leaf worm *Spodoptera littoralis* (Boisduval) and the black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) in vitro before in vivo study. The susceptibility of both larval species to the entomopathogenic nematode species, *Steinernema monticolum* and *Heterorhabditis bacteriophora*, was evaluated under laboratory conditions. The concentration of 400 IJs/dish for *S. monticolum* achieved up to 97.77 and 95.55% mortality rates of the 5 larval instars from 2nd to 6th instars of *S. littoralis* and *A. ipsilon*, respectively after 72 h. The concentration of 800 IJs/dish recorded larval mortality rates of 41.86 to 100% against 2nd to 6th instars of *A. ipsilon* larvae, after 72 h. At the lowest concentration (50 IJs/dish), the larvae of *S. littoralis* were more susceptible to *H. bacteriophora* than the larvae of *A. ipsilon*. The data indicated that 200 IJs/dish was the most effective concentration for all larval stages of both insect pests because the mortality percentage was 100%.

Keywords: Entomopathogenic nematode, *Spodoptera littoralis*, *Agrotis ipsilon*, *Steinernema monticolum*, *Heterorhabditis bacteriophora*, Biological control, Basil

Introduction

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.), and the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), are the most important insect pests on many crops as they cause economic losses (El-Sheikh et al. 2013). Extensive studies have been conducted in the field of biological control of insect pests, using many bio-control agents such as entomopathogenic nematodes (EPNs). The Heterorhabditidae and Steinernematidae families live in soils and are deadly parasites to a wide range of insects (Stuart et al. 1997; Orozco R.A. et. al. 2014). They are environmentally safe agent as they do not cause any harmful effects either to humans or farm animals and are beneficial insects (van Zy C. and Malan A.P. 2014). Heterorhabditidae and Steinernematidae have a symbiotic association with the entomopathogenic bacteria genera *Photorhabdus* and *Xenorhabdus*, respectively, and both effectively parasitize and kill their insect hosts (Ehlers 2001). When encountering a suitable host, the infective juveniles (IJs) enter the host via natural openings such as the spiracles, mouth, or anus (Griffin et al. 2005; Atwa A. 2011; Atwa A. 2014; and Gozel and Gozel, 2016). The bacteria grow rapidly in the hemolymph of insect host and produce toxins that kill the host by means of inducing septicemia within 24 to 72 h of infection (Ehlers 2001 and Griffin et al. 2005). Since the first use of the EPN, *Steinernema glaseri* against the white grub *Popillia japonica* in New Jersey (USA) (Glaser and Farrell 1935), no inferior hazards or damages have been recorded by the EPNs to the environment. The application of EPNs is widespread in many parts of the world and could be grown experimentally in large quantities at relatively low costs (Shapiro-Ilan et al. 2006 and Mutegi et al. 2018).

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Numerous issues indicated that the EPNs are also potent and effective for selected insect species; therefore, they are used as a biocontrol agent, instead of pesticides.

Therefore, the present study aimed to evaluate the efficacy of the two EPNs *S. monticolum* and *H. bacteriophora* against *S. littoralis* and *A. ipsilon* larvae under laboratory conditions.

Materials and methods

The study was carried out under laboratory conditions in 2019, to evaluate the efficacy of the EPNs against *S. littoralis* and *A. ipsilon* larvae under laboratory conditions in the Vegetables Pests Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt.

Rearing of *Spodoptera littoralis*

The field strain of *S. littoralis* was obtained from an open-field tomato farm at Giza Governorate, Egypt, was transferred to the laboratory of Vegetables Pests Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt, and was reared at 25 °C ± 2 °C and 65–75 RH% for mass production. *S. littoralis* adults were placed in glass jars and fed on castor bean leaves (*Ricinus communis* L.) (Zhang et al. 2019a). The jars were provided every day with castor bean leaves as a source of food for the larvae. The 6th larval instar was allowed to pupate in larger jars, containing dry saw dust. The pupae were transferred to Petri dishes containing tissue paper and kept in suitable cages for mating after extruding of moths from pupae. The emerging adults were fed on 20% honey solution and allowed to lay their eggs on the provided leaves of *Nerium oleander* as a physical surface for moth mating, oviposition, and resting processes.

Rearing of *Agrotis ipsilon*

Twenty individuals of newly emerged *A. ipsilon* moths were obtained from the cutworm department, transferred to the laboratory of Vegetables Pests Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt, and kept in glass jars covered with pieces of tissue secured in position by rubber bands. Honey solution of 20% concentration was used as food for adults. Females allowed to lay their eggs on muslin strips that were fixed on the top of the jars. These strips were transferred into Petri dishes and, after egg-laying, kept in an incubator under constant temperature of 25 °C ± 1 °C and 70–80 RH% (Zhang et al. 2019b), until hatching. The newly hatched larvae were transferred into small glass jars and provided daily with castor leaves as a source of food. The 4th-instar larvae were separated individually or in small groups in a glass plate to avoid cannibalism.

Susceptibility of *S. littoralis* and *A. ipsilon* larvae to EPNs

Two species of nematodes were used in the present study, *Heterorhabditis bacteriophora* (Poinar 1990) (strain HP88) and a Korean species, *Steinernema monticolum* (Stock et al. 1997), according to Ibraheem (2015). Both nematode species were reared at 26 °C on late-instar larvae of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Galleridae), following the method of Woodring and Kaya (1998). The nematode infective juveniles (IJs) that emerged from insect cadavers were recovered, using modified White traps (Kaya and Stock 1997). After storage at 10 °C for 1 week, they were allowed to acclimatize at room temperature for 45–60 min and their viability was checked by observation of movement under the zoom stereomicroscope (Ibraheem 2015).

Petri dish assays

Petri dishes containing 2 moist filter papers with 5 cm^3 water were used for bioassays of the larvae 2nd, 3rd, 4th, 5th, and 6th instars of *S. littoralis* and *A. ipsilon*. The 1st-instar larvae of both species were excluded in this test due to the difficulty of handling them. Ten individuals/dish were exposed to the IJs of each nematode species. Six nematode concentrations 50, 100, 200, 400, 800, and 1600 IJs/dish were used. The basil leaves were used as food for the larvae. Petri dishes were maintained in an incubator at 26 ± 2 °C. Four hundred and eighty dishes were used in the experiments including 2 nematode species × 6 concentrations × 2 insect species × 5 instars × 4 Petri dishes/concentration (3 replicates + 1 control). The control was exposed to the same laboratory conditions of the treatments, except that no nematode IJs were added to the control. Inspection was carried out at 24, 48, and 72 h to record the mortality percentage. The presence of the nematodes inside the insect cadavers were ensured by inspection to confirm the nematode's infection. The mortality was corrected using Abbott's formula (Abbott 1925).

Statistical analysis

Recorded data of the mortality rates were corrected, using Abbott's correction (Abbott 1925). Statistical analysis was done using analysis of variance (ANOVA) by SAS program 1999.

Results and discussion

Susceptibility of immature stages of *S. littoralis* and *A. ipsilon* to EPNs

The bioassay of Steinernema monticolum

The data obtained from Table 1 show that the susceptibility of *S. littoralis* 2nd larval instar to infection with the nematode *S. monticolum*, after 72 h with the concentrations 50, 100, 200, 400, 800, and 1600 IJs/dish, was 77.78, 83.33, 88.66, 91.11, 100, and 100% of percentage mortalities, respectively. However, Table 2 shows that

Table 1 Efficacy of different concentrations of the entomopathogenic nematode *Steinernema monticolum* against larval instars of *Spodoptera littoralis* in the laboratory

Nematode concentrations	Mean % mortality of larval instars at different exposure times																			
IJs/dish	2nd instar				3rd instar				4th instar				5th instar				6th instar			
	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average
50	22.22	46.66	77.78	48.88	15.55	31.11	55.55	34.07	2.22	60	75.55	45.92	2.32	27.9	58.13	29.45	4.76	21.42	45.23	23.80
100	28.88	60	93.33	60.71	16.27	37.2	69.76	41.07	11.11	46.66	82.22	46.66	6.66	42.22	75.55	41.47	6.66	35.55	66.66	36.29
200	31.11	53.33	86.66	57.03	17.77	53.33	71.11	47.40	15.55	57.77	80	51.11	17.7	55.55	82.22	51.82	9.52	38.09	64.28	37.29
400	35.55	51.11	91.11	59.25	22.72	65.9	90.9	59.84	17.77	73.33	95.54	62.21	26.66	64.44	97.77	62.95	24.44	60	91.1	58.51
800	44.44	46.66	100	63.7	24.44	60	95.55	59.99	6.66	93.33	100	66.66	30.23	72.09	100	67.44	35.71	52.38	100	62.69
1600	31.11	57.77	100	62.96	40	55.55	100	65.18	17.77	84.44	100	67.40	34.88	67.44	100	67.44	42.85	61.9	100	68.25
F value	2.54				7.58				3.38				61.11				25.26			
LSD	10.75				14.09				17.01				6.25				11.123			

Table 2 Efficacy of different concentrations of the entomopathogenic nematode *Steinernema monticolum* against larval instars of *Agrotis ipsilon* in the laboratory

Nematode concentrations	Mean % mortality of larval instars at different exposure times																			
IJs/dish	2nd instar				3rd instar				4th instar				5th instar				6th instar			
	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average
50	6.67	15.55	51.11	24.44	24.44	35.55	35.56	31.85	4.44	33.33	46.67	28.15	0	2.22	6.66	2.96	0	17.77	26.66	14.81
100	38.63	50	59.09	49.24	45.45	70.45	81.81	65.90	22	66.66	73.33	53.99	0	20	33.33	17.77	0	22.22	28.88	17.03
200	51.11	57.77	68.88	59.25	55.55	66.66	73.33	65.18	28.88	44.44	82.22	51.85	0	37.77	53.33	30.37	0	35.55	48.88	28.14
400	62.22	68.88	73.33	68.14	77.77	88.88	95.55	87.4	37.77	75.55	95.55	69.63	2.22	35.55	55.55	31.11	6.66	37.77	55.55	33.33
800	77.77	97.77	100	91.84	95.55	100	100	98.52	37.77	95.55	100	77.77	2.32	58.13	76.74	45.73	13.95	25.58	41.86	27.13
1600	80	95.55	100	91.85	95.55	100	100	98.52	71.11	95.55	100	88.88	11.35	61.36	81.81	51.51	13.95	58.13	81.39	51.16
F value	35.72				53.67				15.56				5.39				6.83			
LSD	13.694				11.049				17.29				24.207				15.792			

Table 3 Efficacy of different concentrations of the entomopathogenic nematode *Heterorhabditis bacteriophora* (HP88) against larval instars of *Spodoptera littoralis* under laboratory condition

Nematode concentrations	Mean % mortality of larval instars at different exposure times																			
IJs/dish	2nd instar				3rd instar				4th instar				5th instar				6th instar			
	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average
50	**44.44**	**68.88**	**100**	**71.11**	**51.11**	**80**	**100**	**77.04**	**17.78**	**73.33**	**100**	**63.70**	**20**	**77.77**	**97.77**	**65.18**	**22.22**	**48.88**	**68.89**	**46.66**
100	**57.77**	**82.22**	**100**	**80**	**44.18**	**72.09**	**100**	**72.09**	**28.89**	**73.33**	**100**	**67.41**	**31.82**	**100**	**100**	**77.27**	**13.33**	**35.55**	**84.44**	**44.44**
200	**64.44**	**100**	**100**	**88.15**	**71.11**	**100**	**100**	**90.37**	**60**	**71.11**	**100**	**77.04**	**70.46**	**100**	**100**	**90.15**	**22.22**	**66.66**	**100**	**62.96**
400	**97.78**	**100**	**100**	**99.26**	**100**	**100**	**100**	**100**	**88.89**	**100**	**100**	**96.30**	**100**	**100**	**100**	**100.00**	**24.44**	**86.67**	**100**	**70.37**
800	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100.00**	**48.89**	**100**	**100**	**82.97**
1600	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100.00**	**37.78**	**84.44**	**100**	**74.07**
F value	2.59				2.64				2.39				1.66 insig.				6.19			
LSD	23.895				24.378				34.072								19.498			

Table 4 Efficacy of different concentrations of the entomopathogenic nematode *Heterorhabditis bacteriophora* (HP88) against larval instars of *Agrotis ipsilon* under laboratory condition

Nematode concentrations	Mean % of larval instars at different exposure times																			
IJs/dish	2nd instar				3rd instar				4th instar				5th instar				6th instar			
	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average
50	**31.11**	**57.77**	**73.33**	54.07	**28.88**	**55.55**	**64.44**	49.63	**8.88**	**73.33**	**84.44**	55.55	**15.55**	**31.11**	**48.89**	31.85	**15.9**	**40.9**	**68.18**	41.66
100	**42.22**	**75.55**	**97.77**	71.85	**32.55**	**44.18**	**86.04**	54.26	**18.18**	**47.72**	**90.9**	52.27	**6.81**	**52.27**	**84**	47.69	**13.33**	**35.55**	**80**	42.96
200	**55.55**	**75.55**	**100**	77.03	**62.22**	**73.33**	**100**	78.52	**28.88**	**64.44**	**100**	64.44	**27.27**	**54.54**	**100**	60.60	**17.77**	**40**	**97.78**	51.85
400	**35.55**	**51.11**	**100**	62.22	**22.72**	**65.9**	**100**	62.87	**17.77**	**73.33**	**100**	63.7	**26.66**	**64.44**	**100**	63.7	**24.44**	**60**	**100**	61.48
800	**82.22**	**95.55**	**100**	92.59	**53.33**	**97.77**	**100**	83.7	**15.9**	**95.45**	**100**	70.45	**58.13**	**81.39**	**100**	79.84	**46.67**	**64.44**	**100**	70.37
1600	**73.33**	**93.33**	**100**	88.89	**55.55**	**91.11**	**100**	82.22	**35.55**	**86.66**	**100**	74.07	**44.18**	**100**	**100**	81.39	**42.85**	**90.47**	**100**	77.77
F value	6.51				5.64				2.02				7.92				7.37			
LSD	18.452				19.774				18.548				21.274				17.114			

the susceptibility of *A. ipsilon* 2nd larval instar to infection with the nematode *S. monticolum*, after 72 h with the concentrations 50, 100, 200, 400, 800, and 1600 IJs/dish, was 51.11, 59.09, 68.88, 73.33, 100, and 100% of percentage mortalities, respectively.

The obtained results indicated that the 2nd instar of both larval insects was of high susceptibility than the other larval instars, in addition to the ones under the tested concentrations of EPNs.

Mortality rates were much higher in *S. littoralis* than in *A. ipsilon* after nematode application. This indicated that the 5th and 6th larval instars of *S. littoralis* were highly susceptible to *S. monticolum* infection than that of *A. ipsilon* under the same conditions.

The susceptibility of immature stages of *A. ipsilon* to *H. bacteriophora* was studied by Ebssa and Koppenhöfer (2012), who recorded a high mortality (90%) of *A. ipsilon* larval in laboratory. The mechanisms of nematode infection to insect larvae has been illustrated by Shapiro-Ilan (2009) who stated that once a host is located, the nematodes enter the host through natural opening (spiracles, anus, and mouth) or by directly penetrating through thin layers in the cuticle. Therefore, the direct penetration of the hosts' cuticle commonly occurs in Heterorhabditis that are equipped with a dorsal tooth. Moreover, the *H. bacteriophora* nematode individuals are hermaphrodite; if one is able to enter the cavity of the body insect, it can continue the life cycle and cause death, and this may be discussed in the variety between the larval mortality rates, in the case of using *H. bacteriophora* (HP88) suspension as compared to *S. monticolum.*

The bioassay of Heterorhabditis bacteriophora (HP88)

The data obtained from Tables 3 and 4 show that the susceptibility of *S. littoralis* 2nd, 3rd, 4th, 5th, and 6th larval instars infected by *H. bacteriophora*, after 72 h with the concentrations 50, 100, 200, 400, 800, and 1600 IJs/dish, was almost 100% of percentage mortalities. The results were recorded for *A. ipsilon* 2nd, 3rd, 4th, 5th, and 6th larval instars to infection above 80% by *H. bacteriophora*. Concerning the efficacy of different concentrations of the EPNs, *S. monticolum* and *H. bacteriophora* (HP88), against immature stages of *S. littoralis* and *A. ipsilon* in the laboratory, it could be clear that the 2nd-instar larvae of *S. littoralis* were more susceptible to the infection with EPNs. Because the 2nd-instar larvae have a thin cuticle surface, this leads to ease the direct penetration of nematodes; therefore, the full infection requires a low concentration of EPNs to cause death.

Based on the obtained results, in the nematode species of *H. bacteriophora* (HP88), it is found that the concentration of 200 IJs/dish caused the highest mortality (100%) for all larval instars of both insects, which agreed with those of Abdel-Razek and Abd-Elgawad (2007) who reported that *Heterorhabditis* sp. *ELG*., *H. indica*, and *Heterorhabditis* sp. *ELB*. were with the highest activity, giving a 100% mortality to *S. littoralis* larvae in a Petri dish assay after 24 h post exposure, while *S. monticolum* caused the highest mortality rate for larvae (100%) at the concentration of 1600 IJs/dish for all larval instars. Despite the results obtained from Hassan et al. (2016) who reported that the effect of *S. glaseri* was greater than the nematode, *H. bacteriophora*, the obtained results agree with those of Shairra and Nouh (2014) who reported that higher concentrations of nematodes caused an acute effect, while the latent effect was observed in the case of lower ones and Shairra (2007) who found a positive relationship between concentration and larval mortality, mainly due to the concentration of IJs. However, the defense reactions against the nematodes and their associated bacteria may play an important role. EL-Bishry et al. (2002) demonstrated that nematode dose, IJs age, expos. Similar results were obtained by Shamseldean et al. (1995) who recorded that *H. bacteriophora* (HP88) achieved 64% at 35 °C to 100% at 25 °C mortality of *S. littoralis*.

Conclusion

The main objective of this research is to work on producing medicinal and aromatic crops (basil), which are in great demand, whether for export of pharmaceutical and aromatic industries, which is necessary to non-use of chemicals in production processes, especially for pest control. Therefore, extensive studies have been conducted in the field of biological control of insect pests, using many bio-control agents such as (EPNs). It is possible to rely on the results of this research in preparation for use in field application for safe control of pests.

Abbreviations

GDP: Gross domestic product; BCW: Black cutworm; EPNs: Entomopathogenic nematodes; IJs: Infective juveniles; RH%: Relative humidity; HP88: Strain of *Heterorhabditis bacteriophora*; EPN: Entomopathogenic nematode; *n*: Insect numbers; T: Treated; Co: Control

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Authors' contributions

All authors contributed 100% participation. The authors Hassan M. Sobhy, Nagwa A. Abdel-Bary, Farid A. Harras, Farha H. Faragalla, and Hussein I. Husseinen contributed the following: suggesting and putting the idea, preparing the manuscript writing and finishing the paper, and data analysis. The authors read and approved the final manuscript.

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