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

Hassan M. Sobhy 1, Nagwa A. Abdel-Bary 2, Farid A. Harras 3, Farha H. Faragalla 3 and Hussein I. Husseinen 3*

**Abstract**

The study aimed to evaluate the efficacy of the EPNs against the larvae of Egyptian cotton leaf worm *Spodoptera littoralis* (Boisd.) 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 septicaemia 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 Mutegei et al. 2018).

© The Author(s). 2020 Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.
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 cm³ 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 tests 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 *Steinemema monticolum* against larval instars of *Spodoptera littoralis* in the laboratory

| Nematode concentrations | Mean % mortality of larval instars at different exposure times | 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 |
| 50                      |                                                               | 22.22 | 46.66 | 77.78 | 48.88 | 15.55 | 31.11 | 55.55 | 34.07 | 2.22 | 60 | 75.55 | 45.92 | 2.22 | 60 | 75.55 | 45.92 | 2.22 | 60 | 75.55 | 45.92 |
| 100                     |                                                               | 28.88 | 60 | 93.33 | 60.71 | 16.27 | 37.2 | 69.76 | 41.07 | 11.11 | 46.66 | 82.22 | 46.66 | 666 | 42.22 | 75.55 | 41.47 | 666 | 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 | 82.22 | 51.11 | 17.7 | 55.55 | 82.22 | 51.82 | 952 | 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 | 62.11 | 2666 | 64.44 | 97.77 | 62.95 | 2444 | 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 | 3023 | 72.09 | 100 | 67.44 | 3571 | 52.38 | 100 | 62.69 |
| 1600                    |                                                               | 31.11 | 57.77 | 100 | 62.96 | 40 | 55.55 | 100 | 65.18 | 17.77 | 84.44 | 100 | 67.4 | 3488 | 67.44 | 100 | 67.44 | 4285 | 61.9 | 100 | 68.25 |
| F value                 |                                                               | 2.54 | 7.58 | 3.38 | 61.11 | 25.26 |
| LSD                     |                                                               | 10.75 | 14.09 | 17.01 | 625 | 11.123 |
Table 2 Efficacy of different concentrations of the entomopathogenic nematode *Steinemema monticulum* against larval instars of *Agrotis ipsilon* in the laboratory

| Nematode concentrations | Mean % mortality of larval instars at different exposure times |
|-------------------------|---------------------------------------------------------------|
|                         | 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 | 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 | 232 | 58.13 | 76.74 | 45.73 | 1395 | 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 | 1135 | 61.36 | 81.81 | 51.51 | 1395 | 58.13 | 81.39 | 51.16 |

$F$ value: 35.72 53.67 15.56 539 683

LSD: 13.694 11.049 17.29 24.207 15.792
| Nematode concentrations | Mean % mortality of larval instars at different exposure times | 2nd instar | 3rd instar | 4th instar | 5th instar | 6th instar |
|------------------------|---------------------------------------------------------------|------------|------------|------------|------------|------------|
|                        |                                                               | 24 h       | 48 h       | 72 h       | 24 h       | 48 h       | 72 h       | 24 h       | 48 h       | 72 h       | 24 h       | 48 h       | 72 h       | Average |
| 50                     |                                                               | 44.44      | 68.88      | 100        | 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        | 100        | 100        | 88.89      | 100        | 100        | 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        | 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        | 100.00    | 37.78      | 84.44      | 100        | 74.07     |
| F value                |                                                               | 2.59       | 2.64       | 2.39       | 1.66 insig.| 6.19       | 23.895     | 24.378     | 34.072     | 19.498     | 200        | 44.44      | 100        | 100        | 100        | 100       | 100        | 100.00    | 48.89      | 100        | 100        | 82.97     |
| LSD                    |                                                               | 19.498     | 24.378     | 34.072     | 19.498     | 200        | 44.44      | 100        | 100        | 100        | 100        | 100        | 100        | 100        | 100        | 100       | 100        | 100.00    | 48.89      | 100        | 100        | 82.97     |
| Nematode concentrations | Mean % of larval instars at different exposure times |
|-------------------------|-----------------------------------------------|
|                         | 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 |
| 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

**LSD**: 18.452
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 Shamseldan 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

**Acknowledgements**
Not applicable

**Authors’ contributions**
All authors contributed 100% participation. The authors Hassan M. Sobhy, Nagwa A. Abdel-Bary, Farid A. Haras, Farha H. Faragalla, and Hussein I. Hussein 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.

**Funding**
No funding

**Availability of data and materials**
Not applicable
Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declared that the present study was performed in the absence of any conflict of interest or competing interests.

Author details
1Department of Natural Resource, Faculty of African Post Graduate Studies, Cairo University, Giza, Egypt. 2Department of Zoology and Agricultural Nematology, Cairo University, Faculty of Agriculture, Giza, Egypt. 3Department of Vegetable, Medicinal, Aromatic and Ornamental Plant Pests, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt.

Received: 21 March 2020 Accepted: 17 May 2020
Published online: 15 June 2020

References
Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265–267
Abdel-Razek AS, Abd-El-Gawad MM (2007) Investigation on the efficacy of entomopathogenic nematodes against Spodoptera litura (Bioso) and Galleria mellonella (L.). Arch Phytopathol Plant Protect 40(6):414–422
Atwa, AA; (2011). Mode of actions and field applications of entomopathogenic nematodes. In "Microbial insecticides: principles and applications" (Eds.) L. Francis Borgio, K. Sahayaraj & L. Alper Susuruk, pp. 211 - 236, Nova Science Publishers, Inc. New York, USA.
Atwa AA (2014) Entomopathogenic nematodes as biopesticides. In: Sahayaraj K (ed) Basic and applied aspects of biopesticides. Springer, India, pp 69–98. https://doi.org/10.1007/978-81-322-1877-7_5
Ebisa, L. and Koppenhöfer, A.M. (2012). Entomopathogenic nematodes for the management of Agrotis ipsilon: effect of instar, nematode species and nematode production method. 68: 947-957.
Ehlers R (2001) Mass production of entomopathogenic nematodes for plant protection. Appl Microb Technol 56:623–633
El-Bishay MF, El-Assal FM, Abd El-Rahman RM (2002) Factors affecting penetration rate of the entomopathogenic nematodes Heterorhabditis spp. Egypt J Biol Pest Cont 12(1):25–38
El-Sheikh TAA, Rafea HS, El-Aasar AM, Ali SH (2013) Biological and biochemical effects of bacillus thuringiensis, Serata mansorensis and Teflubenzuron on cotton leafworm. Egypt J Agric Res 91(4):1327–1345
Glaser RW, Farrell CC (1935) Field experiments with the Japanese beetle and its nematode parasite. Journal New York Entomology, Society 43:345–371
Usur Gozel and Cigdem Gozel (2016) Entomopathogenic nematodes in pest management. In: Harsimran Gill, Gaurav Goyal (eds) Integrated Pest Management (IPM): Environmentally Sound Pest Management. BoD – Books on Demand.
Griffin CT, Boermere NE, Lewis EE (2005) Biology and behaviour. In: Grewal PS, Ehlers R-U, Shapiro-Ilan DI (eds) Nematodes as biocontrol agents. CAB Publishing, Wallingford, UK, pp 47–75
Hassan HA, Shairra SA, Ibrahim SS (2016) Virulence of entomopathogenic nematodes Steinernema glaseri and Heterorhabditis bacteriophora Poinar (HP88 strain) against the black cutworm, Agrotis ipsilon. Egypt Acad J Biol Sci 9(1):33–48
Ibraheem, H. I. H. (2015) Entomopathogenic nematodes as biocontrol agents of Tuta absoluta on tomato plants M.Sc. Thesis Fac. Agric., Cairo Univ.80 pp.
Kaya HK, Stock SP (1997) Techniques in insect nematology. In: Lacey LA (ed) Manual of techniques in insect pathology. Academic Press, San Diego, pp 281–324
Mutegi, D. M.; Dora, K; John, W. K. and Charles, W. (2018). Integrated use of Kenyan entomopathogenic nematodes (Steinernema species) and neem against Tuta absoluta on tomato. International Journal of Research in Agricultural Sciences(4): 2348 – 3997.
Orozco, RA; Lee M. and Stock S. P. (2014). Soil sampling and isolation of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae). J. Vis. Exp. (89): e52083 | Page 1 of 8
SAS Institute. 1999. SAS/STAT: guide for personal computers. SAS Institute.

Shairra, S. A. (2007). Effects of entomopathogenic nematodes and some pharmaceutical inhibitors of eicosanoid biosynthesis on the desert locust Schistocerca gregaria (Forsk.) I. Ph. D. Thesis, Fac. Sci., Cairo Univ., Egypt, 65.
Shairra SA, Nouh GM (2014) Efficacy of entomopathogenic nematodes and fungi as biological control agent against the cotton leaf worm, Spodoptera littoralis (Boisd). Egyptian Journal of Biological Pest Control 24(1):247–253
Shamseldeen MM, Abd-El-Gawad MM, Atwa AA (1995) Evaluation of four entomopathogenic nematodes against Spodoptera littoralis (Lepid., Noctuidiae) larvae under different temperatures. Anzeiger Schadlingskde, Pflanzenschutz, Umweltschutz 69:111–113
Shapiro-Ilan DI (2009) Characterization of biological traits in the entomopathogenic nematode Heterorhabditis georgiana (Kesha strain), and phylogeny analysis of the nematode’s symbiotic bacteria. Biol Control 51:387
Shapiro-Ilan DI, Gough DH, Piggott SJ, Patterson Fife J (2006) Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. Biol Control 38:124–133
Shams el Deeb M.K. (2004) Steinernema carpocapsae (Heterorhabditidae: Steinernematidae) and its effect on the tomato leafworm Spodoptera littoralis. Egypt Acad J Biolog Sci 9(1):33–48
Stuart RJ, Polavarapu S, Lewis EE, Gaugler R (1997) Differential susceptibility of Dymiscoccus vaccini (Homoptera: Pseudococcidae) to entomopathogenic nematodes (Heterorhabditidae and Steinemematidae). Econ Entomol 90:925–932
van Vy C, Malan AP (2014) The role of entomopathogenic nematodes as biological control agents of insect pests, with emphasis on the history of their mass culturing and in vivo production. African Entomology 22(2):255–249
Woodring JL, Kaya HK (1998) Steinernematid and heterorhabditid nematodes: a handbook of techniques. Arkansas Agricultural Experiment Station, Fayetteville, Arkansas. South Cooper Bull 331:1–30
Zhang M, Demeshko Y, Dumbart R, Iven T, Feusner L, Lebedov G, Ghanim M, Barg R, Ben-Hayyim G (2019a) Elevated α-linolenic acid content in extra-plantidial membranes of tomato accelerates wound-induced jasmonate generation and improves tolerance to the herbivorous insects Heliothis peltigera and Spodoptera littoralis. J Plant Growth Regul 38:223–738
Zhang Z, Xu C, Ding J, Zhao Y, Lin J, Liu F, Mu J (2019b) Cyantraniliprole seed treatment efficiency against Agrotis ipsilon (Lepidoptera: cutidae) and residue concentrations in corn plants and soil. Pest Manag Sci 75:464-1472

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen journal and benefit from:  
- Convenient online submission  
- Rigorous peer review  
- Open access: articles freely available online  
- High visibility within the field  
- Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com