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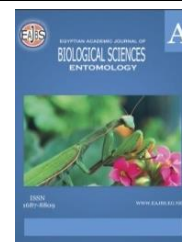
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Laboratory Valuation of The Efficacy of Entomopathogenic Nematodes Against Some Insect Pests of the Potato crop (*Solanum tuberosum* L.)

Gehan M. Nouh

Biological Control Dept., Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt

E-mail:gehannoah@yahoo.com

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ABSTRACT

The potato crop (*Solanum tuberosum* L.) is one of Egypt's most fundamental food crops and global. It is an onslaught by many insect pests that as the potato tuber moth, *Phthorimaea operculella* (Zeller), the hard black beetle, *Pentodon bispinosus* (Kuster), and the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval). The current research aims to study the efficacy of entomopathogenic nematodes (EPNs); *Heterorhabditis bacteriophora*, *Steinernema glaseri*, and *Steinernema carpocapsae*, against some major pest species that infest the potato crop. The experiment of *P. operculella* 4th larval instars and pupae treated with *H. bacteriophora* and *S. carpocapsae* gave the highest mortality compared with *S. glaseri*. The LC₅₀ values of *H. bacteriophora* and *S. carpocapsae* were calculated in the 4th instar larvae of *P. operculella* as 127.1496 and 148.5994 IJs/larva, respectively. While the LC₅₀ value of *S. glaseri* recorded, was 247.2687 IJs/larva. Treatment of *P. bispinosus* larvae *S. glaseri* showed the highest efficacy against the 2nd and 3rd instars larvae of *P. bispinosus* compared with *H. bacteriophora* and *S. carpocapsae*. The LC₅₀ values of *S. glaseri* were 1073.640 and 1330.312 IJs/larva to combat the 2nd and 3rd instars larvae of *P. bispinosus*. In the experiment of *S. littoralis* on 3rd and 5th instar larvae, the *H. bacteriophora* and *S. carpocapsae* showed higher mortality than *S. glaseri*. LC₅₀ values of *H. bacteriophora* and *S. carpocapsae* were (38.489 and 40.791) and (42.149 and 51.932) IJs/larva in the 3rd and 5th instar larvae of *S. littoralis*, respectively. While in the case of *S. glaseri*, correspondent LC₅₀ values recorded were 70.106 and 95.295 IJs/larva. *H. bacteriophora* recorded the most elevated mortality in (the 4th larval instars and pupae of *P. operculella*) and (3rd and 5th instar larvae of *S. littoralis*), followed by *S. carpocapsae*. *S. glaseri* recorded the highest mortality (100%) of 2nd and 3rd instars larvae of *P. bispinosus* compared with *H. bacteriophora* and *S. carpocapsae*. The results also concluded that *S. littoralis* was the most sensitive to the use of EPNs, followed by *P. operculella* and then *P. bispinosus*. EPNs can be relied on as successful biocontrol agents against soil-dwelling pests and add nematodes to pest control programs.

INTRODUCTION

Potato *Solanum tuberosum* L. is a principal food crop in Egypt and many countries of the world. Egypt is among the world's largest potato exporters (Mohamed *et*

al., 2013). In the field, potato plants are onslaught by several insect pests like the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), the hard black beetle, *Pentodon bispinosus* (Kuster) (Coleoptera: Scarabaeidae), and cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) (Kepenekci *et al.*, 2013). Potato tuber moth, *P. operculella* is an extremely dangerous pest in storerooms and potato fields. The female *P. operculella* lays its eggs on the underside of the potato leaves or the revealed tubers, and the invasion rate may be as high as 100% (Alyokhin, 2012). Larvae infest leaves, stems, and tubers, and the infestation of tubers and leaves may be equal (Abd El-Salam and Teixeira da Silva, 2010). During storage, *P. operculella* causes a partial or a complete rot with infestation due to larval feeding on tubers making them unmarketable (Sarhan, 2004). The white grubs' *P. bispinosus* is an important root-feeding pest (Koppenhofer and Eugene, 2008). In Egypt, white grubs are one of the main pests of the potato crop. The first instar larvae feed on organic substances, while the second and third instar larvae migrate under the soil to feed on roots or tubers (Frew *et al.*, 2016). Third-instar larvae are responsible for causing damage to the tubers (Coy *et al.*, 2019). The white grubs can cause injuries to tubers and reduce the crop quality, causing infection in tubers with many fungal and bacterial diseases (Giordanengo *et al.*, 2013). The Egyptian cotton leafworm, *S. littoralis* (Boisd.) (Lepidoptera: Noctuidae), is one of the most economic agricultural pests (Quero, 2002) that feeds on the leaves of several field crops and vegetables in Egypt and many nations in the world (El-Kholy and Rahouma, 2018). The potato crop (*Solanum tuberosum*) is one of the major vegetables on which *S. littoralis* larvae feed, and adults prefer to lay eggs on its leaves (Fowler and Lakin, 2001). Continuing utilization of insecticides against insect pests resulted in resistant generations to the insecticides used (Rizk *et al.*, 2010). Therefore, natural control agents, including the entomopathogenic nematodes (EPNs), can be applied to dominate these pests, as they do not pollute the environment and do not cause resistance to the insect pests (Shamseldean *et al.*, 2009). Nematodes kill the insect pests in the soil by septicemia within 24 to 72 h. of infestation (Griffin *et al.* 2005).

The study aspired to evaluate the effectiveness of EPNs, against some major pests that infest the potato crop under laboratory conditions.

MATERIALS AND METHODS

Target Insects:

The Potato Tuber Moth, *Phthorimaea operculella*:

The potato tuber moth, *P. operculella* used in this investigation was obtained from the Department of Crop Pests, Plant Protection Research Institute, ARC, Giza Egypt. The insect was grown under laboratory conditions (30±2°C and 65-70% R.H). The insect was offered potato (*S. tuberosum*) as a source of diet inside plastic jars with sawdust in the bottom to soak up humidity and covered with a cotton cloth installed with a rubber band until the pupation.

The Hard Black Beetle, *Pentodon bispinosus*:

The 2nd and 3rd instar larvae of the white grubs, *Pentodon bispinosus* were collected from a potato farm in the Noubaria area, Beheira Governorate, Egypt. White grubs were found by digging under the potato plants, and around their roots. The collected white grubs were positioned in a plastic container (3 kg capacity, 25 cm height, and 12 cm diameter), half-filled with moistened sterile sandy soil, wrapped with a cotton cloth, and were fed for a week on the roots of the potato plants before employing the larvae in lab tests. White grubs were examined after that, and the healthy 2nd and 3rd instar larvae were selected for utilization in lab tests.

The Egyptian Cotton Leafworm, *Spodoptera littoralis*:

Larvae of *S. littoralis* were compiled from potato fields in the Noubaria area, Beheira Governorate, Egypt, and reared in the laboratory and fed on castor (*Ricinus communis* L.) leaves until pupae emerged. The pupae were transferred to plastic jars (2 kg capacity) until adults emerged. While the emerging adults were fed on 20% honey solution and even mate and lay eggs (Zhang *et al.* 2019a).

Entomopathogenic Nematode Species:

The EPN, *H. bacteriophora* Pionar was isolated by Pionar (1975) and *S. glaseri* was isolated by (Steiner, 1929). Meanwhile, *S. carpocapsae* Weiser was isolated by Weiser (1955). Nematode species were cultivated *in vivo* on the full-grown larvae of the greater wax moth *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae), according to the methods of (Dutky *et al.*, 1964). Larvae of *G. mellonella* were collected from infested beehives and raised, following the technique of (Birah *et al.*, 2008).

Laboratory Experiments:

Entomopathogenic nematode species were efficacy tested against some potato pests at different concentrations by mixing the concentrations of nematodes with sterilized and moistened sandy soil. The experiments were conducted at temperatures of ($27^{\circ}\text{C}\pm 2$ and $55\text{-}60\pm 2\%$ R.H.) in plastic containers (15-9-7cm), filled (1 cm high) with soil mixed with nematodes, and covered with plastic lids the water content was continually at 20%. The plastic containers were examined after 6 days of treatments and daily to record the numbers of larval mortality and dissect them to confirm the nematode infection. Distilled water was used to treat the controls. The concentration of nematodes needed varies by the insect host. The concentrations used for each insect were determined according to the insect's sensitivity to nematodes after initial experiments.

***Phthorimaea operculella*:** Ten 4th larval instars and pupae of *P. operculella*, were treated with 3 species of nematodes and using the concentrations 50, 100, 200, 400, 800 IJs/ larva/ plastic container/ 5 replicates. The larvae and pupae were placed in plastic containers and treated with nematodes, and covered with plastic lids.

***Pentodon bispinosus*:** Five larvae from the 2nd and 3rd instar of *P. bispinosus* were treated with 3 species of nematodes in plastic containers. Roots of the potato plants were added as a source of food and covered with plastic lids. The cups were treated with 5 concentrations of 500, 1000, 2000, 4000, and 8000 (IJs/larva). Five larvae/ tested larval instar/ plastic container/ 10 replicates/ concentration.

***Spodoptera littoralis*:** Ten, 3rd, and 5th instars' larvae of *S. littoralis* were treated by 3 species of nematodes at the concentrations of 15, 30, 60, 120, and 240 IJs/larva/ plastic container/ 10 replicates were used. The treatment was placed in plastic containers at the concentrations of the 3 species of nematodes, provided with castor leaves in the experiment of larvae as a food source and the containers were covered by plastic lids.

Statistical Analysis:

Mortality rates were corrected according to Abbott's formula (Abbott 1925). LC_{50} was calculated for the 3 species of nematodes for all experiments according to Finney (1971).

RESULTS***Phthorimaea operculella*:**

The data in Table (1) presents mortality percentages of the 4th instar larvae and pupae of *P. operculella* after 6 days of applications of *H. bacteriophora*, *S. glaseri*, and *S. carpocapsae* at various tested concentrations. Larvae were more sensitive to the nematodes than the pupae at all concentrations. The *H. bacteriophora* and *S.*

carpocapsae, LC₅₀ values in 4thlarval instars of *P. operculella* were (127.1496 and 148.5994 IJs/larva, respectively). While for *P. operculella* pupae, a lower mortality rate was given by the treatment with nematodes than larvae where, the LC₅₀ values were 209.5566 and 234.0717 IJs/pupae, respectively. *S. glaseri* was less effective for *P. operculella*, larvae, and pupae than *H. bacteriophora* and *S. carpocapsae*, and the LC₅₀ values were 247.2687 IJs/larva and 545.6909 IJs/pupa, respectively. *H. bacteriophora* was given the highest mortality rates in the 4thlarval instar and pupae than *S. carpocapsae* and *S. glaseri*.

Table 1: Mortality percentages with different concentrations of *Heterorhabditis bacteriophora*, *Steinernema glaseri*, and *Steinernema carpocapsae* to control larvae and pupae of *Phthorimaea operculella*.

Con. IJs/ larvae	<i>Heterorhabditis bacteriophora</i>		<i>Steinernema glaseri</i>		<i>Steinernema carpocapsae</i>	
	Larvae	pupae	Larvae	pupae	Larvae	pupae
50	26	14	10	6	20	16
100	40	30	28	20	36	28
200	64	48	42	30	58	46
400	80	68	62	44	80	64
800	100	88	80	58	100	86
Average	62	49.6	44.4	31.6	58.8	48
Slope	1.678	1.6444	1.5846	1.1563	1.8603	1.5613
LC ₅₀	127.1496	209.5566	247.2687	545.6909	148.5994	234.0717

***Pentodon bispinosus*:**

The data in Table (2) presents rates of mortality for 2nd and 3rd instars larvae of the white grub, *P. bispinosus* after 6 days of treatments with *H. bacteriophora*, *S. glaseri*, and *S. carpocapsae* at 5 concentrations. *S. glaseri* was the highest mortality rate (100%) for the 2nd and 3rd instars larvae of *P. bispinosus* compared with *H. bacteriophora* and *S. carpocapsae*. LC₅₀ values of *S. glaseri* against 2nd and 3rd instars larvae were 1073.640 and 1330.312 IJs/larva, respectively. *H. bacteriophora* was better influential than *S. carpocapsae*, in which LC₅₀ were 1649.990 and 2074.811 IJs/larva, while LC₅₀ values of *S. carpocapsae* were 3195.939, and 5058,809 IJs/larva against the 2nd and 3rd instars larvae of the *P. bispinosus*, respectively.

Table 2: Percentage mortality with different concentrations of *Heterorhabditis bacteriophora*, *Steinernema glaseri*, and *Steinernema carpocapsae* to control of second and the third instar larvae of *Pentodon bispinosus*

Con. IJs/ larvae	<i>Heterorhabditis bacteriophora</i>		<i>Steinernema glaseri</i>		<i>Steinernema carpocapsae</i>	
	2 nd instar larvae	3 rd instar larvae	2 nd instar larvae	3 rd instar larvae	2 nd instar larvae	3 rd instar larvae
500	20	16	30	24	10	4
1000	38	32	48	42	26	18
2000	56	50	66	60	40	34
4000	72	64	82	80	54	46
8000	90	82	100	100	70	58
Average	55.2	48.8	65.2	61.2	40	32
Slope	1.568	1.505	1.588	1.694	1.272	1.0132
LC ₅₀	1649.990	2074.811	1073.640	1330.312	3195.939	5058.809

***Spodoptera littoralis*:**

The data in Table (3) presents the mortality percentages of 3rd and 5th instars instar larvae of *S. littoralis* after 6 days of treatments for *H. bacteriophora*, *S. glaseri*, and *S. carpocapsae* at different concentrations. Mortality percentages of *H. bacteriophora* and *S. carpocapsae* were higher than *S. glaseri*. The third instar larvae of *S. littoralis* were more sensitive to infection of nematode concentrations than the 5th instar larvae. LC₅₀ values of *H. bacteriophora* were 38.489 and 40.791, While LC₅₀ values of *S. carpocapsae* were 42.149 and 51.932 with 3rd and 5th instar larvae of *S. littoralis*, respectively. LC₅₀ values for *S. glaseri* on 3rd and 5th instar larvae of *S. littoralis* were 70.106 and 95.295, respectively.

Treatments of *P. operculella* with *H. bacteriophora* and *S. carpocapsae* were effective, where LC₅₀ values in 4th larval instars recorded were 127.1496 and 148.5994 IJs/larva, respectively. Larvae were more sensitive to the nematodes than the pupae at all concentrations. The highest mortality rate of 100% was recorded in the treatment of *P. bispinosus* compared with *H. bacteriophora* and *S. carpocapsae*. LC₅₀ values of *S. glaseri* against the 2nd and 3rd instars larvae recorded 1073.640 and 1330.312 IJs/larva, respectively. In the treatment of *S. littoralis*, the mortality percentages of *H. bacteriophora* and *S. carpocapsae* were higher than with *S. glaseri*. The third instar larvae of *S. littoralis* were more sensitive to nematode concentrations than the 5th instar larvae. The results concluded that *S. littoralis* was the most sensitive pest species to the use of EPNs, followed by *P. operculella* and then *P. bispinosus*. According to the positive results, can be recommended to add the EPNs to the integrated pest management programs for controlling the potato crop pests.

Table 3: Percentage mortality with different concentrations of *Heterorhabditis bacteriophora*, *Steinernema glaseri*, and *Steinernema carpocapsae* to control of third and the fifth instar larvae of *Spodoptera littoralis*

Con. IJs/ larvae	<i>Heterorhabditis bacteriophora</i>		<i>Steinernema glaseri</i>		<i>Steinernema carpocapsae</i>	
	3 rd instar larvae	5 th instar larvae	3 rd instar larvae	5 th instar larvae	3 rd instar larvae	5 th instar larvae
15	24	20	14	10	22	16
30	44	42	28	24	40	34
60	62	60	46	38	58	52
120	86	82	64	56	80	76
240	100	100	80	72	100	94
Average	63.2	60.8	46.4	40	60	54.4
Slope	1.694	1.904	1.568	1.438	1.754	1.860
LC₅₀	38.489	40.791	70.106	95.295	42.149	51.932

DISCUSSION

Hassani-Kakhki *et al.* (2013) studied the ability of EPNs, strains *S. carpocapsae*, *S. feltiae*, *S. glaseri*, and *H. bacteriophora* against *P. operculella* larvae and pupae. The results appeared that *S. carpocapsae* and *H. bacteriophora* recorded the most elevated mortality rates in the larval stage and pre-pupae of *P. operculella* compared with *S. feltiae* and *S. glaseri*. This study is compatible with the research under study, wherever the effectiveness of *H. bacteriophora* and *S. carpocapsae* attained higher mortality rates against *P. operculella* larvae and pupae than *S. glaseri*. Al-Ghnam and Heikal (2017) studied the activity of the EPNs, for *S. carpocapsae* and *H. bacteriophora* as one of the

biological control methods against potato tuber moth *P. operculella* larvae and pupae under laboratory conditions. *H. bacteriophora* was the highest mortality rate compared to *S. carpocapsae* in all the concentrations. The results of this study were in agreement with the studied research, as *H. bacteriophora* was given higher mortality rates in larvae and pupae than *S. carpocapsae*. Abdelmonem *et al.* (2018) studied the effectiveness of (EPNs); *S. carpocapsae* and *H. bacteriophora* against 4th instar larvae of *P. perculella* under laboratory conditions. The effectiveness of EPNs revealed the LC₅₀ rates for nematodes *S. carpocapsae* and *H. bacteriophora* against 4th instar larvae were 105.96 and 121.78, respectively. This study is consistent with the present study, where the *H. bacteriophora* LC₅₀ rate was 127.1496. (Yan *et al.*, 2020) who studied the *S. Carpocapsae* was utilized at different doses to combat 2nd, 3rd, and 4th instar larvae of the potato tuber moth, *P. operculella*, under lab ambience. The LC₅₀ of *S. carpocapsae* of the 4th instar larvae (181 IJs, and the 4th instar) were more sensitive for all doses. While the record *S. carpocapsae* in this study on the 4th instar larvae LC₅₀ was 148.5994. (Kary *et al.*, 2021) investigated the effectiveness of EPNs, *H. bacteriophora*, and *S. feltiae* against larvae, pre-pupa, and pupa of *P. operculella*. The results obtained for *S. feltiae* were more effective than *H. bacteriophora* against larvae stages tested. The pupae were less sensitive to EPN than pre-pupae. *H. bacteriophora* was more effective on pupae than on *S. feltiae*. This study was consistent with the results of the present research, in which the larvae were more sensitive to nematodes than the pupae, and *H. bacteriophora* was more effective on the pupae than *Steinernema* sp.

Ibrahim, (2005) studied the efficacy of nematodes strains, *S. glaseri*, *S. carpocapsae*, and *Heterorhabditis* sp., to control the 3rd instar larvae of *P. bispinosus*. The data recorded that after a week of exposure to nematodes at 27°C, *S. glaseri* recorded the highest mortality percentages (72%), followed by *Heterorhabditis* sp. recorded (56%) and *S. carpocapsae* (52%). The results of this research agree with the results of the present study when used at the same temperature, *S. glaseri* was more effective on 3rd instar larvae of *P. bispinosus*, followed by *H. bacteriophora*, and then *S. carpocapsae*. Ibrahim *et al.*, (2010) examined the role of EPNs, for *S. glaseri*, *S. carpocapsae*, and *H. bacteriophora* in controlling the 3rd larval instar of *P. bispinosus*. The results proved that *S. glaseri* attained the most efficiency (mortality percentages 90%) against the 3rd instar larval of white grub followed by *H. bacteriophora* (83%) and *S. carpocapsae* (56%). Mortality rates increased with increasing the nematode concentrations and when mixed with the soil. This study agrees with the present research that *S. glaseri* was the highest effective against the 3rd instar larva, while *H. bacteriophora* and *S. carpocapsae*, recorded 82 and 58%, respectively. El-Ashry and Ramadan, (2021) used EPNs against the 3rd instar larvae of the white grub *P. bispinosus*. Results recorded after one week of treatments revealed that mortality rates were only 100%, with *S. glaseri*, 92 and 92 %, with *H. bacteriophora* and *S. carpocapsae* sequentially. This study matched with the present evaluation, in which mortality rates of 3rd instar larvae were 100% with *S. glaseri* only. Atwa and Hassan, (2014) studied the impact of EPNs against *S. littoralis* using 2 species *H. bacteriophora*, and *S. glaseri*. *H. bacteriophora* exhibited the highest impact on the 5th instar larvae of *S. littoralis* (80-100%) as compared to treating with *S. glaseri* (2-20%). The results of this study agree that *H. bacteriophora* was more effective on the 5th instar larvae than *S. glaseri*. Gomaa *et al.*, (2020) estimated the effectiveness of treatments of the 2 EPNs *H. bacteriophora*, and *S. carpocapsae*, on the 3rd instar larvae of *S. littoralis*. The data showed that the nematode *H. bacteriophora* was higher effective than *S. carpocapsae*, where LC₅₀ values later 72h. from the application were 53.3 and 81.41 IJs/ml, respectively. Taha, (2021) studied the sensibility of the 5th instar larvae of *S. littoralis* to various concentrations of species of EPNs; *S. carpocapsae*, and *H.*

bacteriophora. The data proved that *H. bacteriophora* was the higher effective on *S. littoralis* larvae was 100% after 48h. of treatments than *S. carpocapsae*. These 2 studies agree with this study in that *H. bacteriophora* was the highest effective than *S. carpocapsae*.

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