Protective and curative treatments of entomopathogenic nematodes against the potato tuber moth, *Phthorimaea operculella* (zell.)

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The potato tuber moth, *Phthorimaea operculella* (Zell.) is a destructive pest of potato and other crops of the family Solanaceae. Larvae attack potato leaves, stems and more importantly tubers and cause rapid rotting to the infested tubers. Entomopathogenic nematodes being safe to environment and food supply may offer control alternative to chemical insecticides. This work included protective (against insects outside the tubers) and curative (against insects inside the tubers) treatments of EPNs *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* for the biological control of *P. operculella* infesting potato tubers in the soil. In the protective treatments the nematodes succeeded in decreasing up to 100% of the pest population and succeeded in development inside the infected cadavers in rates of 50-97%. In the curative treatments, the nematodes could cure mostly all the damage caused by the pest larvae (0-5% damage). An integrated control program comprising EPNs would be effective against the PTM immature stages outside or inside the potato tubers may eliminate the dependence on chemical insecticides in controlling the PTM.

**Keywords:** *Phthorimaea operculella; Steinernema carpocapsae; Heterorhabditis bacteriophora; protective; curative treatments*

**INTRODUCTION**

Potato, *Solanum tuberosum* production ranks fourth among agricultural crops. A large number of insect pests attack potatoes. Worldwide, the potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) is the most destructive potato pest (Shelton and Wayman 1979; Mandour 1997; Keasar and Sadeh 2007). This pest occurs in Asia, Africa and America (Kroschel and Sporleder 2006). The insect adults lay eggs in soil cracks near potato stems and on exposed tubers. After hatching, the larvae mine directly into the tubers or mine the foliage and stems (Rondon et al., 2007). Mining of the tubers causes the greatest damage and happens both in the field and in storage. The insect larvae cause blotches in leaves and fold leaves during feeding (Mandour 1997). At harvest, tubers do not always show signs of damage but may bear eggs and early-instar larvae (Caedo et al., 1999). Pupation takes place on the potato leaves or tubers or mostly in the soil. Transportation of infested tubers to potato stores leads to more propagation of the pest and increased damage of tubers.

Insecticides are still used in controlling the PTM either in the field or in storage. As widely known the overuse of conventional insecticides may cause destruction of many natural biological control agents and may build up a cross resistance to target insect pests (Richardson and Rose, 1967; Cisneros, 1984). The integrated pest management (IPM) including the biological control is the best alternative to hazardous chemicals. Using of parasitoids, predators and microbial
Entomopathogenic nematodes

Steinernema carpocapsae (S2) (Nematoda: Steinernematidae), isolated and identified by Shamseldean et al., (1996).

Heterorhabditis bacteriophora (HP88) (Nematoda: Heterorhabditidae), imported from Kiel University, Germany.

The EPNs were maintained on last instar larvae of the wax moth, Galleria mellonella according to Kaya and Stock (1997).

Protective treatments

Assays were conducted to determine the role of the entomopathogenic nematodes S. carpocapsae (S2) and H. bacteriophora (HP88) in protection of the potato tubers against the PTM. The treatments were applied on the soil containing potato tubers artificially infested with eggs or first larval instar (L1) or prepupaepae in the soil while still outside the potato tubers.

Egg treatments

The experiments were carried out under 25°C in 150cc plastic cups, 80cm² surface area filled with fine sterilized moistened (10% water content) sand and covered with plastic mesh. The potato tubers were inserted in the sand then artificially infested with the insect eggs (1day old) at a rate of 15 eggs/tuber then the nematodes were applied at two rates (5 infective juveniles (IJs) and 10 IJs/cm² of soil surface. Control plots received only water. Experiments were replicated 4 times. After 20 days numbers of alive insects found in each treatment were recorded. Rates of development of nematodes in infected cadavers were determined in subsamples of 4 insect cadavers as available. Cadavers were dissected under a stereomicroscope and only cadavers with alive nematodes inside them were recorded as cadavers with developed nematodes.

First instar larvae treatments

As the aforementioned procedures in “egg treatments”, the L1 treatments were conducted except that the tubers were artificially infested with L1 larvae instead of eggs. The infestation rate was 15 L1-larvae/tuber. Nematode development in insect cadavers was determined when cadavers were available.

Prepupae treatments

In case of prepupaep treatments, the soil in aforementioned cups contained 15 PTM prepupaep/ cup. The nematodes S. carpocapsae (S2) and H. bacteriophora (HP88) were applied at
5 and 10 IJs/cm² on soil surface. Control plots received only water. Experiments were replicated 4 times. After 7 days, numbers of alive insects in each treatment were recorded. Nematode development was determined in insect cadavers as mentioned above.

Curative treatments
In the curative treatments, the nematodes were applied on soil containing potato tubers that already infested with the insect. The treatments were carried out under 25°C in 150cc plastic cups, 80cm² surface area filled with fine sterilized moistened (10% water content) sand and covered with plastic mesh. The potato tubers were inserted in the sand then artificially infested with the insect eggs (1d old) at a rate of 10 eggs/tuber. The nematodes were applied 10 days after artificially infestation when the hatched larvae started mining in the tubers. The nematodes were applied at two rates (5 infective juveniles (IJ)s and 10 IJs/cm² of soil surface. Control plots received only water. Experiments were replicated 4 times. After 10 days of nematode treatments, the damage caused by the PTM to potato tubers was evaluated according to the categories in (Table 1) based on the degree of visible larval tunneling (Fenemore 1980).

Table 1. Visible categories of damage in potato tubers caused by the potato tuber moth Phthorimaea operculella required for calculation of damage index according to Fenemore (1980).

<table>
<thead>
<tr>
<th>Infestation category</th>
<th>Damage category</th>
<th>Weighing factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean</td>
<td>No visible sign of infestation</td>
<td>0</td>
</tr>
<tr>
<td>Slight</td>
<td>one or two mines/tuber that could be removed readily on peeling</td>
<td>X1</td>
</tr>
<tr>
<td>Moderate</td>
<td>More than two mines presented and up to one third of surface showing</td>
<td>X2</td>
</tr>
<tr>
<td>Severe</td>
<td>More than one-third of the tuber surface showing damage</td>
<td>X3</td>
</tr>
</tbody>
</table>

Data analysis
Data of the protective treatments of the nematodes against the PTM were calculated according to the equation of Henderson and Tilton (1955) which assesses the percent reduction in the insect population as a result of nematode treatments as follows:

\[
\% \text{Reduction} = 100 \times \left(1 - \frac{Ta \times Cb}{Tb \times Ca}\right)
\]

Where:
- \(Tb\) = number of alive insects in treatment plots before treatment
- \(Ta\) = number of alive insects in treatment plots after treatment
- \(Cb\) = number of alive insects in control plots before treatment
- \(Ca\) = number of alive insects in control plots after treatment

The mean damage index of tubers (DI) for each treatment was calculated by the formula given by Fenemore (1980) as follows:

\[
DI = \frac{(no. \text{slight} X1) + (no. \text{moderate} X2) + (no. \text{severe} X3)}{ \text{Total numbers of tubers} }. 
\]

Rates of nematode development in insect cadavers were statistically compared with ANOVA.

RESULTS
Protective treatments
The protective nematode treatments were applied in the soil on immature stages of the PTM (Eggs, \(L_1\) and prepupae) while these stages were still outside the potato tubers. Table (2) shows the numbers of alive insects after treatments. It also shows the percent reduction in the PTM population due to the nematode treatments calculated according to the equation of (Henderson and Tilton1955). It is clearly shown that all nematode treatments were highly effective against immature stages of the pest. At the end of the experiment, the mean alive insects remained in the control plots were 5.8, 10 and 13.6 insects in eggs, \(L_1\) and prepupae plots, respectively. At this time, the mean alive insects at the lower concentration (5IJs/cm²) of \(S. \text{carpocapsae}\) was only 0.8, 0.2 and 3.5 insects in eggs, \(L_1\) and prepupae treatments, respectively.

For the other treatments, \(S. \text{carpocapsae}\) (10 IJs/cm²) or \(H. \text{bacteriophora}\) (5 or 10 IJs/cm²) the mean alive insects after treatments ranged 0-2.75, 0-0.2 and 2.0-7.5 insects, respectively. Complete control (no alive insects left) of the pest population was obtained at \(S. \text{carpocapsae}\) (10 IJs/cm²) egg treatment or \(H. \text{bacteriophora}\) (10 IJs/cm²), \(L_1\) treatment.

Population reduction percentages of the pest after nematode treatments were remarkably high. The lower concentration (5 IJs/cm²) of \(S. \text{carpocapsae}\) caused 74.26-98% population reduction while the same concentration of \(H. \text{bacteriophora}\) caused 92.21-97.25%. However, the higher concentration of both nematodes caused 92.21-100% population reduction. Always the effect of nematodes on the pest population was higher when applied on early insect stages i.e. eggs and \(L_1\) (86.21-100% population...
reduction) than the prepupal stage 74.26-97.83%. This was due to the difference in length of the exposure period of insects to the nematodes.

**Development of nematodes inside insect cadavers**

The insect cadavers were found decayed and partially or totally disappeared after nematode treatments especially in early stage treatments (eggs and L1) when the period between the treatment and the evaluation was long. All cadavers in eggs and L1 treatments of *S. carpocapsae*, 10 IJs/cm², *H. bacteriophora* 5 or 10 IJs/cm² were found decayed at the evaluation time.

**Table (2): Population reduction of Phthorimaea operculella after treatments of Steinernema carpocapsae or Heterorhabditis bacteriophora on immature insect stages outside the potato tubers**

<table>
<thead>
<tr>
<th>Nematode</th>
<th>Concentration</th>
<th>Insect stage</th>
<th>Alive insects Mean± SE</th>
<th>% Population reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Steinernema carpocapsae</em></td>
<td>5IJs/cm²</td>
<td>Eggs</td>
<td>0.8 ± 0.37</td>
<td>86.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L1</td>
<td>0.2 ± 0.2</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prepupae</td>
<td>3.5 ± 1.84</td>
<td>74.26</td>
</tr>
<tr>
<td></td>
<td>10 IJs/cm²</td>
<td>Eggs</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L1</td>
<td>0.2 ± 0.2</td>
<td>99.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prepupae</td>
<td>1.75 ± 1.7</td>
<td>96.29</td>
</tr>
<tr>
<td><em>Heterorhabditis bacteriophora</em></td>
<td>5IJs/cm²</td>
<td>Eggs</td>
<td>2.75 ± 1.1</td>
<td>97.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L1</td>
<td>0.2 ± 0.2</td>
<td>99.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prepupae</td>
<td>7.5 ± 3.12</td>
<td>92.21</td>
</tr>
<tr>
<td></td>
<td>10 IJs/cm²</td>
<td>Eggs</td>
<td>0.8 ± 0.37</td>
<td>99.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prepupae</td>
<td>2.0 ± 0.54</td>
<td>97.83</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>Eggs</td>
<td>5.8 ± 0.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prepupae</td>
<td>13.6 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

Subsamples of cadavers remained after treatments were inspected under the microscope for nematode presence and development and the percentages of nematode development at all treatments were illustrated in (Fig1). Nematodes have succeeded in development in insect cadavers after treatments at all cases. The percent of nematode development in cadavers ranged from 50 to 97.5%. The experimental error (SE) was relatively high because the numbers of insects available for inspection (n=4) was not high enough to statistically express the mathematical differences in nematode development rates among the treatments. ANOVA showed insignificant differences (F= 2.029, P≥ 0.122) among all nematode treatments. A photograph of nematode development inside the PTM cadaver in the tunnel in a potato tuber is shown in (Fig 2).

**Curative treatments**

As mentioned before, the curative nematode treatments were applied on soil containing infested potato tubers. At this time, one – two mines/ tuber can be detected and they can be removed with peeling the potato tubers. The damage symptoms were categorized and recorded 10 days after nematode treatments then the damage index was calculated according the equation of Fenemore (1980). As shown from (Fig 3), all nematode treatments were highly effective in curing the potato tubers infested with the PTM. The damage index was 25.5% in untreated plots and 5% in plots treated with *S. carpocapsae*, (5IJs/cm²).
E*= Egg treatment, L1*= First instar larvae treatment, PP*= Preapuae treatment

Figure. 1 Development of *Steinernema carpocapsae* or *Heterorhabditis bacteriophora* in *Phthorimaea operculella* cadavers treated as immature stages

Figure. 2. A photograph showing development of *Steinernema carpocapsae* inside a *Phthorimaea operculella* larva in a treated potato tuber

Figure. 3. Damage index of potato tubers previously infested with *Phthorimaea operculella* in the soil after treatments with *Steinernema carpocapsae* or *Heterorhabditis bacteriophora*
In the higher concentration of *S. carpocapsae* i.e. 10 IJs/cm² or both concentrations of *H. bacteriophora*, the damage index was zero. That means that the nematodes have succeeded in curing mostly all the damage caused by the insect.

**DISCUSSION**

The potato tuber moth, *P. operculella* is an injurious pest on potato crops. Larvae attack potato leaves, stems and more importantly tubers and cause rapid rotting to the infested tubers. Entomopathogenic nematodes being safe to environment and food supply may offer control alternative to chemical insecticides. This work included protective and curative treatments of EPNs *S. carpocapsae* and *H. bacteriophora* for the biological control of *P. operculella* infesting potato tubers in the soil. In the protective treatments the nematodes succeeded in decreasing the pest population while outside the potato tubers. The population reduction of the pest reached 100% after nematode treatment. Citations – mostly laboratory studies- reported susceptibility of immature stages of the pest for infection of either *S. carpocapsae* or *H. bacteriophora* with differences in results according the nematode species, the concentration, the insect stage and the soil type (Ivanova et al., 1994, Siegel et al., 2004; Lacey and Kroschel 2009, Barbosa-Negrisoli et al., 2010, Kakhki et al., 2012).

Our results also showed that the nematodes succeeded in development inside the infected insect cadavers by rates of 50-97%. Such high rates of nematode development could give indications about the future of nematode recycling and persistence in the field after treatment. Similar conclusion was reported by Elawad et al., (2001) who generally studied the recycling potential of the entomopathogenic nematodes in lepidoptran larvae.

Strong (2002) mentioned that among factors affecting the field persistence of EPNs are their ability to fiend and infect hosts and to produce offspring. Susurluk & Ehlers, 2008 added that the availability of hosts also influences the persistence of EPNs in the field.

The curative nematode treatments were applied on soil already containing infested potato tubers when one to two mines/tuber could be detected and can be removed with peeling the potato tubers. When the damage index in the control plots was 25.5%, the nematodes treated plots have only 0-5% damage. That means that our nematode treatments have succeeded to cure the tubers already infested with PTM. Fenemore (1980) reported that the maximum sever damage could be caused by the PTM in potato tubers is 30 when all tubers fall into the severe category. In this respect, it is worth mentioning that, Ramane 1987, Moawad (1995 & 2000), Moawad and Ebadh (2007) and Sharaby et al., (2009) used the same damage index equation in evaluating the role of plant powders in curing the damage caused by the PTM in potato tubers.

An integrated control program comprising EPNs is effective against the PTM immature stages outside and inside the potato tubers may eliminate the dependence on chemical insecticides in controlling the PTM in both field and storage.

**CONCLUSION**

Our study included protective and curative treatments of EPNs *S. carpocapsae* and *H. bacteriophora* for the biological control of *P. operculella* infesting potato tubers in the soil. In the protective treatments the nematodes succeeded in decreasing the pest population while outside the potato tubers. The reduction of the pest reached 100%. This work also showed that the nematodes succeeded in development inside the infected insect cadavers at high rates. Such high rates of nematode development could give indications about the future of nematode recycling and persistence in the field after treatment. Also, the nematode treatments have succeeded to cure the tubers already infested with PTM. Future work is necessary to determine the efficacy and feasibility of entomopathogenic nematodes against *P. operculella* under the field condition.

**CONFLICT OF INTEREST**

No conflict of interest

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**AUTHOR CONTRIBUTIONS**

SS Moawad, MME Saleh and YA Mahmoud suggested the idea and designed the research. SS Moawad, IM Ebadh and HM Metwally conducted the protective and curative treatments. MME Saleh analyzed the data. MME Saleh and HM Metwally wrote the manuscript. All authors contributed to the writing and approved the
manuscript.

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