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Evaluating the Role of Entomopathogenic Nematodes for the Biological Control of the Potato Tuber Moth, *Phthorimaeao perculella* under Laboratory Conditions.

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ABSTRACT

In this study we evaluated the control potential effect of five doses of the entomopathogenic nematode, *Steinernema carpocapsae* (All) and *Heterorhabditis bcteriophora* (HP88) as a biocontrol agent against potato tuber moth, *Phthorimaeao perculella* under laboratory conditions. Using five concentrations of each nematode species (500, 1000, 1500, 2000, and 2500 infective juveniles, IJs) that inoculated in two stages(larvae and pupae) of the potato tuber moth. Mortality percentages were recorded along 5 days for all doses and calculated for each insect stage at different dose of entomopathogenic nematode. The larval mortality percentages was 90% and 100% at dose 2000IJs/ 10 individualsat the second and the third days,respectively, by *S. carpocapsae* and *H. bcteriophora*. The highest mortality percentages obtained from the dose 2500 IJs/10individuals by using *H. bcteriophora* 50%, 90% and 100% at the first, second and third days, respectively. At dose 2500 and 2000 IJsapplied for pupal stage not exhibit more than 40% and 50% by using *S. carpocapsae* and 50% and 70 % by using *H. bcteriophora*, respectively mortality percentages at the fifth day of the experiment. Our work indicates that *P. operculella* larvae were achieving high mortality percentages and it can applying entomopathogenic nematodes as biocontrol agents against *P. operculella*.

Keywords: Biocontrol, Insect host, Entomopathogenic nematode, *Steinernema carpocapsae, Heterorhabditis bcteriophora*.

INTRODUCTION

Potato (*Solanum tuberosum* L) is one of the most important crops allover the world especially in Egypt. Several insect species attacking the potato plants, but the potato tuber moth, *Phthorimaeao perculella* (Zeller) (Lepidopterous: Gelechiidae) is the most destructive insect pest (Mandour, 1997; Islam *et al.,* 1990). The entomopathogenic nematodes (EPNs) consider one of the most effective control groups of soil insect pests that belong to the two families Steinernematidae and Heterorhabditidae. These two families are obligated insect-parasitic organisms and mutualistically related with bacteria from genera *Photorhabdus* (Heterorhabditidae) and *Xenorhabdus* (Steinernematidae). As reported by Kaya and Gaugler (1993), these bacteria are carried within the digestive tracts of nematodes. These bacteria when released from the nematode intestine multiple rapidly in the host haemolymph and cause septicemia within 24-48 hours (Gaugler, 1988). The nematodes feed upon the bacterial cells and host tissues to produce progeny which emerge from the cadaver as IJs begin searching for new hosts which they live in the soil and enter the insect host through natural openings (mouth, anus and spiracles) or through the cuticle. Sweelam*et al.,* (2010) evaluated the pathogenicity of the ENPs species, *S. carpocapsae* and *H. hebcteriophora*, against all stages of the red palm weevil, *Rhynchophorus ferrugineus* Oliver (Coleoptera: Curculionidae) at 25 ± 2ºC and 65±5 %RH. The highest percentile of mortality was recorded on the egg stages (98.2 %) at 5000 IJs/ 10 individuals, followed by 95.5% for larval, 66.5% for adult and 40.0% for pupal stages. Ilker *et al.,* (2013) assessed three local Turkish isolates of *S. carpocapsae, S. feltiae* and *H. bcteriophora* against the last instar of *P. operculella* under laboratory conditions. They found at 25ºC and 1000 IJs concentration, the mortality of larval stage was 96 and 80% for *S. carpocapsae* and *H. bcteriophora*, respectively. However, at all temperature and concentrations, *S. feltiae* exhibited lower than 40% mortality, except when the nematodes are applied in infected insect host cadavers. At 25ºC, infected cadavers showed 97, 83 and 67% mortality by *S. carpocapsae, H. bcteriophora* and *S. feltiae,* respectively. Sweelam *et al.,* (2011) performed the biological control of *P. operculella* by ENPs, *S. carpocapsae* in different stages of host (larvae, pupae, adults) when exposed to five concentrations of the nematode (500, 1000, 1500, 2000, and 2500 IJs). The results showed that *S. carpocapsae* nematode greatly reduced the larvae of *P. operculella* after five days of the exposure, where it recorded 74% as grand mean mortality percentages at 2000 IJs/10 Individcuals.

This study aimed to determine the entomopathogenic nematode species that could be increase the success of biological control of *P. operculella*.

MATERIALS AND METHODS

Entomopathogenic nematode:

The two species of ENPs *S. carpocapsae*(All) and *H. bcteriophora*(HP88) were obtained from regular culture in the Department of Pest Physiology, Plant Protection Research Institute, Agricultural Research Centre (ARC) Dokki, Giza., Egypt.,which reared on greater wax moth *Galleria mellonella* at 25 ±2ºC.

Potato tuber moth:

The two stages (larvae and pupae) of the potato tuber moth, *Phthorimaeao perculella* were obtained from the laboratory colony maintained from Department of Vegetable Pests Research and Medical and Aromatic herbs, Plant Protection Research Inst. Agricultural Research Centre (ARC) Dokki, Giza., Egypt.

Application of nematode on the potato tuber moth stages:

The two stages of insect larvae and pupae of the potato tuber moth, *P. operculella* were obtained from the culture reared in the laboratory in glass jars on potato tubers. Thirty individuals of each stages tested to each concentration (500, 1000, 1500, 2000 and 2500 IJs/5ml/Petri dish) of two species of ENPs *S. carpocapsae* and *H .hebcteriophora* to evaluated their effects against potato tuber moth insect under laboratory conditions at 25 ± 2ºC.

Each ten individuals of each stages of the potato tuber moth were kept in Petri dish, each of 5 cm diameter containing 2 moist filter papers where individuals were put between them, and exposed to doses of the entomopathogenic nematodes. Doses of nematodes each of them were sprayed on the individuals as 5 ml distilled water containing nematodes. As control treatment, individuals were sprayed with 5 ml distilled water without nematodes. Each treatment was replicated four times. Mortality of *P. operculella* was daily obtained for five days for all concentration, and percentages of mortality wereestimated for each species of ENPs at the five doses. Mortality percentage was modified by (Abbott 1925). The data were analyzed by one-way ANOVA. The means were separated by Duncan's multiple range test (P<0.05) (Colman, 2001) according to the statistical methods of Snedecor (1956).

RESULTS AND DISCUSSION

Influence of entomopathogenic nematode

- **On larval stage :**

Data in table (1) revealed that the higher mortality percentages 100% was obtained non significant different for concentration 2000 and 2500 IJs/10 individuals at the third day of the experiment. Where at the fourth day non significant different mortality percentages 100% was exhibited for concentration 500, 1000 and 1500 IJs/10 individuals. While 50% mortality percentages significantly different was obtained for the concentration 1000 IJ at the second day.Finally the lowest percentage mortality was 10% significant different at the second day of the experiment for concentration 500 IJs/10 individuals.

Data obtained in table (2) show that *H. hebcteriophora* provided high of mortality percentages 100% non significant differences between at concentrates 1500, 2000 and 2500 IJs/10individuals at the third day of the experiment, Where at the fourth day non significant differences between mortality percentages 100% was obtained for concentration 500 and 1000 IJs/10 individuals, While 50% mortality percentage was recorded for the dose 500 at the third day. And the same percentage was obtained for the dose 2500 at the first day. A lowest mortality percentage was exhibited forthe dose 1500 IJs/10 individuals significantly different at the first day of the experiment.

Table 1. Mortality percentage of larval stage of the potato tuber moth *P. operculella* application of entomopathogenic nematodes *S. carpocapsae* .

Nematode concentrations	%Mortality percentages ± SE				
IJs/10 larvae	1 Day	2 Day	3 Day	4 Day	5 Day
500	0 b	10 c ± 0.577	40 b ± 5.773	100 a ± 5.773	--------
1000	0 b	50 b ± 2.886	80 a ± 5.773	100 a ± 5.773	-------
1500	0 b	80 a ± 5.773	90 a ± 2.886	100 a ± 0	----------
2000	0 b	90 a ± 5.773	100 a ± 11.54	---------	---------
2500	20 a ± 1.1154	90 a ± 2.886	100 a ± 5.77	---------	----------
Check	0.0	0.0	0.0	0.0	
LSD	1.627	12.88	21.90	21.525	

Values in the same column with different letters were significantly different (Duncan's multiple range; P<0.05).

Table 2. Mortality percentage of larval stage of the potato tuber moth *P. operculella* application of entomopathogenic nematodes *H. bcteriophora*.

Nematode concentrations	Mortality percentages ± SE				
IJs/10 larvae	1 Day	2 Day	3 Day	4 Day	5 Day
500	0 d	40 b ± 2.886	50 b ± 0	100 a ± 5.773	---------
1000	0 d	50 b ± 5.773	90 a ± 5.773	100 a ± 11.547	----------
1500	10 c ± 5.773	80 a ± 5.773	100 a 0	---------	---------
2000	40 b ± 5.773	90 a ± 5.773	100 a 0	----------	---------
2500	50 a ± 2.886	90 a ± 0	100 a ± 5.773	----------	----------
Check	0.0	0.0	0.0	0.0	
LSD	9.132	4.667	11.506	30.442	

Values in the same column with different letters were significantly different. (Duncan's multiple range; P<0.05).

2- On pupal stage:

From table (3) it is evident that *S. carpocapsae* have a low effect on the pupal stage than larvae stage of the host insect potato tuber moth *P. operculella*. Non significant different (10%) mortality percentages was obtained at concentrates 2000 and 2500IJs/ 10 individuals at the third day and the same percentage significantly different at the fifth day for1000 IJs/10 individuals was recorded. while the 50%mortaility percentage non significant differently was obtained for the dose 2500 IJs/10individuals at the fifth day and this is the highest mortality percentage.

Data in table (4) indicated that the *H. hebcteriophora* nematode was achieved zero mortality percentage at the dose 500 for all days of the experiment. While the dose 1000 IJs/10 individuals was obtained significant mortality percentage (10%), and the dose 2000 IJs/10individuals recorded significant different mortality percentage 50% at the fifth day of the experiment. Nons ignificant different highly mortality percentage (70%) was obtained at the fifth day of the experiment for dose 2500 IJs/10individuals.

Table 3. Mortality percentage of pupal stage of the potato tuber moth *P. operculella* application of entomopathogenic nematodes *S. carpocapsae*.

Nematode concentrations	Mortality percentages ± SE				
IJs/10 pupal	1 Day	2 Day	3 Day	4 Day	5 Day
500	٠	٠	0 b	0 c	0 b ± 0.577
1000	٠	٠	0 b	0 c	10 b ± 5.773
1500	٠	٠	0 b	0 c	40 a ± 5.773
2000	٠	٠	10 a ± 0.577	10 b ± 0.577	40 a ± 5.773
2500	٠	٠	10 a ± 1.154	30 a ± 2.586	50 a ± 5.773
Check	٠	٠	٠	٠	٠
LSD	-	-	1.819	4.148	14.115

Values in the same column with different letters were significantly different. (Duncan's multiple range; P<0.05).

Table 4. Mortality percentage of pupal stage of the potato tuber moth *P. operculella* application of entomopathogenic nematodes *H. bcteriophora*.

Nematode concentrations	Mortality percentages ± SE				
IJs/10 pupal	1 Day	2 Day	3 Day	4 Day	5 Day
500	0	0	0 b	0 b	0 d
1000	0	0	0 b	0 b	10 d ± 5.773
1500	٠	٠	0 b	10 b ± 0.773	30 c ± 5.773
2000	٠	٠	0 b	10 b ± 1.154	50 b ± 5.773
2500	٠	٠	20 a ± 5.773	30 a ± 14.294	70 a ± 5.773
Check	٠	٠	٠	٠	٠
LSD	-	-	8.136	20.22	14.115

Values in the same column with different letters were significantly different. (Duncan's multiple range; P<0.05).

Our results indicate that the entomopathogenic nematode *H. bcteriophora* are highly effect in mortality percentages on the insect potato tuber moth in the two stages of the insect host more than *S. carpocapsae*. The results agree with (Sorial, 2001; Keila, 2004; Mohamed and Osman, 2007; Shamseldean*et al.,* 2008 and Sweelam*et al.,* 2011).

Many studies have been carried out to test the effect of ENPs against *P. operculella* larvae, prepupa, pupa and adults stages. It was reported that the larva and prepupa were susceptible whereas, the pupae and adults stages were the resistant to nematode infection (Ivanova*et al.,* 1994; Sweelam*et al.,* 2010 and Hassani-Kakhki*et al.,* 2013).

From this study, the tested entomopathogenic nematodes can be applied in aqueous suspension through irrigation systems or agricultural spray methods (Shapiro-llan*et al.,* 2003).

CONCULSION

This study revealed that the entomopathogenic nematodes *H. bcteriophora*and *S. carpocapsae*, as a biocontrol agent, have greatly effect against the larval stage of the potato tuber moth more than the pupal stage after the five days of exposure, since they recorded high percentages of reduction in the host insect population. From this work, it could be concluded that both species of entomopathogenic nematodes can be sprayed against *P. operculella* under the field condition using aqueous suspension or infected formulations.

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تقييم دور النيماتودا الممرضة للحشرات فى المكافحة الحيوية لفراشة درنات البطاطس تحت الظروف المعملية.

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فى هذه الدراسة تم إختبار مستويات عدوى مختلفة من النيماتودا الممرضة للحشرات وإستخدامها كعامل حيوى فى مكافحة حشرة فراشة درنات البطاطس *perculella Phthorimaeao* على الطورين اليرقة والعذراء وذلك تحت ظروف المعمل حيث تم إستخدام نوعين من النيماتودا الممرضة للحشرات وهما *Steinernema carpocapsae*و *Heterorhabditis bcteriophora* بخمس تركيزات مختلفة وهى (٥٠٠و١٠٠٠و١٥٠٠و٢٠٠٠و٢٥٠٠) وسجلت متوسط نسبة موت لمدة التجربة وهى خمس أيام حيث أظهرت أفضل النتائج وذلك عند معاملة الطور اليرقى للحشرة بالنيماتودا الممرضة للحشرات ٩٠%و١٠٠% على الترتيب وذلك فى اليوم الثانى والثالث عند مستوى عدوى ٢٠٠٠يرقة معدية/للحشرة وذلك باستخدام النوع *S. carpocapsae* وسجلت نفس النتائج ٩٠% و١٠٠% على الترتيب باستخدام النوع *H. bcteriophora* . بينما استخدام النوع *H. bcteriophora* عند تركيز٢٥٠٠يرقة معدية/للحشرة سجل نتائج جيدة من اليوم الاول وكانت كالتالى ٥٠%و ٩٠%و١٠٠% لليوم الاول والثانى و الثالث على التوالى . اظهرت النتائج عند استخدام مستويات العدوى من النيماتودا الممرضة للحشرات مع طور العذراء حيث سجلت نسبة موت ٤٠% و٥٠% عند مستوى٢٥٠٠و٢٠٠٠يرقة معدية/للحشرة على التوالى باستخدام النوع *S. carpocapsae*وسجلت٥٠% و٧٠% نسبة موت عند مستوى عدوى ٢٠٠٠و ٢٥٠٠ يرقة معدية/للحشرة للنوع *H. bcteriophor*ولذلك ينصح باستخدام النيماتودا الممرضة للحشرات كعامل حيوى فى مكافحة حشرة فراشة درنات البطاطس.