Minufiya J. Agric. Res. Vol.36 No. 2:427-435 (2011) "http://www.mujar.net"

BIOLOGICAL CONTROL OF POTATO TUBER MOTH, *PHTHORIMAEA OPERCULELLA* BY ENTOMOPATHOGENIC NEMATODE, *STEINERNEMA CARPOCAPSAE*

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(Received: Mar. 5, 2011)

ABSTRACT: *This research was conducted to study the potential effect of different doses of the entomopathogenic nematode, Steinernema carpocapsae in the biological control of the potato tuber moth, Phthorimaea operculella Zeller under laboratory conditions. Different stages of the potato tuber moth (larvae, pupae, adults) were exposed to five doses of the nematode (500, 1000, 1500, 2000, 2500 IJS). Mortality of insect stages was checked along 5 days for all concentrations, and percentage of mortality was calculated for each insect stage at different concentrations. Results reported that the S. carpocapsae nematode greatly controlled the larvae of the potato tuber moth along the five days of the exposure of larvae to nematodes, where it gave 74 % as grand mean of mortality percentages at 2000 infective juvenile individuals per 10 larvae . Regarding to pupal stage, nematode did not have any effect (0%) at all concentrations of nematode. In addition, it did not satisfactorily control the adult stage of the potato tuber moth, where it gave only 16 % as grand mean of mortality percentages at all doses. It could be concluded that the use of the entomopathogenic nematode Steinernema carpocapsae in the control of potato tuber moth stages gave the best mortality percentages against larval stage (74%) at the dose of 2000 infective juveniles per ten individuals of larvae.*

Key words: *Potato tuber moth, Entomopathogenic nematode, Steinernema carpocapsae, Phthorimaea operculella, biological control.*

INTRODUCTION

Beneficial nematodes are an example of live natural enemies that are inundatively released. These nematodes travel either through the soil or on the soil surface, and actively attack their insect hosts. Once inside, they release symbiotic bacteria, which multiply and kill the host. The nematodes feed on the bacteria and insect tissue, then mate and reproduce. After one to two weeks, young nematodes emerge from the insect cadaver to seek new hosts. Nematodes are highly susceptible to desiccation, exposure to

ultraviolet light and temperature extremes. They are most useful against insects living on or in the soil, or in other protected environments (such as tunneling inside plants).

Gaugler and Molloy (1981) found that the susceptibility of *Simulium vittatum* to *Neoaplectana carpocapsae* increased with successive larval instars by laboratory bioassays. First, second, and third instar larvae were resistant to infection, while seventh instars were highly susceptible.

Ghally *et al.*, (1991) studied the histopathological effects of different dosages (50, 100, 200, 500 and 1000) of *Stinernema feltiae* larvae on the larval tissues of *M. domestica*. It was found that these nematode larvae invade the fat tissue, gut, cuticle and muscular tissues of the host. All of these tissues showed signs of disintegration before death of the host. The tissues of the gut and the fat body are most severely damaged, and the damage depends largely on the timing and intensity of the infection.

Renn (1995) encapsulated entomopathogenic nematodes Steinernema feltiae (= S. bibionis) and Heterorhabditis megidis in calcium alginate and their efficacy was tested against immature houseflies.

El-Sooud *et al.*, (2001) tested Infectivity of four isolates (BA1, ES1, GF and SA2) belonging to the insect nematode species *Heterorhabditis bacteriophora* against the housefly larvae, *Musca domestica*. Data revealed that the most infective isolate at a dosage level of 5 IJs/ml per larva was SA2 isolate, causing 40% mortality after 72 h, followed by ES1, GF and BA1 isolates which caused 32.5, 30.0 and 25.0% mortality after 96 h, respectively.

Also, Mahmoud (2007) evaluated botanical insecticides based on azadirachtin and the entomopathogenic nematode *Steinernema feltiae* for their control of the peach fruit fly, *Bactrocera zonata*.

Mahmoud and Osman (2007) performed laboratory experiments to assess the efficiency of the entomopathogenic nematode *Steinernema feltiae* Cross N 33 against second and third instar larvae and 1, 4 and 6 days old pupae of the peach fruit fly *Bactrocera zonata*. Mortality rates after 3 exposure times ranged from nil to 24%, nil to 40% and 8 to 56% for 2nd instar larvae and 8 to 72%, 28 to 84% and 32 to 88% for 3rd instar larvae.

Sweelam *et al.*, (2010) evaluated the pathogenicity of the entomopathogenic nematode species *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* to the red palm weevil, *Rhynchophorus ferrugineus* Oliver (Coleoptera: Curculionidae) stages (eggs, larvae, pupae ,and adults) under laboratory conditions 25 ± 2°C and 65± 5 % RH. The highest mortality percentages were recorded with egg stages of the weevil (98.2 %) at 5000 IJs / 10 individuals , followed by larvae (95.5%) , while the mortality percentages were only (66.5%) for adults and (40.0%) for pupae.

From theses points of view, the aim of this research is to evaluate the potential effect of entomopathogenic nematode, *Steinernema carpocapsae* doses against different stages of the potato tuber moth, *Phthorimaea operculella* Zeller under laboratory conditions.

MATERIALS AND METHODS

Rearing of the entomopathogenic nematode:

The entomopathogenic nematode, *Steinernema carpocapsae* was extracted from the soil of the mango trees of the Experimental Station of the Faculty of Agriculture Shebin El-Kom, Minufiya Governorate.

Soil samples were taken from the rhizosphere of mango trees at depth of 15-20 cm using a handle trowel. From each sampling site, a number of sub samples were collected and thoroughly mixed to form a composite sample. Ten composite soil samples each 1kg were kept in polyethylene bags and directly sent to the nematode laboratory of the Faculty of Agriculture, Economic Entomology and Agriculture Zoology Department, Minufiya University. About 250 g of each soil sample was put in modified Baermann funnel (Southey, 1970) for 72 hrs under laboratory condition 25±3 °C and 65±5% R.H. to extract nematodes. For nematode infection by *Steinernema* nematode to the late instar larvae of the greater wax moth, *Galleria mellonella*, Petri dishes 15 cm were used where 10 wax mature larvae were placed on a filter paper Whitman No.1 in Petri dish and 20 cm^3 of the extracted nematodes were added, then covered with another filter paper to avoid the escaping of the larvae. Dishes were covered by the cap of each dish to maintain the water from evaporation and incubated under 25°C, three days later, infected larvae were recognized by their appearance often flaccid and their color is changed to orange, yellow or brown, which were placed in modified white traps (White, 1927) to harvest emerging infective Juveniles (IJS) (Woodring and Kaya, 1988). Modified white trap was consists of a petri dish 7 cm potted on its opposite side in another Petri dish 10 cm, then a filter paper (10 cm) was covered the small dish where 10 of infective larvae arranged in a circle on it, 50 ml of distilled water (0.1% formalin were added as some drops to avoid the protozoan contamination) were added in large Petri dish and tightly capped with its cover to avoid the entry of organisms. After incubation at 25°C for 7 days, young nematodes started to emerge from the *Galleria* cadavers. Nematodes were then collected from the Petri dishes and filtrated using filter paper to collect pure and active infective stages of nematodes, which were stored in distilled water with 0.1% formalin.

Permanent mounts for nematodes were carried out as follows: killing nematodes by adding hot water 80°C to an equal amount of nematode solution in a plastic tube (10 × 1cm) (Pionar,1975). After killing nematodes, it must be fixed for 4-5 days in TAF solution and then processed to glycerin via the evaporation method (15 parts 95% ethanol, 1 part glycerin, 5 parts water), then nematodes were lift by small needle to the glass slides in one drop of acid fuchsin lactophenol and covered with a glass cover which was fixed by finger-nail polish.

Keys of nematodes have been used (Pionar, 1975, 1979, 1990). Keys of characters are normally visible under a light microscope (70-280 x) and all

required measurements for the identification process were carried out as proposed by Nguyen and Smart (1992).

The greater wax moth, *Galleria mellonella* were used for culturing of the entomopathogenic nematode. They were starved for 2 hours before being infect with nematodes. Modified white traps (as previously described) were used in large numbers to obtain sufficient numbers of nematodes for the present experiments. Collected nematodes were stored in plastic tubes (50 ml) in a refrigerator adjusted to 10 °C temperature.

Application of nematodes on the potato tuber moth stages:

Larvae, pupae and adults of the potato tuber moth, *Phthorimaea operculella* were collected from the culture reared in the laboratory in glass jars (30 x 30 x 60 cm) on potato tubers. Thirty individuals of each stage subjected to each concentration (500, 1000, 1500, 2000 and 2500 IJS) of *Steinernema carpocapsae* nematodes to determine their effects against potato tuber moth insect under laboratory conditions of (25 ±5 C°& 60±5% RH).

Each ten individuals of each stage 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. Every nematode concentration was sprayed on the individuals as 5 ml distilled water containing nematodes. At control treatment, individuals were sprayed with 5 ml distilled water without nematodes. Each treatment was replicated three times. Mortality was daily checked along 5 days for all concentrations, and percentage of mortality was calculated for each nematode species at different concentrations. Mortality percentage was modified by Abbott's formula (Abbott 1925).

RESULTS AND DISCUSSION

Effect of the nematode, *Steinernema carpocapsae* against different stages of the potato tuber moth, *Phthorimaea operculella* Zeller under laboratory conditions.

1 - Effect on larval stage of the potato tuber moth

Data presented in Table (1) indicated that the *Steinernema carpocapsae* nematode greatly controlled the larvae of the potato tuber moth along the five days of the exposure of larvae to nematodes, where it gave 74 % as grand mean of mortality percentages at 2000 infective juvenile individuals per 10 larvae, and resulted 70 % mortality at 1500 & 2500 infective juveniles of nematode. At 1000 invective juveniles per ten larvae, the grand mean of the mortality percentages was 64 %, while it was only 44 % with the concentration of 500 infective juveniles per 10 larvae of potato tuber moth.

Table (1): Mortality percentages of larval stage of the potato tuber Moth *Phthorimaea operculella* as influenced by *Steinernema carpocapsae* nematode under laboratory conditions.

Nematode concentrations IJs/ 10 larvae	Mortality percentages of larvae					
	1 day	2 Day	3 Day	4 day	5 Day	Grand mean
500	0.0	0.0	20.0	100.0	100.0	44.0
1000	0.0	40.0	80.0	100.0	100.0	64.0
1500	0.0	80.0	80.0	90.0	100.0	70.0
2000	0.0	80.0	90.0	100.0	100.0	74.0
2500	0.0	70.0	80.0	100.0	100.0	70.0
Check	0.0	0.0	0.0	0.0	0.0	0.0

2- Effect on pupal stage of the potato tuber moth

Data presented in Table (2) indicated that the *Steinernema carpocapsae* nematode did not have any effect on the pupal stage of the potato tuber moth at different concentrations of nematode, where the mortality percentages were zero along the five days of the experiment.

Table (2); Mortality percentages of pupal stage of the potato tuber Moth *Phthorimaea operculella* as influenced by *Steinernema carpocapsae* nematode under laboratory conditions.

Nematode concentrations IJs/ 10 pupae	Mortality percentages of pupae					
	1 day	2 Day	3 Day	4 day	5 Day	Grand mean
500	0.0	0.0	0.0	0.0	0.0	0.0
1000	0.0	0.0	0.0	0.0	0.0	0.0
1500	0.0	0.0	0.0	0.0	0.0	0.0
2000	0.0	0.0	0.0	0.0	0.0	0.0
2500	0.0	0.0	0.0	0.0	0.0	0.0
Check	0.0	0.0	0.0	0.0	0.0	0.0

3- Effect on adult stage of the potato tuber moth

Data presented in Table (3) indicated that the *Steinernema carpocapsae* nematode did not satisfactory control the adult stage of the potato tuber moth along the five days of the exposure of adults to nematodes, where it gave only 16 % as grand mean as mortality percentages at 500, 1000, 2000, and 2500 infective juvenile individuals per 10 adults, and resulted only 12 % mortality of potato tuber adults at 1500 infective juveniles of nematodes.

Table (3): Mortality percentages of adult stage of the potato tuber Moth Phthorimaea *operculella* as influenced by *Steinernema carpocapsae* nematode under laboratory conditions.

Nematode concentrations IJs/ 10 adults	Mortality percentages of adults					
	1 Day	2 Day	3 Day	4 day	5 Day	Grand mean
500	0.0	20.0	20.0	20.0	20.0	16.0
1000	0.0	20.0	20.0	20.0	20.0	16.0
1500	0.0	0.0	20.0	20.0	20.0	12.0
2000	0.0	20.0	20.0	20.0	20.0	16.0
2500	0.0	20.0	20.0	20.0	20.0	16.0
Check	0.0	0.0	0.0	0.0	0.0	0.0

Finally, data indicated that the use of the entomopathogenic nematode *Steinernema carpocapsae* in the control of potato tuber moth stages gave different results according to the target stage of the moth as well as the used concentration of the nematodes, where the highest mortality percentages were recorded with larva stage (74%), followed by adult stage (16%), while it was zero% with pupal stage. The most suitable concentration of *Steinernema carpocapsae* nematode was 2000 infective juveniles per ten individuals of the pest.

These results are in harmony with those obtained by Belton *et al.*, (1987), Sorial (2001), Keila (2004), Mahmoud (2007) , Mahmoud and Osman (2007), Shamseldean *et al.*, (2009) , Divya *et al.*, (2010), and Sweelam *et al.*, (2010) who applied different genera of entomopathogenic nematode i.e. *Heterorhabditis indica, Heterorhabditis bacteriophora, Heterorhabditis megidis, Steinernema feltiae, Steinernema carpocapsae* against many of insect pests i.e. red palm weevil, *Rhynchophorus ferrugineus* gram pod borer, *Helicoverpa armigera*, tobacco caterpillar, *Spodoptera litura* , greater wax moth, *Galleria mellonella* , the Egyptian cotton leafworm, *Spodoptera littoralis*, the greasy cutworm, *Agrotis ipsilon* , peach fruit fly, *Bactrocera zonata* , tephritid fruit fly *Anastrepha ludens*, mole cricket, *Gryllotalpa africana* and house fly *Musca domestica.*

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المكافحة البيولوجية لدودة درنات البطاطس بالنيماتودا الممرضة للحشرات

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الملخص العربي

تم اجراء هذه التجربة بمعمل النيماتودا بكلية الزراعة جامعة المنوفية وذلك لاختبار كفاءة النيماتودا الممرضة للحشرات فى مكافحة اطوار فراشة درنات البطاطس وتناول البحث دراسة التاثيرات المحتلفة للنيماتودا ***Steinernema carpocapsae*** فى المكافحة البيولوجية لفراشة درنات البطاطس ***Phthorimaea operculella*** تحت ظروف المعمل وتم تعريض الاطوارالمختلفة من الحشرة وهى (اليرقات– العذارى– الحشرات الكاملة) الى تركيزات محتلفة من النوع السابق من النيماتودا (IJS 2500 -2000 -1500 -1000 -500) وسجلت النتائج على مدى خمسة أيام من المعاملة ووجد ان التاثير الفعال للنيماتودا كان على الطور اليرقى للحشرة حيث اعطت متوسط نسبة موت ٧٤% عند التركيز I.J.S/2000/١٠يرقات وكان ليس لها تاثير على العذارى حيث كانت نسبة الموت صفر% وكان التاثير على الطور الكامل للحشرة ضعيفا حيث اعطت متوسط نسبة موت ١٦% لذا نفضل استخدام Steinernema carpocapsae فى المكافحة البيولوجية ليرقات فراشة درنات البطاطس.