Minufiya J. Agric. Res. Vol.39 No. 6: 1935 - 1941 (2014) "http://www.mujar.net"

IMPACT OF ENTOMOPATHOGENIC NEMATODES (HETERORHABDITIDAE AND STEINERNEMATIDAE) IN THE BIOLOGICAL CONTROL OF THE GREATER WAX MOTH, *GALLERIA MELLONELLA*

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(Received Sept., 22, 2014)

ABSTRACT: *This work was conducted in the laboratory and the experimental apiary of the Faculty of Agriculture, Menoufia University, Egypt. The main purpose of this work was to study the effect of two entomopathogenic nematodes Heterorhabditis bacteriophora, and Steinernema carpocapsae in the control of the greater wax moth using two methods of application. The obtained results indicated that the highest reduction percentages of the wax moth larvae were recorded at the treatment of direct exposure of 2000 infective juveniles of Heterorhabditis bacteriophora after 6 days reaching 85.3 %, while the least percentages were recorded with the treatment of 500 infective juveniles after 6 days reaching 45.2 %. The highest reduction percentages of the wax moth larvae were recorded at the treatment of direct exposure of 1500 infective juveniles of Steinernema carpocapsae after 6 days reaching 88.8 %, while the least percentages were recorded with the treatment of 500 infective juveniles after 6 days reaching 47.7 %. The method of spraying bee wax with nematodes and let wax larvae to fed on it , gave un satisfactory reduction results in comparison with the direct exposure of wax moth larvae to nematodes.*

Key words: *Heterorhabditis bacteriophora, Steinernema carpocapsae, entomopathogenic nematode, Galleria mellonella, biological control.*

INTRODUCTION

Entomopathogenic nematodes from the family Steinernematidae and Heterorhabditidae (Chitwood and Chitwood) are obligate and lethal parasites of insects. They occur in soil and epigeal habitats and are effective biological control agents for several pests (Abbas *et al.,* 2000; Ebssa *et al.,* 2001; Journey and Ostlie, 2000; Lacey and Chauvin, 1999). The only free-living stage of Steinernematidae is the infective juvenile (IJ), the third larval stage. Steinernematids are symbiotically associated with enteric bacteria of the genus *Xenorhabdus* Thomas and Poinar, 1979 (Stock and Koppenhöfer, 2003). IJs enter the insect host through natural orifices (mouth, anus and spiracles) (Lewis and Clarke 2012; Gaugler , 2002) or through thin sections of the cuticle (Peters and Ehlers, 1994). When the IJs reach the hemocoel of the host, the bacterial cells are released resulting in insect death within 48 h (Boemare and Akhurst, 1988). The bacterium serves as a food source for the nematode and is required for nematode growth and reproduction (Griffin *et al.,* 2005). The nematodes undergo one to three amphimictic generations within a host and when the nutrients of the host are depleted, whereupon adult development is suppressed and IJ accumulate and emerge into the soil where they may survive for several months waiting for another suitable host (Stock and Koppenhöfer, 2003).

Interest in entomopathogenic nematodes has increased significantly in recent years as a biological control mechanism for insect pests as well as in basic research areas such as ecology, biodiversity, evolution, biochemistry, symbiosis and molecular genetics (Burnell and Stock, 2000; Shapiro-Ilan *et al.,* 2012). The successful application and commercialization of nematodes as

biological control agents has stimulated research to improve their efficacy against pests and to isolate new and more virulent strains (Gaugler, 2002; Gungor *et al*., 2006; Shapiro-Ilan *et al*., 2012). Therefore, considerable effort is being made to evaluate these nematodes to be used as biological control agents for pests.

From these points of view the aim of this study was to evaluate the efficacy of two different entomopathogenic nematode genera in the biological control of the greater wax moth, Galleria mellonella , under laboratory conditions.

MATERIALS AND METHODS:

1- Rearing of wax moth:

Second instar larvae of the greater wax moth were collected from naturally infested honey bee hives. Wood boxes 40 x 30 x 30cm were used in the rearing process under laboratory conditions of 25 ± 5 ºC and 70 ± 5 % Relative Humidity. Collected larvae were putted in the boxes with infested wax combs and left to feed and grow. Boxes were covered with polyethylene plastic. Wax combs were added as needed until pupation process, then after emergency of moths which laid eggs, hatched to larvae. Fourth instar larvae were used in the toxicological tests.

2- Rearing of entomopathogenic nematodes:

Two species of entomopathogenic nematodes (*Heterorhabditis bacteriophora* and *Steinernema carpocapsae*) were obtained from the biological laboratory of the Economic Entomology and Agricultural Zoology of the Faculty of Agriculture Shebin El-Kom, Menoufia Governorate.

Modified White traps (1927) were used in the rearing process of nematodes, where the trap 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. The greater wax moth, Galleria mellonella were used for culturing of both entomopathogenic nematodes. They were starved for 2 hours before being infect with nematodes. Modified white traps 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.

Experiments were conducted at 10 cm Petri dishes where 15 larvae of the greater wax moth were put at each dish , then 10 ml water solution contain 500, 1000, 1500, 2000 infective juveniles of each nematode species. Pots were covered with its cover , and each treatment was replicated 10 times . Reduction percentages of wax larvae using Abbott formula (1925) was determined daily for six days.

Another method of nematode application was conducted at 10 cm Petri dishes , where 1 ml water solution contain 500, 1000, 1500, 2000 infective juveniles of each nematode species was sprayed on about 5 grams of wax pieces putted in the dish and covered with its cover . Treatments were repeated ten times , and the reduction percentages of wax larvae using Abbott formula (1925) was determined daily for six days.

RESULTS AND DISCUSSION:

Efficacy of entomopathogenic nematodes against the greater wax moth larvae under laboratory conditions :

The efficacy of different doses of the two entomopathogenic nematode species (*Heterorhabditis bacteriophora* , *Steinernema carpocapsae*) applied at two methods , were evaluated against the greater wax moth larvae under laboratory

conditions 27± 3 C° &70 ± 5% relative humidity .

Data presented in Table (1) show the effect of direct exposure (Direct spraying) of different doses of the entomopathogenic nematode , *Heterorhabditis bacteriophora* on the sixth instar larvae of the greater wax moth , *Galleria mellonella*, under laboratory conditions.

Results indicated that the highest reduction percentages of the sixth instar larvae of the greater wax moth , *Galleria mellonella*, was observed at the treatment of exposure of 15 larvae to a water solution of 2000 Infective juveniles of *H. bacteriophora* recording 85.31 % , followed by the treatment of 1500 infective juveniles giving 75.32% , and the dose of 1000 infective juvenile gave 70.47 % , while the dose of 50 nematode infective juveniles gave only 45.16 % reduction percentages.

Data presented in Table (2) show the effect of feeding the sixth instar larvae of the greater wax moth , *Galleria mellonella* on pieces of bee wax treated with different doses of the entomopathogenic nematode , *Heterorhabditis bacteriophora* , under laboratory conditions.

Results indicated that the highest reduction percentages of the sixth instar larvae of the greater wax moth , *Galleria mellonella*, was observed at the treatment of feeding 15 larvae to pieces of bee wax treated with 2000 Infective juveniles of *H. bacteriophora* recording 39.99 % , followed by the treatment of 1500 infective juveniles giving 33.32 % , and the dose of 1000 infective juvenile gave 22.22 % , while the dose of 50 nematode infective juveniles gave only 17.77 % reduction percentages.

Data presented in Table (3) show the effect of direct exposure (Direct spraying) of different doses of the entomopathogenic nematode , *Steinernema carpocapsae* on the sixth instar larvae of the greater wax moth , *Galleria mellonella*, under laboratory conditions.

Results indicated that the highest reduction percentages of the sixth instar larvae of the greater wax moth , *Galleria mellonella*, was observed at the treatment of exposure of 15 larvae to a water solution of 1500 Infective juveniles of *S. carpocapsae* recording 88.73 % , followed by the treatment of 2000 infective juveniles giving 80.71 % , and the dose of 1000 infective juvenile gave 59.28 % , while the dose of 500 infective nematode juveniles gave only 47.62 % reduction percentages.

Table (1): Reduction percentages of the sixth instar larvae of the greater wax moth , Galleria mellonella as influenced by direct exposure of entomopathogenic nematode , *Heterorhabditis bacteriophora* , under laboratory conditions (Direct spraying).

Nematode dose	Reduction percentages after						Over all reduction
	One day	Two days	Three days	Four days	Five days	Six days	
500	33.33	33.33	40	42.85	42.85	78.57	45.16
1000	46.66	66.66	66.66	78.57	78.57	85.71	70.47
1500	33.33	60.0	80	85.71	92.85	100	75.32
2000	53.33	86.66	93.33	92.85	92.85	92.85	85.31

Table (2): Reduction percentages of the sixth instar larvae of the greater wax moth , *Galleria mellonella* as influenced by feeding on bee wax treated with entomopathogenic nematode , *Heterorhabditis bacteriophora* , under laboratory conditions (sprayed on wax pieces) .

Wax treatments	Reduction percentages after						Over all reduction
	One day	Two days	Three days	Four days	Five days	Six days	
500	0.0	6.66	20	26.66	26.66	26.66	17.77
1000	0.0	13.33	20	33.33	33.33	33.33	22.22
1500	13.33	26.66	33.33	33.33	46.66	46.66	33.32
2000	6.66	33.33	46.66	46.66	53.33	53.33	39.99

Table (3): Reduction percentages of the sixth instar larvae of the greater wax moth , *Galleria mellonella* as influenced by direct exposure of entomopathogenic nematode , *Steinernema carpocapsae*, under laboratory conditions (Direct spraying).

Nematode dose	Reduction percentages after						Over all reduction
	One day	Two days	Three days	Four days	Five days	Six days	
500	13.33	40	46.66	64.28	42.85	78.57	47.62
1000	26.66	40	53.33	64.28	85.71	85.71	59.28
1500	80	80	86.66	92.85	92.85	100	88.73
2000	66.66	73.33	80	78.57	92.85	92.85	80.71

Data presented in Table (4) show the effect of feeding the sixth instar larvae of the greater wax moth , *Galleria mellonella* on pieces of bee wax treated with different doses of the entomopathogenic nematode , *Steinernema carpocapsae*, under laboratory conditions.

Results indicated that the highest reduction percentages of the sixth instar larvae of the greater wax moth , *Galleria mellonella*, was observed at the treatments of feeding 15 larvae to pieces of bee wax treated with 2000 or 1500 infective juveniles of *S. carpocapsae* recording 38.88 % , while the treatments of 500 and 1000 infective juveniles gave only 23.32 and 27.77 % .

Table (4): Reduction percentages of the sixth instar larvae of the greater wax moth , *Galleria mellonella* as influenced by feeding on bee wax treated with entomopathogenic nematode , *Steinernema carpocapsae*, under laboratory conditions (sprayed on wax pieces) .

Wax treatments	Reduction percentages after						Over all reduction
	One day	Two days	Three days	Four days	Five days	Six days	
500	6.66	26.66	26.66	26.66	26.66	26.66	23.32
1000	0.0	20.0	33.33	33.33	40.0	40.0	27.77
1500	13.33	26.66	40.0	46.66	53.33	53.33	38.88
2000	6.66	20.0	33.33	53.33	60.0	60.0	38.88

The obtained results are in harmony with those obtained by Ramos-Rodriguez, *et al*., (2007), Shahina and Salma (2009), Athanassiou, *et al*., (2010), Shahina, and Salma (2010) , and Shrestha, and Kim Yong Gyun (2010) ,Sweelam *et al*. (2010) whom used entomopathogenic nematodes, *Heterorhabditis bacteriophora* and *Steinernema feltiae* in the control of different insects i. e. house fly, *Musca domestica* , the rice weevil, ***Sitophilus oryzae*** , the red flour beetle, *Tribolium castaneum* , the lesser grain borer, *Rhyzopertha dominica* (F.), the Mediterranean flour moth, *Ephestia kuehniella* (Zeller) , and the pulse beetle, *Callosobruchus chinensis* (L.).

It could be concluded that the use of entomopathogenic nematodes, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* in the control of insects registered good results, but it needs more studies.

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أهمية النيماتودا الممرضة للحشرات فى المكافحة البيولوجية لدودة الشمع الكبرى

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الملخص العربى

أجرى هذا البحث تحت الظروف المعملية لإستخدام النيماتودا فى مكافحة ديدان الشمع الكبيرة فى المعامل البحثية والمنحل البحثى التابع لقسم الحشرات الاقتصادية والحيوان الزراعى بكلية الزراعة جامعة المنوفية .

أشارت نتائج مكافحة دودة الشمع الكبيرة بتركيزات مختلفة لنوعين من النيماتودا الممرضة للحشرات عن طريق المعاملة المباشر على اليرقات الى النتائج التالية :

١-بالنسبة للنوع النيماتودا *Heterorhabditis bacteriophora* أوضحت النتائج أن أعلى معدل موت ليرقات ديدان الشمع بعد ٦ أيام من تنفيذ التجربة كانت عند إستخدام تركيز (٢٠٠٠) بنسبة بلغت ٨٥.٣ ٪ ، يليها التركيز ١٥٠٠بنسبة ٧٥.٣ ٪ ونسبة ٧٠.٤ ٪ لتركيز (١٠٠٠) ، بينما كانت أقل نسبة موت للتركيز (٥٠٠) حيث بلغت ٤٥.٢ ٪.

٢-وبالنسبة لنوع النيماتودا *Steinernema carpocapsae* أشارت النتائج الى أن أعلى معدل موت ليرقات دودة الشمع الكبيرة كانت عند إستخدام التركيز (١٥٠٠)حيث بلغت نسبتها ٨٨.٨ ٪ ، يليها التركيز (٢٠٠٠) بسبة ٨٠.٧ ٪ وجاء تتركيز (١٠٠٠) في المركز الثالث بنسبة ٥٩.٣ ٪ بينما احتل التركيز (٥٠٠)المركز الأخير بنسبة ٤٧.٧ ٪ .

كما أوضحت نتائج مكافحة دودة الشمع الكبيرة بتراكيز مختلفة من النيماتودا الممرضة للحشرات السابق ذكرها عن طريق معاملة قطع من شمع النحل وتقديمه لليرقات للتغذية عليه الى النتائج التالية :

١- أعلى نسبة موت ليرقات دودة الشمع الكبيرة عند إستخدام جنس *Heterorhabditis bacteriophora* ، كان عند إستخدام التركيز (٢٠٠٠) حيث بلغت ٣٩.٩٪ ، يليها التركيز (١٥٠٠) بنسبة ٣٣.٣ ٪ ، وكانت النسبة منخفظة جدا للتراكيز (١٠٠٠) ، (٥٠٠) بنسبة ٢٢.٢ ، ١٧.٨ ٪ على التوالي .

٢- لم تختلف النتائج كثيرا عند إستخدام النيماتودا *Steinernema carpocapsae* حيث تساوى معدل موت يرقات ديدان الشمع عند إستخدام التراكيز (٢٠٠٠ ، ١٥٠٠) بنسبة ٣٨.٩٪، بينما بلغت نسبة موت يرقات ديدان الشمع للتركيز (١٠٠٠) نسبة ٢٧.٨ ٪ والتركيز (٥٠٠) ٢٣.٣ ٪.

ومن خلال نتائج البحث يتضح أن إستخدام لنوعى النيماتودا تحت الاختبار عن طريق المعاملة المباشرة على اليرقات أدت الى نتائج أفضل من رش النيماتودا على أقراص الشمع المستخدم في تغذية اليرقات .