ENTOMOPATHOGENIC NEMATODES AS A MODEL OF FIELD APPLICATION AGAINST SOME IMPORTANT INSECT PESTS IN EGYPT

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ABSTRACT

Entomopathogenic Nematodes (EPN) from the families Steinernematidae and Heterorhabditidae proved to be effective as biological control agents. Virulence and field efficacy of different species and/or strains of entomopathogenic nematodes of both families were tested against insect pests of fruits and vegetables in Egypt. Local isolate, EGB20, belonging to the genus Steinernema, Steinernema glaseri (NJ strains), and Heterorhabditis bacteriophora (EGB13), Heterorhabditis indica (EBN16), were tested against the leopard moth, Zeuzera pyrina, the Egyptian cotton leafworm, Spodoptera littoralis, the scarab beetle, Temnorhynchus baal, and the cabbage looper, Pieris brassica in the field. The results indicated that all the tested nematodes at 1000 IJs/ml were more effective against Z. pyrina. The data showed the superiority of Steinernema sp. (EGB20) strain over Heterorhabditis bacteriophora (EGB13) and H. indica (EBN16). Injection of the tested nematode suspension into the insect galleries of Zeuzera pyrina was more effective than the spray technique. The addition of an evaporation retardant and sticker agent was associated with efficient insect control. Moreover, Steinernema glaseri was tested in the field against T. baal infestation on strawberry plants with a percentage of population reduction varying from 89.2% to 96.8% after four field applications. The overall percentage of population reduction after eight field applications was 96.3% to 99.1%. The results also showed that; both EGB13 and EGB20 nematode isolates were more effective in reducing larval population of S. littoralis and P. brassica on cabbage plants than the isolate EBN16.

Key words: *Entomopathogenic nematodes, Steinernema and Heterorhabditis genera, Temnorhynchus baal (Reiche), Steinernema glaseri, Galleria mellonella, biological control, Egyptian cotton leafworm, fruit insect pests, vegetable insect pests.*

INTRODUCTION

Sustainable agriculture in the 21st century will rely increasingly on alternative interventions for pest management that are environmentally friendly and reduce the amount of human contact with chemical pesticides. The role of microbial pesticides in the integrated management of insect pests has been recently reviewed for agriculture forestry and public health. Nematodes from the families Steinernematidae and Heterorhabditidae have proven to be the most effective as biological control agents. They are soil-inhabiting agents and can be used effectively to control soilborne insect pests and insect in cryptic habitats (Jansson, *et al.*, 1991). As a group of nearly 45 species, each with its own suite of preferred hosts, beneficial nematodes can be used to control a wide range of insect pests including a variety of caterpillars, cutworms, grubs, and weevils. Beneficial nematodes have been released extensively in the field with negligible effects on nontarget insects and are regarded as exceptionally safe to the environment (Bedding 1976).

Field application showed that the entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* are effective biological control agents against a wide variety of soil insect pests and for various cropping systems, such as the black vine weevil, *Otiorhynchus sulcatus* F. in cranberry bogs and strawberry fields, citrus root weevils (*Diaprepes abbrevatus* L. *Pachnaeus litus* Germar) in citrus groves, or the alfalfa fields (Fife, *et al.*, 2003). The inoculate release of nematode-based biological control agents are thought to succeed when: 1) the pest is present throughout most of the year, 2) the pest has a high economic threshold, and 3) soil conditions are favourable to nematodes survival. All these criteria can be met in turf system, in which the univoltine scarabs have larvae present in the soil for most of the year and the turf is irrigated during dray conditions favourable to nematodes. These conditions are close to conditions of strawberry fields in Egypt. In this study, focus will be concentrated on using entomopathogenic nematode as a model under field conditions for controlling the leopard moth, *Zeuzera pyrina*, the Egyptian cotton leafworm, *Spodoptera littoralis*, the scarab beetle, *Temnorhynchus baal*, and the cabbage looper, *Pieris brassica*. All these insects are important as pests of crops, fruits and vegetables in Egypt.

MATERIALS AND METHODS

Experimental nematodes:

Local isolate EGB20, belonging to the genus *Steinernema*, *Steinernema glaseri* (NJ strains), *Heterorhabditis bacteriophora* (EGB13) and *Heterorhabditis indica* (EBN16) were used in this study. All these local isolates were cultured on the last instar larvae of *Galleria mellonella* according to the method by Dutky *et al.* (1964) and IJs were harvested from nematode traps as described by White (1927) at 25 ± 2°C. A stock suspension of IJs in sterilized

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distilled water was stored at 10°C until needed. All nematodes were used within 2 weeks of harvest and a new infection cycle and a stock of IJs was made every 2 weeks.

1- Effect of Successive Applications on major insect pests:

***1-1- Zeuzera pyrina* Infestation:**

In this experiment, injection of 1000 IJs/ml (3 ml/active gallery) of EGB13, EBN 16, and EGB20 was practiced and the data were conducted with oriented spraying using a 10 liter portable sprayer of the same nematodes 1000 IJs/ml during the insect activity in April 2005. These treatments were tested to study the possibility to control the leopard moth, *Z. pyrina* in an apple orchard at El-Sidkiah village, Kaliubia Governorate during the insect active season. Control trees were exposed to water or super film solution. Experimental units consisted of individual apple trees in five replicates per treatment. All treatments were applied just before sunset. At the time of application, active galleries of *Z. pyrina* larvae were marked and frasses were removed for further two days. One week after treatment, individual galleries were examined to estimate larval mortality. The dead larvae were extracted by breaking the small branches or by using a flexible hooked wire. These dead larvae were dissected to ascertain nematode infection as mentioned before. The percentage of reduced mortality in the population was calculated using Henderson and Tilton formula (1955).

1-2– *Temnorhynchus baal* Infestation:

The Nematodes were applied to the soil surface within the barriers at 4:00 PM before dusk, from October,2004 to March, 2005. The EPN were used through the fertilizing system via the net of drip irrigation (Atwa, 2003). The nematode concentrations were adjusted to reach 1000 IJs per each nozzle in the drip irrigation system and 20,000 nozzles were used to irrigate one feddan (ca 4,200 m^2). Meanwhile, 40,000 plants were grown in this feddan in rows, 1 m. wide, and 50 m. long. The nematode viability was checked just before application by pouring samples of the nematode solution into a glass beaker. These samples were examined for nematode movement and curled nematodes with a 10X hand lens immediately before application to verify that the nematodes were alive before the application. The nematodes were also collected from dripping nozzles to make sure that they were not affected by either the drip pressure or any contaminations in the irrigation system (Atwa, 2003). The pathogenecity of the nematode *Steinernema glaseri* after going through the drip irrigation system was determined by dripping 5 random nematode samples from each plot during the application, the suspension was examined by placing into a 15-cm petri dish lined with filter paper. The efficacy was examined using *G. mellonella*. The percentage in population reduction was calculated using Henderson and Tilton formula (1955).

1-3– *Spodoptera littoralis* Infestations:

In this test, all materials were applied in the field by spraying using 10 litters portable spraying on naturally-infested cabbage plants with *S. littoralis*. These materials included a suspension of superfilm (2 ml/liter) mixed with a suspension of EGB13, EBN16 or EGB20 strains (1000IJs/ml).

A cabbage *Brassica oleracea* var. capitata field of about ½ feddan naturally infested in the field with *S. littoralis* (5-7 larvae/plant) at Abou-Elnomros region, Giza governorate was selected for the present investigation. The research area was divided into plots (5x4m). Each plot contained 70 plants and represented one replicate. At sunset on the 1st of October, 2004 and 2005, three replicates were used per treatment. Three days after the applications, *S. littoralis* larvae were collected. Numbers of alive and dead larvae were determined. Additionally, the dead larvae were dissected to assure nematode infection. The percentage in population reduction was calculated using Henderson and Tilton formula (1955).

1-4– *Pieris brassicae* Infestation:

In this experiment a suspension of superfilm (2 ml/liter) mixed with a suspension of EGB13, EBN16 or EGB20 (1000 IJs/ml) were sprayed, using a 10 liter portable sprayer, at sunset to avoid excessive evaporation. These control agents were sprayed on cabbage *Brassica oleracea* var. capitata plants naturally-infested with *P. brassicae* larvae under field conditions at Abou-Elnomros, Giza Governorate on 1st November, 2004 and 2005.

The experimental area (½ feddan) was divided into many plots (20m^2 each). Each plot contained 70 cabbage plants. In this test, each plot was considered as one replicate. Three replicates were used per treatment. Treated plots were arranged in a randomized complete-block design. Three days after application, alive and dead larvae of *P. brassicae* per plot and per plant were determined. The dead larvae were collected and transported to the laboratory to be dissected for infectivity (Atwa, 1999). The percentage in population reduction was calculated using Henderson and Tilton formula (1955).

RESULTS AND DISCUSSION

1- Field evaluation of entomopathogenic nematodes on major inset pests

1-1- *Zeuzera pyrina* infesting apple trees

The efficacy data of tested entomopathogenic nematodes against *Z. pyrina* are presented in table (1). The percentage of infestation reduction was computed by Henderson and Tilton equation (1955).

The records were highly reduced under the two methods of treatment and also with or without evaporation retardant agent superfilm (tricitn polymers). The results showed the superiority of EGB20 strain against *Z. pyrina* infestation over both other strains especially with injection methods. This strain reduces the population by about 5 and 18% more than EGB13 and EBN16 respectively. In general, the data indicated that the efficacy of the tested nematode was more pronounced with injection methods and with superfilm technique than the other treatments.

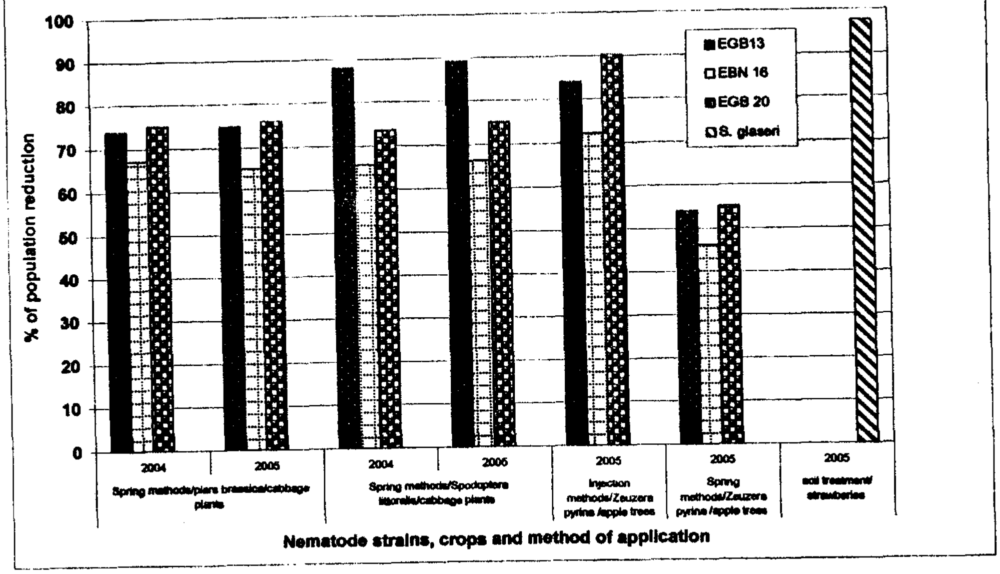
Concerning both methods of application (injection and spraying), it was found that injection of tested agents into galleries was the most effective method against *Z. pyrina* larvae. Apparently, these galleries protected the injected IJs from the unfavourable conditions such as desiccation and ultraviolet. In contrast, spraying nematodes on the smooth surface of the bark of the infested trees caused a great loss of nematode inoculums by dripping and desiccation. Furthermore the concealed tunnels of *Z. pyrina* larvae protected them from spraying applications (Abdel-Kawy *et al.*, 1988). It was noted that, application of superfilm (an evaporation retardant and sticker agent) used in the control of the leopard moth was associated with efficient insect control as shown in table (1). Apparently, this agent succeeded in prolonging nematode survival which leads to improving insect control. Similar mechanisms of other agents were reported by Welch and Briand (1961) who tested glycine, honey, glucose, sorbitol, urea and agar, Webster and Bronshill (1968) checked the water thicker Gelgard- M (0.13%), the evaporation retardant Folicote 351 (0.2%) and the surfactant A vlatone-T (0.1%). Bedding (1976) evaluated a variety of inert organic solvents including paraffin oils and Abdel Kawy *et al.* (1988) applied the water thicker SGP-104 starch graft polymer and Penolene as a spreader and sticker agents.

1-2– *Temnorhynchus baal* infesting strawberries.

The data in table (2) present the corrected percentage mortality of the scarab insect pest (Henderson and Tilton, 1955). The data indicated that the tested entomopathogenic nematode species *Steinernema glaseri* showed a high virulence against *Temnorhynchus baal* infestation. As cited in table (2), there was a significant variation between the six treated field plots and the untreated one (control). Meanwhile there is no significant differences between the six treated fields. The percentages of population reduction ranged from 89.2% to 96.8% after four field applications, while the overall percentage of population reduction after eight field applications varied from 96.3% to 99.1% (table 2). The use of inoculative release for the four and eight field applications gave almost the same degree of percent reduction of larval population with slight superiority of *S. glaseri*, but the insect population after the four applications was still high enough to cause economic damages to strawberry plants. Consequently, the application continued till the eighth application. The reduction percent ages were also recorded during the growing season and throughout the period of nematode application. This high level of reduction in pest population occurred due to the use of entomopathogenic nematode (crop / insect / nematodes, complex) application in cryptic habitat such as the soil. Once applied to the soil surface, the nematodes locate their insect host by detecting movement and following CO_2 emissions. They enter the pest larvae via natural openings such as the mouth, anus or breathing spiracles, or hack their way directly through weak spots in the hosts outer covering. Inside the insect larva, they release bacteria that multiply up on the insect tissue, quickly killing it. The nematode then feeds off the bacteria, and starts producing thousands of infective juvenile offspring. When the host insect cadaver finally disintegrates, these infective juveniles then move into the soil to locate a new host, and start the process over again. The short persistence of entomopathogenic nematodes in sandy soil is attributable to the quick lose of humidity in the sandy soils. The effect of "soil / pest (scarabs and weevils) / nematodes" systems probably facilitates the long persistence of entomopathogenic nematodes (Jansson, *et al.*, 1991). The drip irrigation system wash the nematodes quickly from the root area (rhizosphere) and at the same time, the daily irrigation induced a high levels of nematode recovery throughout the application period. Relative humidity, a critical nematode persistence factor in controlled environment, is also important in the soil environment and influences nematode survival (Booth, *et al.*, 2002).

Bedding and Nickson (1999) used *Heterorhabditis* sp. to control the African black beetle with nematode concentrations of 50000, 100000, 150000, and 200000 Infective Juveniles (IJs) / m^2 as inundative release. They obtained 42, 65, 80, and 88 % of insect mortality, respectively. Meanwhile, in the present study, we used 10000 IJs / m^2 in an inoculative release were used every 3 weeks (8 treatments, with a total number of 80000 IJs / m^2). The nematodes function extremely well with the presence of large numbers of whitegrubs in the soil (high larval population), because, the more dense of the grubs population, the greater chance of entomopathogenic nematodes to find their hosts, and the better chance of a second wave of parasitism (where thousands of infective juveniles are produced in the cadavers of whitegrubs killed by the initial application move back into the soil, looking for new hosts). Meanwhile, comparing efficacy of entomopathogenic nematodes with any other control

Figure 1. Comparative of population reduction required to different application methods and environment conditions.



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الملخص العربى

النيماتودا الممرضة للحشرات كنموذج للتطبيق الحقلي ضد بعض الآفات الحشرية الهامة فى مصر

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النيماتودا الممرضة للحشرات والتابعة لعائلتي شتينرنيما و هيتيرورابديس لها القدرة أن تكــون فعالــة كأحــد عوامــل المكافحــة البيولوجية للحشرات وقد تم إختبار القدرة والفعالية الحقلية للأنواع والسلالات المختلفة من هذه النيماتودا الممرضة للحشرات التابعــة للعــائلتين المذكورتين ضد العديد من الآفات الحشرية التي تصيب الخضر والفاكهة فى جمهورية مصر العربية. العزلة المحلية للسلالة إي جــي بــي ٢٠ التابعة لعائلة شتينرنيما والنوع شتينرنيما جلسري المعزول من ولاية نيو جيرسي والنوعين المعزولين من التربــة المحليــة هيتيرورابديــدس باكتيريوفورا (إي جي بي ١٣) و هيتيرورابديس إنديكا (إي بي إن ١٦) ، كل هذه العزلات و الأنواع تم إختبارها حقلياً ضد حفار ساق التفــاح (زيوزيرا بيرينا) ودودة ورق القطن المصرية (سبودوبترا ليتوراليس) وجعل الفراولة (تيمنورهينكس بال) ودودة الكرنب أو أبي دقيق الكرنــب (بيريس براسيكا) . وقد أثبتت التجارب أن كل سلالات وعزلات النيماتودا المختبرة عند تركيز ١٠٠٠ طور معدي / ملل فعاليــة عاليــة ضــد حفار ساق التفاح ، كما أظهرت النتائج تفوق العزلة إي جي بي ٢٠ التابعة لجنس شتينرنيما اكثر من العزلة هيتيرورابديس بــاكتيريوفورا (إي جي بي ١٣) والعزلة هيتيرورابديس إنديكا (إي بي إن ١٦). وقد أعطت عملية المكافحة عن طريق حقن محلول النيماتودا تفوقاً واضــحاً فــى مكافحة حفار ساق التفاح مقارنةً بعملية الرش الموجه لأنفاق الحشرة بهدف المكافحة ، كما أن إضافة مادة لاصق إلى محلول النيماتودا زاد مــن فعالية النيماتودا فى عملية المكافحة. علاوة على ذلك فإنه عند إستخدام النوع النيماتودى شتينرنيما جلسري لمكافحة جعل الفراولة (تيمنــورينكس بال) فإن عملية المكافحة عن طريق الإطلاق المحدود (ثماني إطلاقات) ، فإن الخفض في تعداد الآفة يتراوح تقريبا بين ٨٩ ، ٩٧ % بعد أربــع إطلاقات في حين أن الخفض فى التعداد تراوح تقريباً بين ٩٦ ، ٩٩ % للحقول المعاملة ، وعلى الرغم من أنه لا توجد فــروق معنويــة بــين الإطلاق المحدود لأربع مرات وثماني مرات إلا أن الإطلاق لثمان مرات يمثل أهمية خاصة للحفاظ على خفض تعداد الآفــة. كمــا أوضــحت النتائج أيضاً أن كلاً من السلالتين النيماتودتين إي جي بي ١٣ و إي جي بي ٢٠ كانتا ذات تأثير في خفض تعداد يرقات دودة ورق القطن وأبي دقيق الكرنب على نباتات الكرنب أكثر من العزلة أي بي إن ١٦.

الكلمات الدالة: النيماتودا الممرضة للحشرات ، شتينرنيما و هيتيرورابديس ، تيمنورينكس بال، شــتينرنيما جلــسري ، جاليريــا ميلــونيللا ، المكافحة الحيوية ، دودة ورق القطن المصرية ، آفات الفاكهة والخضر.