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EFFICIENCY OF ENTOMOPATHOGENIC NEMATODES, HTERORHABDITIDAE AND STEINERNEMATIDAE ON THE SNAIL *Theba pisana*(Muller) IN RAPHAH, NORTH SINAI, EGYPT

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ABSTRACT

The efficiency of entomopathogenic nematodes on the terrestrial snail,*Theba pisana*, was studied in the laboratory and under semi-field conditions.The percentage of mortalities were recorded when four nematode species, i.e.; *Heterorhabditis indicus* (Hi),*H. bacteriophora* (Hb),*H.bacteriophora* (HP88),and *Steinernema riobravis* were used at 1000,2000, 3000,4000,and 5000 IJs/pot. The infected snails were observed for 8 days. At the highest inoculums level, *H. indicus* ,*H.bacteriophora riobravis* (Hb),*H.bacteriophora* (HP88)gave mortality percentages up to 100% after 4 days of infection, while the species *S . riobravae* (Sr) gave 65% mortality at the same inoculum levels and after the same time. No development of the nematodes occurred inside the cadavers of snails. In addition, all cadavers were decomposed completely. In the semi field test, two species ,*H. indicus* and *S. glaseri* were sprayed on colonies of snails *Theba pisana* on the tree branches at three inoculum levels (1000,2000,3000 IJs/ml)with direct spraying. No mortalities were obtained at the two lower concentrations of both species. At the highest level (3000IJs/ml), *H. indicus*(Hi) gave 57.89% mortality after 3days of application only in the small individuals which ranged between 2-7mm. in length, while *S. glaseri*(Sg) gave 77.27% after the same time, and same inoculum level.

INTRODUCTION

Terrestrial mollusks represent an important economic problem in the world. In Egypt, they infest ornamental plants, vegetables, orchards, and field crops causing severe damage (El-Okda, 1979, 1980, 1984, and Hassanein and Hamed, 1989). In the present study, the snail *Theba pisana*(Muller) was found and collected from many plants such as olive and plum trees and grass. The entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae are promising bio-control agents for a wide range of pests. They are safe to non target organisms, exempting from registration in many countries and easy for production and application. Besides, they have the ability to search for hosts (like parasitoids) and kill them within 24 – 48 hours (Woodring & Kaya, 1988). The aim of present study is to investigate the efficiency of entomopathogenic nematodes on the snail,*Theba pisana* (Muller)in Raphah, North Saini Governorate, Egypt.

MATERIAL AND METHODS

Entomopathogenic nematodes tested in this work were *Steinernema riobravis* (Sr) and *Heterorhabditis indicus* (Hi) (isolated from Ras Al-Khima, United Arab Emirates(Abbas *et al.*, 2001) , *H. bacteriophora* (Hp88) and *S.*

glaseri (Sg) obtained from Dr. M.M.E, Saleh, Department of Pests and Plant Protection, National Research Center, Giza, Egypt, *H. bacteriophora*, (Hb) local strain isolated by Dr. Randa,M.Abd El-Rhman, Dept. of Pest Physiology, Plant Protection Research Institute, Giza, Egypt.
All nematode species were propagated in the laboratory on larvae of the greater wax moth, *Galleria mellonella* at 25 + 2C.

Laboratory Studies:

Snails were collected from Rafah area at north of Sinai. Twenty individuals were placed in plastic pots (15x 10x 6 cm). The pots were filled with sterile soil and sprayed with distilled water. Infective juveniles of nematodes in suspensions containing 1000, 2000,3000,4000,5000 IJs were applied into each pot. The pots were kept at 25+ 2 C and observed daily. Cadavers were obtained by gently brushing the shell removing its fragments and examined under stereomicroscope. The nematodes used in this trial were *Steinernema riobravis* (Sr), *H. indicus* (Hi), *H. bacteriophora* (Hp88) and *H. bacteriophora*, (Hb).

Semi –Field Studies:

Small tree branches about 20-30 cm. in length carrying individuals of snails were cut and fixed in plastic pots filled with soil .Three inoculum levels of infective nematodes juveniles , i.e.,1000, 2000, 3000 IJs/ml of *H. indicus* (HI) , and *S. glaseri* (Sg) were applied on the branches with colonies of snails directly. The pots were placed in muslin cages 30x30x50 cm and were kept out door at 35 C and 70+ 5 % R.H. Infected snails were examined daily for 8 days.

RESULTS AND DISCUSSION

1- Laboratory Studies:

Data in Table (1,2and3) show the infectivity of different nematode species to the snail. It was obvious that *H. indicus* was the most effective nematode species at all inoculum levels. The mortality percentages by all tested nematodes at the highest concentration (5000 IJs/pot) were 100 % after 4 and6 days of treatment. At the lowest inoculum (1000 Ijs/pot) they were50, 100 % after 4 and 6 days of infection, respectively. The % mortality reached 100% after 4 days by *H. indicus* and after 8 days by *H. bacteriophora*, (Hb). However, the highest mortality caused by *S. riobravis* (Sr) and *H. bacterophora* (Hp88) atthe same concentration were 50 and 75% , respectively after 8 days of infestation.

The LC50 values were (3097, 2165, 16457, 3.58 E +06) IJs after 4 days for the four tested nematodes (Hi, Hb, HP88, and Sr), respectively.

In spite of this relatively high percentage of mortality, no developmental stages of nematode were observed when the cadavers of snails were examined microscopically. Meanwhile, when these cadavers were kept in White - traps no migration of new progeny occurred.

Table(1):The percentage of mortality by different nematode species to the *T. pisane* after 4 days of infestation.

Inoculum level IJS/POT	Percentage of mortality			
	H. indicus	*H. bacteriophora*	*H.bacteriophora*	*S. riobravis*
1000	50	15	35	0
2000	55	25	45	0
3000	75	50	45	35
4000	85	80	75	50
5000	100	100	100	65
Control	0	0	0	0
LC25	27.473	18.095	0.476	37.254
LC50	3097.095	2165.101	16457.91	3.58E+06
LC95	3.13E+08	2.53E+08	1.93E+15	5.09E+18
slope(b)	0.329+/-0.045	0.325+/-0.046	0.149+/-0.977	0.135+/-0.057
c.c®	0.975	0.957	0.977	0.918

Table(2):The percentage of mortality by different nematode species to the *T. pisane* after 6 days of infestation.

Inoculum level IJS/POT	Percentage of mortality			
	H. indicus	*H. bacteriophora*	*H.bacteriophora*	*S. riobravis*
1000	100	75	55	10
2000	100	85	95	10
3000	100	90	95	85
4000	100	90	100	85
5000	-	-	-	85
Control	0	0	0	0
LC25	-	0.0013	-	307.807
LC50	-	24848.51	-	264810
LC95	-	1.38E+22	-	3.8E+12
slope(b)	-	0.093+/-0.04	-	0.23+/-0.04
c.c®	-	0.917	-	0.917

Table(3):The percentage of mortality by different nematode species to the *T. pisane* after 8 days of infestation.

Inoculum level IJS/POT	Percentage of mortality			
	H. indicus	*H. bacteriophora*	*H.bacteriophora*	*S. riobravis*
1000	-	100	75	50
2000	-	100	100	55
3000	-	100	100	100
4000	-	100	-	100
5000	-	-	-	100
Control	0	0	0	0
LC25				
LC50				
LC95				
slope(b)				
c.c®				

The failure of nematodes to develop and propagate in the cadavers of infected snails may be attributed to the reaction of snails against invasion of any organism (phagocytosis or encapsulation) . Encapsolation was described byYousif *et al.*(1980) and Azzam *et al.*(2000) in *Marisa cornaurietis* infected with the nematode *Angiostrongylus cantonensis* ,and in *Eobania vermiculata* infected with the nematode *Rhabditis* sp. The snails mortality might be attributed to the symbiotic bacteria that associate with Heterorhabditids and Steinernematids which are responsible for killing the host (Poinar and Thomas , 1966)

The cadavers of snails were decomposed completely as a result of the multiplication of such associated bacteria.

2-Semi field trial:

No mortalities were obtained at the two lower concentrations (1000, 2000 IJs/ml) of both *H. indicus* (Hi), and *S. glaseri* (Sg). On the other hand, the concentration 3000 JIs/ml of spray gave 57.9 and 77.3% mortality by *H. indicus*(Hi) and *S.glaseri* (Sg), respectively, in small snails only (3-7 mm length). The large snails (1-2 cm length) were not infected and no mortality occurred. This result could be interpreted as the large snails close the shells by a membrane made of dry mucus and are tightly attached to the branches that protect them from nematode invasion.

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تأثير النيماتودا الحشرية الهتيرورابديتيدي و الاشتاينيرنيماتيدي علي القوقع الارضي ثيبا بيسانا في رفح – شمال سيناء – مصر

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تم دراسة تأثير النيماتودا الحشرية علي القوقع الارضي ثيبا بيسانا في المعمل وكذا في تجربة شبة حقلية . تم تسجيل نسبة الموت عند استخدام ٤ انواع من النيماتودا وهي هتيرورابديتيس إنديكاس (Hi) ، هتيرورابديتيس باكتيريوفورا (Hb) ،هتيرورابديتيس باكتيريوفورا (HP88) و شتاينرنيما ريوبرافى (Sr) بتركيزات ١٠٠٠و ٢٠٠٠ و ٣٠٠٠ و ٤٠٠٠ و ٥٠٠٠ طور معدي/ علبة . واحصيت الافراد المعدية من القوقع الارضي بعد ٨ ايام . فعند مستوي اللقاح المرتفع , اعطت الانواع النيماتودية هتيرورابديتيس إنديكاس (Hi)، هتيرورابديتيس باكتيريوفورا (Hb) ،هتيرورابديتيس باكتيريوفورا HP88) نسبة موت ١٠٠% بعد ٤ ايام من العدوي , بينما اعطي النوع شتاينرنيما ريوبرافى (Sr) نسبة موت ٦٥% عند نفس مستوى التركيز و بعد نفس الفترة الزمنية. وقد لوحظ عدم حدوث تطور للأطوار النيماتودية داخل العائل الميت بالرغم من حدوث تحلل كامل لجثث القواقع. وفي التجربة الشبة حقلية تم رش أفرع شجر صغيرة مغطاة بمستعمرات من القوقع الأرضي بالأطوار النيماتودية المعدية للجنسين هتيرورابديتيس إنديكاس(HI) ، شتاينرنيما جلاسيرى (Sg) بتركيزات ١٠٠٠، ٢٠٠٠، و ٣٠٠٠ طورنيماتودى معدي /مل رشا مباشرا .

لم يتم تسجيل موت للقوقع عند استخدام التركيزين ٢٠٠٠،١٠٠٠ طور معدي /مل لكلا الجنسين،بينما بالنسبة للتركيز المرتفع٣٠٠٠طور معدي/ مل فقد أعطى النوع هتيرورابديتيس إنديكاس(HI) نسبة موت ٥٧,٨٩% بعد ٣ ايام من الرش علي القواقع الصغيرة فقط والتي تتراوح ما بين ٢-٧ مم في الطول, بينما اعطي الجنس شتاينرنيما جلاسيرى (Sg) ، نسبة موت ٧٧,٢٧عند نفس التركيز والزمن علي الافراد الصغيرة فقط للقواقع التي يتراوح قطرها بين ٢ – ٧ مم.