HACETTEPE JOURNAL OF BIOLOGY AND CHEMISTRY
Volume 35, Issue 1, January 2007

Research Article

Hacettepe J. Biol. & Chem., 2007, 35 (1), 39-44

Bioassays of Entomopathogen Nematode *Steinernema feltiae* All Type (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Tur-H2 (Rhabditida: Heterorhabditidae)

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Abstract

Steinernema feltiae Filipjev 1934 (Rhabditida: Steinernematidae) which was isolated on the Black Sea coast as the first record of entomopathogenic nematodes, has been cultivated for almost ten years in the Ecological Sciences laboratories in Biology Department of Hacettepe University since its first isolation. In this study, the infection potential of *S. feltiae* All type isolate has been evaluated in different conditions and compared with *Heterorhabditis bacteriophora* Poinar, 1970 (Rhabditida: Heterorhabditidae), another entomopathogenic nematode species. The optimum temperature has been shown to be 25 °C. A longer contact time resulted in higher infection efficiency. A smaller diameter of the petri-dishes where the host and nematodes were in contact, also increased the efficiency.

Among the arthropod hosts, *Plodia interpunctella* (Lepidoptera: Pyralidae) showed the highest susceptibility after *Galleria mellonella* (Lepidoptera: Galleridae) which is the most appropriate host for entomopathogen nematodes. *Musca domestica* (Diptera: Muscidae) and *Aedes aegypti* (Diptera: Culicidae) larva were seen as the least sensitive hosts regarding their susceptibility to both entomopathogenic nematodes.

Key Words: Entomopathogen nematodes, *Steinernema feltiae*, *Heterorhabditis bacteriophora*

Introduction

Entomopathogenic nematodes are insect parasites. The field of entomopathogenic nematology has experienced exponential growth over the past decade. These nematodes of the families Steinernematidae and Heterorhabditidae have been the subject of interest because of their great potential as biological control agents and as the model organisms showing parasitism and mutualism.

Twenty-five thousand hectares of Florida citrus were treated with nematodes originally. Nematodes are applied against the insect organisms harmful to cranberries, turfgrass, artichokes, mushrooms, apples, peaches, ornamentals and many other pests of horticulture, agriculture, home and garden (1,2,3,4,5,6). These nematodes mutualistically associated with bacteria in the genera Xenorhabdus and Photorhabdus are also candidates for studying host-parasite interactions. Advances or breakthroughs have also been made in elucidating foraging strategies, room temperature, formulations, ecological roles, population dynamics and innumerable other facets of entomological nematology (6).

In Turkey, *Steinernema feltiae* Filipjev,1934 (Rhabditida: Steinernematidae) was recorded for the first time in soil samples from the Black Sea coast (7). Then *Heterorhabditis bacteriophora* Poinar, 1976 (8,9), *S. carpocapsae* (10), *S. anatoliense* (11) were isolated in Turkey. Soil survey studies and ecological studies have been going on in Hacettepe University, Science Faculty

from that time.

In this study some bio-ecological experiments have been done on *S. feltiae* All type after its first isolation year, 1995 and *H. bacteriophora* TUR-H2 to assess their biotic potential under different conditions in different host species.

Materials and Methods

Steinernema feltiae has been reared in our laboratories since its first isolation in 1995 in *Galleria mellonella* which is the most appropriate host for most Steinernema species (12).

Entomopathogen nematodes were reared in the greater wax moth larva *G. mellonella* in the laboratory. The host, *G. mellonella* were cultured in the laboratory and kept 25 °C and 86 % humidity (13). All the containers in which the host and infective juveniles of entomopathogen nematode were kept were incubated at 20-25 °C for five days. Then dead larvae were transferred to and incubated individually in modified "White traps" (14) which consisted of a plastic container (90x 15 mm) filled with distilled water to a depth of 10 mm. The bottom of an inverted petri dish (60x15 mm) was placed on another petri dish allowing the edge of a Watman filter paper to come in contact with the water. The dead hosts were placed on the filter paper and incubated at 25 °C until all the nematode progeny had emerged and moved down into the water. Infective juveniles were collected and then kept in sterile distilled water at 4° C until used in the experiments.

The nematodes were identified by morphometric analysis, cross-breeding studies and molecular techniques (6, 14).

Heterorhabditis bacteriophora Tur-H2 isolate has been taken from Plant Protection and Control Research Institute. The biotic potential of the isolate *S. feltiae* and laboratory stock *H. bacteriophora* have been studied by counting all the infective juveniles emerging from the host and the duration of emergence after their exposure to *G. mellonella* larva. All emerging infective juveniles from a single host were recovered, counted and stored in the flasks. The duration of this emergence period was also recorded in our previous studies. The experiments on infection efficiency of *S. feltiae* in different laboratory conditions were done at different temperatures as 4°C, 10 °C, 15 °C, 20 °C, 25 °C and 30 °C, in different diameter petri-dishes and with different dosages and exposure times. For all of these experiments infective juveniles of *S. feltiae* were exposed to *G. mellonella* larvae and their % larval mortality was recorded. The experiments were kept at 25 °C. Host individuals of similar weights were chosen for the experiments.

To see the effect of different species of nematodes on different hosts; host species were challenged with a different number of infective juveniles in 10 cm diameter petri-dishes lined with one piece of filter paper, in the laboratory at 25 °C. *Galleria mellonella* on which the nematodes show the greatest activity were used as the standard organisms. *Musca domestica* (Diptera: Muscidae) and *Aedes aegypti* (Diptera: Culicidae) which are important vector organisms were chosen because of their availabilty in our laboratories eventhough they are not considered as targeted hosts. *Plodia interpunctella* (Lepidoptera: Pyralidae), *Hyponomeuta mallinella* (Lepidoptera: Hyponomeutidae) and *Hyponomeuta padella* (Lepidoptera: Hyponomeutidae) were collected from the trees that they were living on. 50 individuals of each species and of similar weights were chosen for each of the experiment. The hosts were exposed to the nematodes at 25 °C. 100 infective juveniles were inoculated into each individual host, two hosts for the each experiment were kept as control. Mortality were recorded after 48, 72 and 96 hours. The dead host was placed in white-traps to see if the nematod emergence occurs and complete the parasitic cycle. Then they were dissected in Ringer solution to see if the mortality was due to the inoculated nematode.

Results and Discussion

Galleria mellonella is the most appropriate host for the entomopathogen nematodes as recorded in many articles (6). Only one species , *S. scapterisci* has shown low susceptibility to *G .mellonella* up to now (15). In our study, *Galleria mellonella* was found to be the most sensitive host to *S. feltiae* All type among the arthropods tested. The infective juveniles of *S. feltiae* were still active on *G. mellonella* in our laboratory conditions. The ability to continue the nematode culture for ten years in laboratory conditions is dependent on different factors. It is not clear how much longer it will be possible to continue the culture in our laboratory. Infection efficiency has been tested at periods of certain periods with these nematodes.

The population growth of the nematodes mostly depends on the host reserves and partly on the population density in the host body. Other abiotic factors also play a role in this growth. In the same conditions, *H. bacteriophore* showed the faster growth, longer duration of emergence and higher number of infected juveniles emerging from the host in our previous studies. (13). The longer duration enables longer survival in the absence of the appropriate host,because they are safe in the host hoemocoel. These kinds of optimal strategies have been discussed in the latest article in terms of mutualistic evolution (16,17).

The infection efficiencies of *S. feltiae* in different conditions have been shown in Table 1 and 2. Infective juveniles were inoculated in *G. mellonella* larvae at different temperatures in petri-dishes and in test tubes containing substrat. At different temperatures, *S. feltiae* has showed the highest activity at 25°C against *G. mellonella.* In Hazır, et al's study, the development of five geographic isolates of *S. feltiae* at different degrees was examined and, all isolates caused 100 % mortality of wax moth larvae and developed and produced progeny between 8 and 25 °C . At 28 °C, mortality was 100 %, but no progeny was observed (18).

Saunders and Webster (1999) also observed the highest activity at 20-24 °C with *S. feltiae* (19). Mason and Hominick (1995) demonstrated the highest activity at 25 °C with four Heterorhabditis species. It was recorded in the same study that *Steinernema sp*. isolated from

Table 1. Infection efficacy of *Steinernema feltiae* (% mortality) at different temperatures.

	Temperature (°C)					
	4	10	15	20	25	30
	Mortality (%)					
In petri-dishes*	0	0	56.6	80	100	0
In test tubes**	0	13.3	60	76.6	100	10
	LD_{50} 14.62, LD_{90} 21.01* LD_{50} 24.86, LD_{90} 80**					

Table 2. Infection efficacy of *Steinernema feltiae* (% mortality) at different conditions in the laboratory.

Area (cm. parameter)			Exposure time (h.)			Doses (IJ/larva)					
3.5	4.5	8	2	4	6	10	30	50	75	100	150
100	75	75	33.3	46.6	86.6	22.5	55	67.5	82.5	100	100
X^2 : 0.153			LD_{50} 3.17, LD_{90} 9.23			LD_{50} 24.86, LD_{90} 80					

Canada could infect *G. mellonella* larva at 4 °C (20). In our study, no infection was seen at this temperature in either petri-dishes or tubes containing soil. The lowest temperature at which infection occured was 15 °C in petri-dishes and 10 °C in tubes.

According to our results, mortality rates alone do not show the optimal temperatures of the organisms. The temperature has an impact on mortality rates, the emergence time of infective juveniles, the number of emerging infective juveniles, the body size of the juveniles, the survival rate of these free forms. Each isolate tends to be more active at that temperature similar to the temperature of the area where they were originally isolated. In biological control studies, local species are generally more powerful in the soil to which they had adapted. So the efficiency at different temperatures also gives an idea of their distribution. Nevertheless, entomopathogen nematodes have been isolated from soils throughout the world in ecosystems ranging from sub-arctic to arid and tropical climates (6,12,21). Entomopathogen nematode isolation from cold regions indicates that they are capable of withstanding sub-zero conditions. However, little is known about their tolerance of cold temperatures (6). High temperatures (> 32 °C) have an adverse effect on reproduction, growth and survival of many organisms, including nematodes. Heat-shock proteins are known to be involved in survival at elevated temperatures (6,20). The improvement of heat tolerance of entomopathogen nematodes was a prime goal in several research projects conducted in the past few years. They can survive in extreme conditions by means of different adaptation mechanisms but infection may occur to a more limited degree, as seen in this experiment. Before using the entomopathogens in biocontrol studies this kind of infection studies should be carried out.

The infection efficiency of entomopathogen nematode cultures has been evaluated in Petri-dishes with 3.5 cm, 4.5 cm, 8 cm diameters. The smallest petri-dishes provided more chance of exposure, but the other dishes didn't show any difference in infection activity. In the applications inoculating the entomopathogen nematodes at the same location in high numbers and watering after inoculation make the infection chance higher as seen in many applications (5). The area size is a parameter related with the contact between the host and the parasites. For some entomopathogen nematodes which are cruisers, can actively search for their hosts and the area is not as important as it is for ambusher nematodes which wait for their hosts and for which contact becomes more important. It is necesarry to know their foraging behaviour for success in biocontrol studies.

The longer the time of exposure in the experiment, the higher were the infection rates in *G. mellonella* as a result of enhanced contact time. And finally dosages showed positive correlation with the increasing mortality, with 10, 30, 50, 75, 100 and 150 infective juveniles per host. The use of a hundred or 150 juveniles didn't make any difference with 100 % infection rate. Actually one IJ may be enough to infect the host *G. melonella* (5) . But, high numbers also increased the number that reached the host, came in close contact with it, entered its body and grew in its hoemocoel.

As we looked at the infection efficiency of *S. feltiae* against different hosts, *Plodia interpunctella* was seen as the most sensitive (Table 3). *Hyponomeuta mallinella, H. padella, Musca domestica* and *Aedes aegypti* gave poor results against *S. feltiae*. Actually the last two host insects which are of medical importance are not appropriate species for seeing the biological control effect because of separate natural habitats. But, we wanted to see if the parasitic cycle could be completed in different hosts regardless of their usefulness. Because they may be important hosts as presenting safe places for the nematodes to be able to survive in nature while they are waiting for more appropriate hosts.

Table 3. Infection efficacy of *Steinernema feltiae* and *Heterorhabditis bacteriophora* in different host organisms.

Organism tested	Nematod species	% mortality (25°C)		
		48 h	72 h	96 h
Galleria mellonella	*S. feltiae*	68	86	94
	H. bacteriophora	54	94	100
Plodia interpunctella	*S. feltiae*	40	58	32
	H. bacteriophora	40	62	74
Hyponomeuta mallinella	*S. feltiae*	7.5	10	20
	H. bacteriophora	40	12.5	17.5
Hyponomeuta padella	*S. feltiae*	12.5	7.5	42.5
	H. bacteriophora	37.5	15	27.5
Musca domestica	*S. feltiae*	2	2	8
	H. bacteriophora	10	4	10
Aedes aegypti	*S. feltiae*	-	1.48	1.48
	H. bacteriophora	-	1.48	3.7

In the laboratory, entomopathogenic nematodes present high effectiveness in close contact with various arthropods (5,6). But in the field they provide unpredictable field efficacy. Poorer than expected results have been attributed to several aspects of ecology and behaviour which were not understood at the time of the application (6).

After dissection of *H. malinella* individuals which were able to pupate but not to reach adulthood, Heterorhabditis infective juveniles were found at the rate of 33%. This rate was 25% for *S. feltiae* juveniles. After dissection of *H. padella* individuals which were able to pupate but not to reach adulthood, no *S. feltia* was observed, *Heterorhabditis bacteriophora* was found in only one pupa. No larval mortality was observed in *Musca domestica* larva. All were able to pupate.

The completion of the whole cycle is related with parasite-host interactions which took long years during their coevolution. To realize the parasite cycle, several steps are necessary: first entering the host, then the release of bacteria, production of the nematod, septicemia of the bacteria, the death of the host and finally the emergence of infective juveniles. Even if they reach the pupal stage, we have found infective juveniles in some hosts after dissection showing the succesfull completion of the first two steps of the parasitic cycle. Studying different hosts will give us more information on coevolution in parasites and their hosts.

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