THE BIOASSAY OF ENTOMOPATHOGENIC NEMATODES ON AGRICULTURAL INSECT PESTS IN LABORATORY CONDITIONS

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ABSTRACT

Entomopathogenic nematode / bacterium (hereinafter: EPN/EPB) symbiotic complexes have been providing a rather efficient and environmentally friendly way of controlling agricultural insects pests. Since the bacterial partner plays an important role of pathogenecity, we have developed several new EPN/EPB combinations between *Heterorhabditis* spp. *and Photorhabdus* spp. strains at the Genetic Department of the Eötvös Loránd University and tested them on several agricultural insect pests at the Plant Protection Institute of the VE Georgikon Faculty of Agriculture. The results of the previous gnotobiological analyses have been published elsewhere (Böszörményi *et al.*, in preparation).
In 1998 we tested some EPN/EPB symbiotic complexes were applied in three doses (1/1,1/10,1/100 IJ/ caterpillar) on last instar wax moth larvae *Galleria mellonella* as well as in two doses (3000 and 5000 infective juvenile/ white insect) on second stage white grubs of maybeetle (*Melolontha melolontha)* in lab conditions. It was concluded that the effectiveness of the new symbiotic complexes could be compared to that of natural EPN strains.
In 1999 we tested the effectiveness of various *Steinernema* species (*S. anomali, S. serratum, S. riobrave, S. glaserii, S. carpocapsae* Mex., T1, T2, T4, *S. feltiae* Nyíregyháza., IS6, (Israel) on German cockroaches (*Blattella germanica*) and on *Periplaneta americana.* Two tests were applied: (1) filter paper method: 5000 infective juvenile/ 5 cockroach/ plate (2) feeding experiment: wax moth killed by nematodes were given to cockroaches. Wax moth larvae killed by *Heterorhabditis bacteriophora* Brecon and wax moth larvae killed by freezing were used as controls. Conclusions: *S. carpocapsae* gave positive results against cockroaches and the cockroaches ate the wax moth larvae killed by *Steinernema* but avoided those killed by *Heterorhabditis.*

IZVLEČEK

BIOTIČNO PREIZKUŠANJE VPLIVA ENTOMOFAGNIH OGORČIC NA V KMETIJSTVU ŠKODLJIVE ŽUŽELKE V LABORATORIJSKIH RAZMERAH

Znano je, da so entomofagne ogorčice (EFN) odličen substrat za biotično varstvo rastlin pred škodljivimi žuželkami. V mehanizmu patogeneze so ključnega pomena mutualistične bakterije (bakterije, ki žive v združbi z ogorčicami).
Preizkušanja so bila opravljena v sodelovanju Inštituta za varstvo rastlin VE Georgikon Faculty of Agriculture in Oddelka za genetiko Univerze Eötvös Loránd. V laboratorijih ELTE so za gnotobiotske analize razmnožili več novih kombinacij različnih ras ogorčice *Heterorhabditis bacteriophora* in sožitne bakterije *Photorhabdus luminescens.*

V prispevku predstavljamo rezultate biotičnega testiranja vpliva zgoraj omenjenih organizmov na škodljive žuželčje vrste.
Leta 1998 smo testiral vpliv nekaj sožitnih kombinacij: entomopatogena ogorčica/bakterija na zadnji stadij ličink voščene vešče, *Galleria mellonella*, v treh različnih odmerkih (1/1, 10/1, 100/1 infektivnih ličink ogorčic/ličinko voščene vešče) ter na drugi razvojni stadij majskega hrošča, *Melolontha melolontha*, v dveh različnih odmerkih (3000 in 5000 infektivnih ličink ogorčic/majskega hrošča).
Ugotovili smo, da so bile simbiotske kombinacije enako učinkovite kot naravne rase entomofagnih ogorčic.
Leta 1999 smo testirali učinkovitost različnih vrst rodu *Steinernema* (*S. anomali, S. serratum, S. riobravae, S. glaserii, S. carpocapsae* Mex., T1, T2, T4, S. feltiae Nyír., IS6, Umeo) na ščurka *Blattella germanica* in vrsto *Periplaneta americana*. Opravljeni sta bili dve testiranji: (1) metoda s filter papirjem: 5000 IJ/5 ščurkov/ploščo (2) prehranjevalni test: ščurkom so bile za prehranjevanje ponujene ličinke voščene vešče, ki so poginile zaradi parazitiranja entomofagnih ogorčic. Kot kontrola so rabile ličinke voščenega molja, ki so poginile zaradi parazitiranja ogorčice *Heterorhabditis bacteriophora* Brecon in ličinke, ki smo jih ubili z zamrzovanjem.
Sklepi:
Vrsta *S. carpocapsae* je bila učinkovita proti ščurkom.
Ščurki so pojedli ličinke voščene vešče, katere so poginile zaradi napada ogorčic iz rodu *Steinernema*, izogibali pa so se ličink, ki so poginile zaradi napada ogorčic iz rodu *Heterorhabditis*.

1. INTRODUCTION

The aim of biological control is to use the natural enemies of the pests instead of using pesticides that are harmful for the environment. Entomopathogenic nematodes have been proved to be the excellent biological control agents of insects.
These nematodes are belonging to the Steinernematidae and Heterorhabditidae families. The free-living, infective forms of nematodes called infective dauer juvenile (hereinafter: IJ) live associated with bacteria. It is a semi-anabiotic form; neither feed nor defecate and keeps living but non-propagating bacterium cells within its intestine. The infective juvenile migrates into the soil searching for another insect host to colonise. After penetrating into the cavity of the host (Poinar, 1967; Poinar and Himswort, 1967) the nematodes discharge the bacteria into the haemocoel. The bacteria release insect toxins and proteases into the blood of the insect which destroy the host by causing septicaemia (Gaugler and Kaya, 1990). Consequently the pathogenecity of nematode/bacterium symbiotic complexes depends above all on bacterium.
The symbionts of *Steinernema* species belong to the *Xenorhabdus* genus. The symbionts of *Heterorhabditis* species belong to different *Photorhabdus* strains. (Boemare and Akhurst, 1993) of different 16S rDNA sequence (Szállás et al., 1997), which have recently been scored into three species and 5 subspecies (Fischer-Le Saux at all, 1999). The association between the *Steinernema* spp. and its symbiotic bacteria (*Xenorhabdus* spp.) seems rather flexible since symbionts of several species could mutually be exchanged.

2. MATERIALS AND METHODS

Several new combinations of different strains of axenic nematodes *Heterorhabditis bacteriophora* and bacteria (*Photorhabdus spp.*) have been constructed for gnotobiological analysis (Oravecz *et al.*, in preparation; Lengyel *et al.*, and Böszörményi *et al.*, in preparation). In this study we tested the new combinations on two insect pests, *Galleria mellonella* larvae and *Melolontha melolontha* grubs.

The investigations were carried out in the Entomological Laboratory of the Plant Protection Institute of the Veszprém University, Georgikon Faculty of Agriculture, Keszthely. The insects required for the tests were obtained from our own-stocks of *Galleria mellonella, Blattella germanica, Periplaneta americana*, and from natural populations of *Melolontha melolontha*. The IJs of entomopathogenic nematodes were from the Molecular Genetics and Nematode laboratory of Eötvös Loránd University, Department of Genetics (ELTE).
In 1998 we tested several EPN/EPB symbiotic complexes on great wax moth (*Galleria mellonella*) larvae and on white grubs (*M. melolontha*). Last instar larvae of *G. mellonella* were put into Petri-dishes containing vet filter paper or soil and IJ nematode suspension were them added in three different doses: 1/1, 1/10, 1/100 to 1 wax moth larvae, respectively. The controls were treated only with water. At the greatest doses we wanted to know if there were any effect. We intended to compare the pathogenecity of the new combinations the concentration one to ten is the best previously tested natural combinations.
But significant differences were only at concentration one to one in our experiments. Ten larvae per experiments were placed in a Petri-dish in a filter paper. The nematodes were placed by micropipette on the filter paper. The experiments were carried out in four replications. We controlled the mortality up to the 5th day daily, then on the 10th day. With White's method we checked if it really was a nematode that killed the insect (White, 1927).
The new combinations used in these experiments are summarised in Table 1.

Legend to Table 1.: The EPN/EPB symbiotic complexes used in the experiments

NEMATODE STRAINS	NEMATODES	BACTERIA
Heterorhabditis bacteriophora	HP88	HP88
	HP88	SZS1
	HP88	GUADELOUPE
	HP88	BRECON
	MOL	MOL
	MOL	AZ36
	BRECON	AZ29
	AZ36	AZ35
	AZ35	AZ29
	AZ29	BRECON
	AZ29	AZ35
	AZ29	RH1
	SZS1	SZS1
	SZS1	AZ35
	NCI	AZ35
	NCI	A1
	A1	A1
	A1(20)	A1(20)
Heterorhabditis megidis	HL81	HSH1

We tested the effect of these combinations also on second stage grubs (*Melolontha melolontha*) too. One larva per experiment was placed in a culture pot containing 100g sterile soil made of mould and sand mixed in 1:1 (volume). The proportion of White grub to dauer larvae was 1/3000, 1/5000 with control. The experiments were carried out in four replications. Mortality was checked on 4th, 7th, 10th, 14th day. Also this time I used White's method.
In 1999 we tested the effectiveness of various *Steinernema* species on German cockroach (*Blattella germanica*) and on American cockroaches (*Periplaneta americana*).

As the cockroaches are omnivorous they eat perished insects even cadavers of their own species, Harry K. Kaya (1999) was the one who supposed that they would eat insects except those that were infected by *Heterorhabditis* strains. We tried to prove this supposition by experiment. Two tests were applied: (1) filter paper method and (2) feeding experiments. The nematodes used in experiments are summarised in Table 2.

Legend to Table 2.: The nematodes used in the experiments

NEMATODE SPECIES	STRAINS
Steinernema anomali	
Steinernema serratum	
Steinernema riobravae	
Steinernema glaserii	
Steinernema carpocapsae	MEXICANA
Steinernema carpocapsae	T1
Steinernema carpocapsae	T2
Steinernema carpocapsae	T4
Steinernema feltiae	NYÍREGYHÁZA
Steinernema feltiae	IS6
Steinernema feltiae	UMEO
Heterorhabditis bacteriophora (control)	BRECON

2. 1. Filter paper method:

Five cockroaches per experiment were placed in a culture pot to a filter paper and add IJs in 1/1000 cockroaches/IJs doses. The experiments where nematodes proved to be efficient were repeated in four replications. The mortality was checked daily.

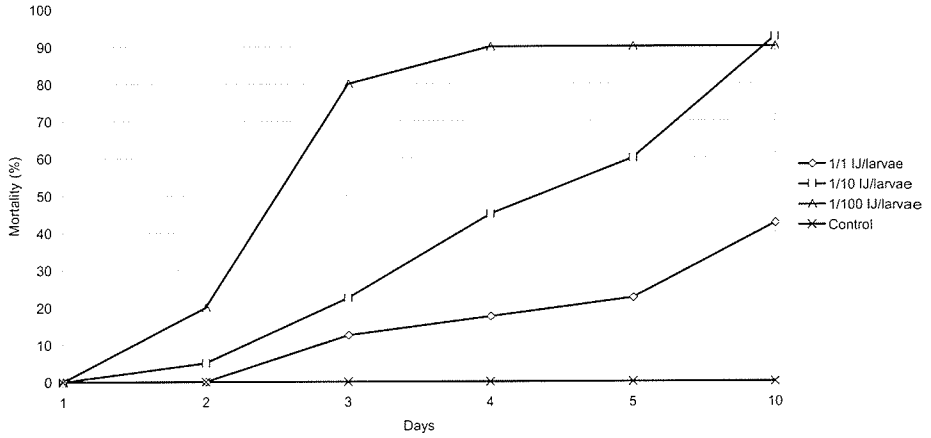
2. 2. Feeding experiment:

G. mellonella larvae were infected by nematode. Three worms killed by nematode were put 4-7 days earlier on a wet filter paper and 5 cockroaches were then transferred to each. As for control, we used worms which had previously infected by *H. bacteriophora* BRECON and, as an absolute control worms killed by freezing were used.

3. RESULTS

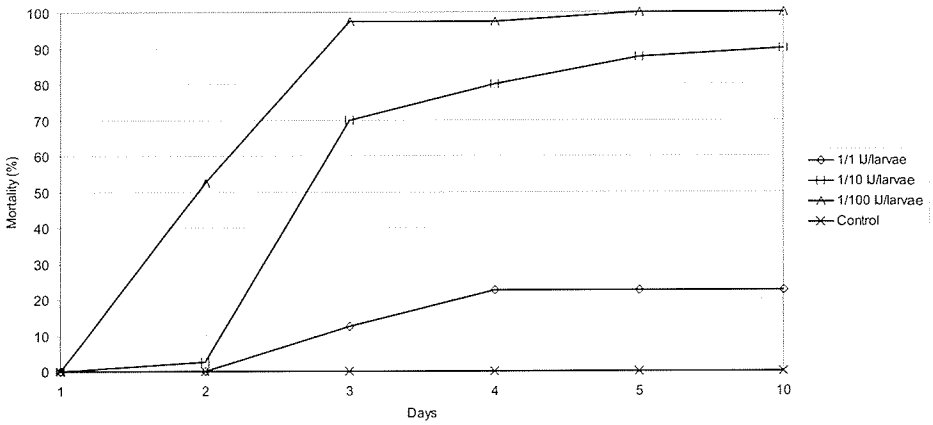
The effectiveness of new EPN/EPB combinations to great wax moth *(Galleria mellonella)*:
Amongst the combinations tested by us we would like to point out the combinations of *H. bacteriophora* strain HP88.
In the control group the rate of mortality was 0.
The one of the poorest result was given by bacterium SZS1. After the process we detected slow mortality. In the greatest doses the mortality were 90% on 4th day. In 1/10 doses the mortality was 92,5% on 10th day. In 1/1 doses the mortality was 42,5% on 10th day (Fig. 1.).

Legend to Fig. 1.The efficiency of *Heterorhabditis bacteriophophora* strain HP88 with *Photorhabdus luminescens* strain SZS1 symbiotic partner on *Galleria mellonella* larvae



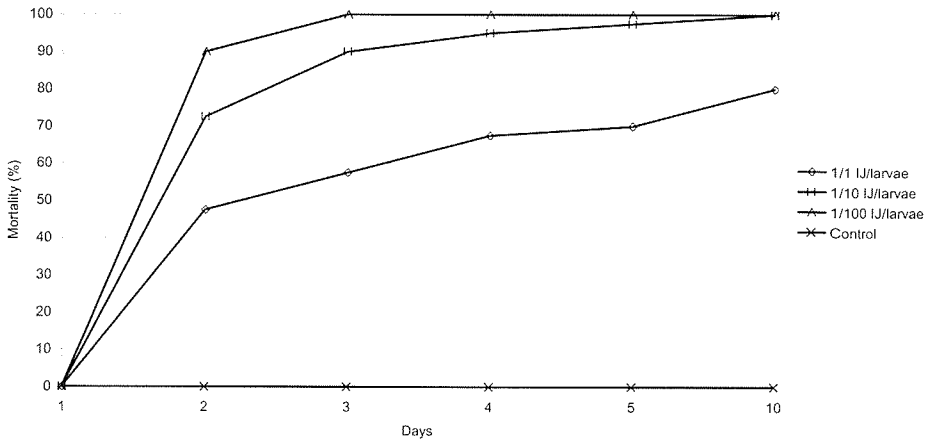
Good result was given by HP88 nematode with its own symbiotic bacteria the mortality were 100% on 3rd day in the greatest doses. In the 1/10 doses the mortality was 90%, in the smallest doses 27,7% on 10th day (Fig. 2.).

Legend to Fig. 2.The efficiency of *Heterorhabditis bacteriophora* strain HP88 and its own symbiotic partner on *Galleria mellonella* larvae



The symbiotic bacterium GUADELOUPE seemed to be even more effective than the HP88 nematode with its symbiotic bacteria. 100% was the mortality on 3rd day in the greatest doses as well but in the 1/1 doses was 80% on 10th day (Fig. 3.).

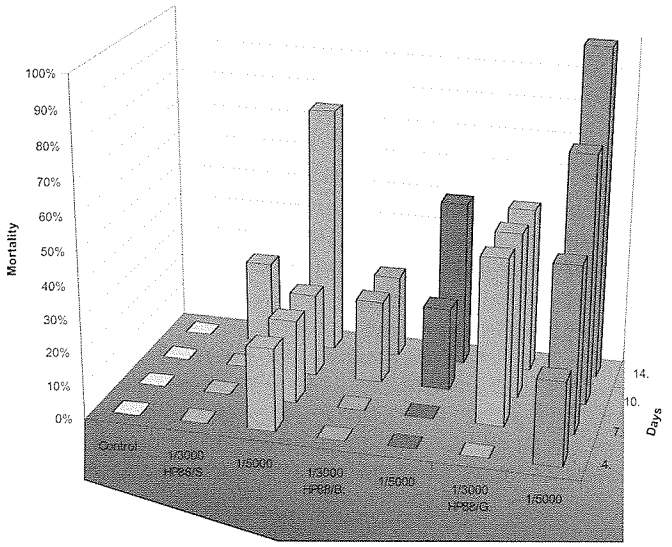
Legend to Fig. 3.The efficiency of *Heterorhabditis bacteriophora* strain HP88 with *Photorhabdus luminescens* strain GUADELOUPE symbiotic partner on *Galleria mellonella* larvae



Effectiveness of new EPN/EPB combinations to white grubs *(Melolontha melolontha)*
In the control group the rate of mortality was 0.
The poorest result was given by symbiotic bacterium BRECON. In the 1/5000 doses the mortality was 50% in the 1/3000 doses were 25% on 14th day.
SZS1. In the 1/5000 doses the mortality was 75%. In the one to three hundred doses were 25% on 14th day (Fig. 4.).

Legend to Fig. 4. : The efficiency of *Heterorhabditis bacteriophora* strain HP88 with different new symbiotic partners on *M. melolontha*

Control
HP88/SZS1 1/3000 larvae/IJ dosis
HP88/SZS1 1/5000 larvae/IJ doses
HP88/Brecon 1/3000 larvae/IJ doses
HP88/Brecon 1/5000 larvae/IJ doses
HP88/Guadeloupe 1/3000 larvae/IJ doses
HP88/Guadeloupe 1/5000 larvae/IJ doses



On the other hand mortality of combination HP88/GUADELOUPE in the largest doses were 100% on 14th day. There was 50% mortality in the lower doses (Fig. 4.).
The efficiency of MOL symbiotic complex was as good as that of HP88/GUADELOUPE (Fig. 4.).
Filter paper method with *Blattella germanica* and *Periplaneta americana*:
The effectiveness of strains MEXICANA, T1, T2, T4 of *Steinernema carpocapsae* had been proved earlier (Appel and Benson, 1994; Koehler at all, 1992). The *S. anomali, S. serratum, S. riobrave, S. glaserii, S. feltiae* strains and *Heterorhabditis bacteriophora* BRECON weren't affective against cockroaches.
At doses 1000 IJs / cockroaches the mortality was 100% with *B. germanica* tested animals. In the 35-80% of the perished insects I found infective juveniles. In the control group there was no mortality (Table 3.).

Legend to Table 3.: The examination of nematode proved to be efficient with Blattella germanica (Keszthely, 2000)

Nematodes	Mortality (%)	Perished cockroaches from which dauer larvae emerged (%)
Steinernema carpocapsae T1	100,0	80,0
Steinernema carpocapsae T2	100,0	35,0
Steinernema carpocapsae T4	100,0	75,0
Steinernema feltiae NYÍREGYHÁZA	0,0	-
Control	0,0	-

The mortality at *P. americana* last instar larvae and adults made 50% by strains T1, T2 of *S. carpocapsae*. *S. feltiae* NYÍREGYHÁZA – isolated in Hungary – proved ineffective, there was no mortality at control group (Table 4.).

Legend to Table 4.: The examination of nematode proved to be efficient with *Periplaneta americana* (Keszthely, 2000)

Nematodes	Cockroaches (example)	Perished cockroaches (example)	Cockroaches from which dauer larvae emerged (example)
Steinernema carpocapsae T1	9	4	4
Steinernema carpocapsae T2	9	5	4
Steinernema feltiae NYÍREGYHÁZA	6	Ø	-
Control	6	Ø	-

In feeding experiment we used the same nematode as at the filter paper method and the rate of the mortality was similar. The *S. carpocapsae* strains proved to be effective against cockroaches. Infective juveniles were emerged from perished insects and these IJs will be able to infect cockroaches (Table 5.)

Legend to Table 5.: The results of the feeding experiments (Keszthely, 1999)

	RESULTS			
Nematodes	Cockroaches (example)	Perished cockroaches (example)	Perished cockroaches from which IJs emerged (example)	Cockroaches re-infected by IJs emerged from the cockroaches (+) with success (-) without success
S. anomali AZORAE	5	1	1	-
S. serratum	5	Ø		
S. riobrave	5	1	Ø	
S. glaserii	5	Ø		
S. carpocapsae MEXICANA	5	5	4	+
S. carpocapsae T1	5	4	2	+
S. carpocapsae T2	5	4	4	+
S. carpocapsae T4	5	5	5	+
S. feltiae NYÍREGYHÁZA	5	Ø		
S. feltiae IS6	5	2	1	+
S. feltiae UMEA	5	1	Ø	
H. bacteriophora BRECON (control)		5	Ø	
Absolute control	5	Ø		

4. DISCUSSION

- The bacteria/nematode combinations we used were affective both against wax moth and grubs. The process of perishing of grubs took a longer time than that of wax moth larvae. It can be explained by the fact that the grubs didn't get in touch immediately with nematode. References say that *H. bacteriophora* is less effective against grubs. Between the complexes made by nematode strain HP88 with different bacteria the HP88/GUADELOUPE seemed to be the most affective but statistically we couldn't express it.
- *Steinernema carpocapsae* strains were affective against cockroaches (*Blattella germanica, Periplaneta americana*). The *S. anomali, S. serratum, S. riobrave, S. glaserii, S. feltiae* strains and *Heterorhabditis bacteriophora* BRECON proved practically ineffective on roaches. Cockroaches ate the worms killed by freezing but didn't even touch the worms killed by *H. bacteriophora* BRECON. Surprisingly they did not really like the worms infected by *Steinernema* either.

5. LITERATURE

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