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NEW POSSIBILITIES OF CONTROLLING LARVAE OF MELOLONTHIDAE AND NOCTUIDAE USING ENTOMOPATHOGENIC NEMATODES

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ABSTRACT

In the Plant Protection Institute of the Pannon University of Agricultural Sciences Georgikon Faculty of Agronomy, Keszthely, investigations have been carried on for several years in cooperation with American researchers (Chitwood, D. J., Klein, M.; Wooster, Ohio) on the effectiveness of entomopathogenic nematodes against various insect pests. In 1996 experiments were conducted under laboratory and insectary conditions to control grubs (*Melolontha melolontha*), a pest of forestry nurseries, as well as larvae of two Noctuidae species (*Helicoverpa armigera* and *Scotia segetum*). In the experiments infective larvae of a Hungarian species and of three American species (*Steinernema glaseri, S. feltiae* and *S. riobravis*) were used. The larvae were treated at two concentrations (1000 and 100/ml nematode, respectively), in four replications. The treatments of *Melolontha* larvae were evaluated on the 7th, those of Noctuidae larvae on the 5th day, by establishing the percentage mortality. In the case of the 100/ml nematode concentration the average mortality was 25.4%, while with the 1000/ml concentration it was nearly 100%. In field insectary treatment with 1000/ml nematode concentration resulted in 42.6% mortality. As regards effectiveness on grubs the order of nematode species was: *H. bacteriophora* HH., *S. glaseri*, *S. riobravis* and *H. bacteriophora* AZ 32. Against Noctuidae larvae *H. bacteriophora* HH was the most effective, resulting in some 50% mortality. As shown by the results, the Hungarian species *H. bacteriophora* HH has proved to be the most effective, which makes it clear that the use in plant protection of a nematode species accommodated to the ecological conditions of Hungary may have a better chance compared to those originating from abroad.

IZVLEČEK

NOVE MOŽNOSTI ZATIRANJA OGRCEV MAJSKEGA HROŠČA (Melolonthidae) IN SOVK Z ENTOMOPATOGENIMI OGORČICAMI

Na Inštitutu za varstvo rastlin Panonske univerze agronomskih znanosti Georgikon Agronomske fakultete v Keszthelyju so v sodelovanju z ameriškimi raziskovalci (Chitwood, D. J., Klein, M.; Ohio University, Wooster) več let preučevali učinkovitost entomopatogenih ogorčic (nematod) proti različnim škodljivim žuželkam. Leta 1996 so opravljali poskuse v laboratorijih in v insektarijih za zatiranje ogrcev majskega hrošča (*Melolontha melolontha*), škodljivca v gozdnih drevesnicah, kot tudi proti gosenicam dveh vrst sovk (*Helicoverpa armigera* in *Scotia segetum*). V poizkusih so uporabljali ličinke ene madžarske in treh ameriških vrst ogorčic (*Steinernema glaseri, S. feltiae* in *S. riobravis*), ki lahko napadajo ogrce oz. gosenice. Ličinke ogorčic so uporabljali v dveh koncentracijah (100 oz. 1000 osebkov/ml) v štirih ponovitvah. Učinkovitost so pri ogrcih ovrednotili 7. dan, pri sovkah pa 5. dan z ugotovitvijo smrtnosti. Pri nižji koncentraciji je bila povprečna smrtnost 25,4%, pri višji koncentraciji pa skoraj 100%. V poljskih poskusih je insektarijska koncentracija nematod povzročila 42,6% smrtnost. Glede na učinkovitost proti ogrcem je bil vrstni red ogorčic tale: *H. bacteriophora* HH., *S. glaseri, S. riobravis in H. bacteriophora* AZ 32. Proti sovkam so bile

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najbolj učinkovite ličinke *H. bacteriophora* HH., ki so povzročile 50% smrtnost. Kot izhaja iz rezultatov, je madžarska vrsta *H. bacteriophora* HH. najbolj učinkovita, kar kaže, da ima uporaba domače vrste, prilagojene na ekološke razmere Madžarske, večje možnosti, da se uveljavi v varstvu rastlin, kot vrste, ki izvirajo od drugod.

For reasons of environment protection and increasing attention is being paid to the elaboration of biotical plant protection methods. Such a method is the use of entomopathogenic nematodes against pests. In Hungary investigations of entomopathogenic nematodes are carried on in several institutions. In this field our Institute has established cooperation with the Biological Centre of the Hungarian Academy of Sciences, Szeged, and the Eötvös Loránd University, Department of Genetics, Budapest, where the genetics and biochemistry of nematodes are studied. Our Institute, in common with the Csongrád County Station for Plant Sanitation and Soil Conservation, has the task of examining the possibilities of employing nematodes in the practice of plant protection. The investigations are carried out in the framework of the American-Hungarian research programme (441/95). On the American side the Beltsville Research Institute of USDA (Maryland) and the Wooster Research Institute of the Ohio University (Ohio) take part in the work.

Out of the entomopathogenic nematodes we have dealt with species belonging to the genera *Steinernema* and *Heterorhabditis.* They have no harmful effect on vertebrata, rapidly reproduce and are easily applied by hand- and machine sprayers.

ACTION MECHANISMS OF ENTOMOPATHOGENIC NEMATODES

The *Steinernema* species live in synbiosis with bacteria belonging to *the Xenorhabdus nematophilus* family (POINAR *et al.*, 1977; POINAR, 1979) while the *Heterorhabditis* species with those from the *Photorhabdus luminescens* family of Enterobacteriaceae (POINAR, 1975). The infective (dauer) juvenile (hereinafter: IJ) penetrates into the cavity of the host (POINAR, 1967; POINAR and HIMSWORT, 1967), then the bacteria are discharged from the nematode and start reproduction. The bacteria destroy the host by subduing its immunosystem. The nematodes feeding on the bacteria and the host's tissues develop and reproduce. The number of generations developing in the host organism depends on the available amount of food. The members of the last generation transform into infective juveniles that abandon the host's carcass to find a new insect host. The insect thus dies of septicaemia, its nutrients are converted by the bacterium so as to become available for the nematode. Hence the term: entomopathogenicity (POINAR and THOMAS, 1966; POINAR and GEORGIS, 1990).

MATERIALS AND METHODS

The investigations were carried out in the entomological laboratory and insectary of the Plant Protection Institute of the Pannon University of Agricultural Sciences, Georgikon Faculty of Agronomy, at the Csongrád County Station for Plant Sanitation and Soil conservation (Hódmezővásárhely) and in the Lábod forestry of the Somogy County forestry and Wood-Working Comp.

In 1995 experiments were carried out with larvae of *Leptinotarsa decemlineata*, *Athalia rosae* (SÁRINGER *et al.*, 1996) and *Scotia segetum* (LUCSKAI *et al.*, 1996). In the course of the 1996 experiments we worked with the larvae of the *Scotia segetum* HÜBNER, *Helicoverpa armigera* HÜBNER, *Melolontha melolontha* L. and *Polyphylla fullo* L. insect pest species. The insects required for the tests were obtained from our own-bred (Noctuidae sp.) and natural populations (Melolonthidae sp.), respectively.

The infective larvae of entomopathogenic nematodes partly were from our own cultures, and partly supplied by the American company BIOSYS, in the form of exhibit preparation. The AZ 32 strain was a present by Prof. N. Simres (V. Azores Ponta Delgada, Portugal).

For the experiments we used infective larvae of a Hungarian species (*Heterorhabditis bacteriophora* HH), of a species originating from the Azores (*Heterorhabditis bacteriophora* AZ 32) and of three American species (*Steinernema glaseri, S. feltiae* and *S. riobravis*). In the laboratory we worked with two concentrations (1000 nema/ml and 100 nema/ml), in insectary and in the field experiments also with two concentrations each (10,000 nema/ml and 1000 nema/ml). The experiments were carried out in four replications. In the laboratory experiments one larva per experiment was placed in a culture pot containing 100 g soil. In the insectary experiments 5 larvae per experiment were placed in a culture pot containing 10,000 g soil. The soils were sterile soils containing 50% black soil and 50% sand. In laboratory the pots were regularly watered in order to maintain a constant humidity. Every two days 14 ml water was poured onto the soils. The nematodes were placed by micropipette on the soil surface. The laboratory and insectary experiments were evaluated on the 4^{th}, 7^{th} and 14^{th} day. The perished larvae were placed in Petri dishes according to the method of WHITE (1927), and after several days the infection by nematodes was proved through the dissection of the dead insects. The results of the experiments are shown in Figs. 1-4. The field experiments were conducted in the nursery of the Lábod forestry of SEFAG, where Turkey oak trees were raised. The soil of the nurseries was highly infected by maybeetle larvae: *Melolontha melolontha* L_2 and *Polyphyla fullo* L_1 larvae occurred. The number of larvae was essentially larger than average, 20-25 grubs of mixed species composition were found in 1 m^2. The size of plots was 20 m^2. The nematode suspension of the given concentration was applied by portable sprayer onto the surface of the plots on 3 October 1996. The evaluation took place in spring.

RESULTS

From the results whown in the tables the following conclusions can be drawn:

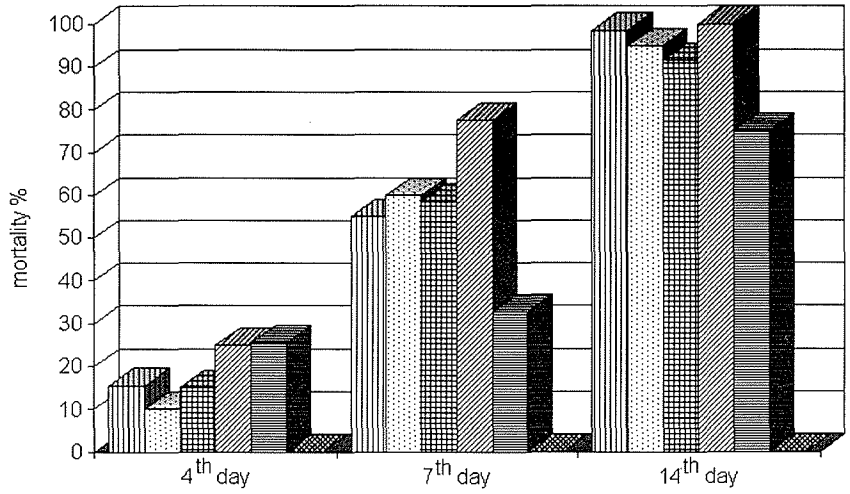
1. Under laboratory conditions at a dose of 1000 nema/ml the entomopathogenic nematodes were successful against the larvae living in the soil. With their application the mortality of *Melolontha melolontha* and *Polyphyla fullo* larvae nearly reached 100%, while the *Scotia segetum* larvae perished in 80%.

2. In the case of the lower concentration of nematode used (100 nema/ml) the mortality was 35%.

3. In the insectary experiments the higher concentration (10,000 nema/ml) resulted in 45% mortality. The lower concentration (1000 nema/ml) on the other hand had no satisfactory effect on the larvae (20% mortality).

4. The entomopathogenic nematodes destroy the larvae only after several days (7 days). Good result can be expected after two weeks only.

1000 nema/ml



100 nema/ml

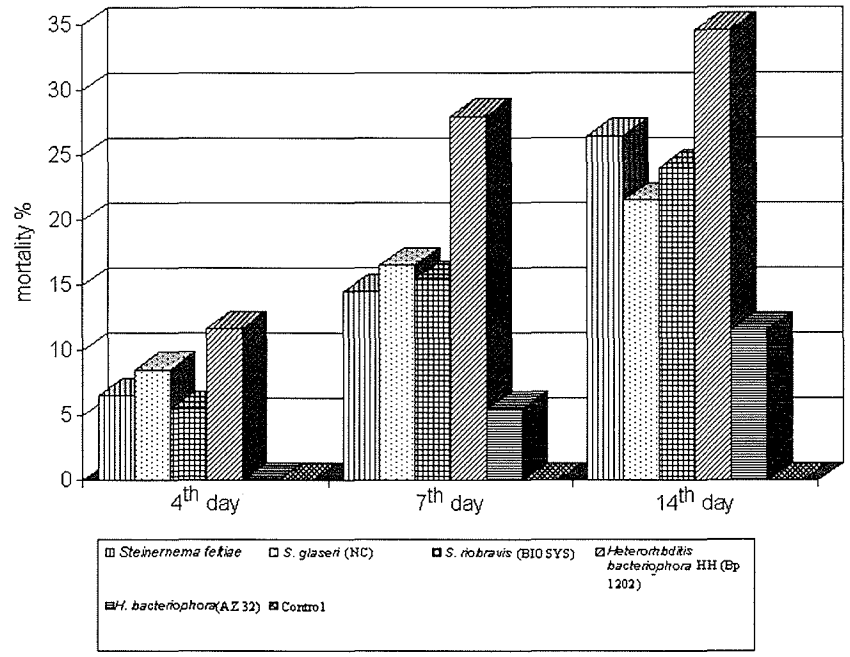


Fig. 1. Effect of entomopathogenic nematodes on L_1 larvae of *Polyphylla fullo* in laboratory

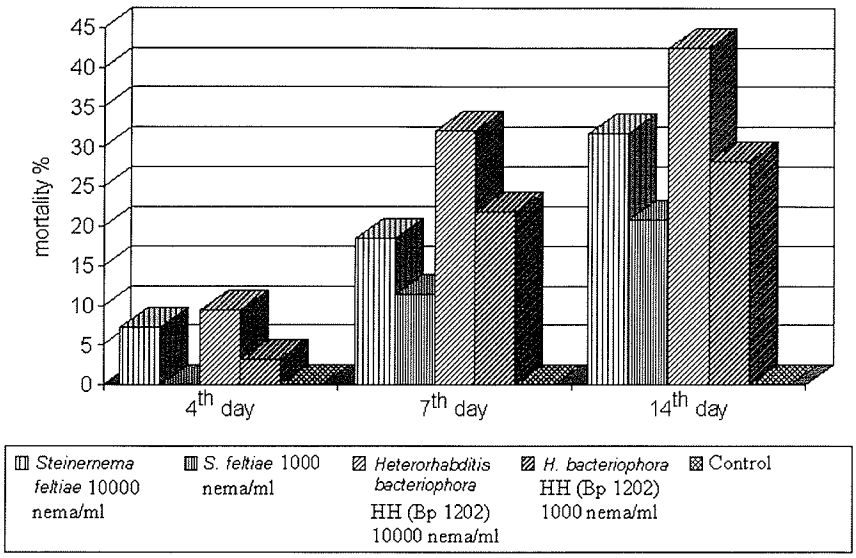
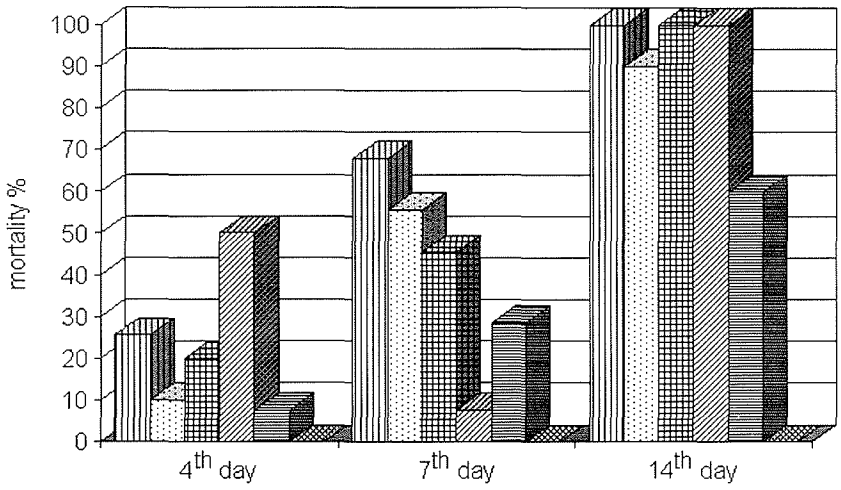


Fig. 2. Effect of entomopathogenic nematodes on L_2 larvae of *Melolontha melolontha* in insectary

1000 nema/ml



100 nema/ml

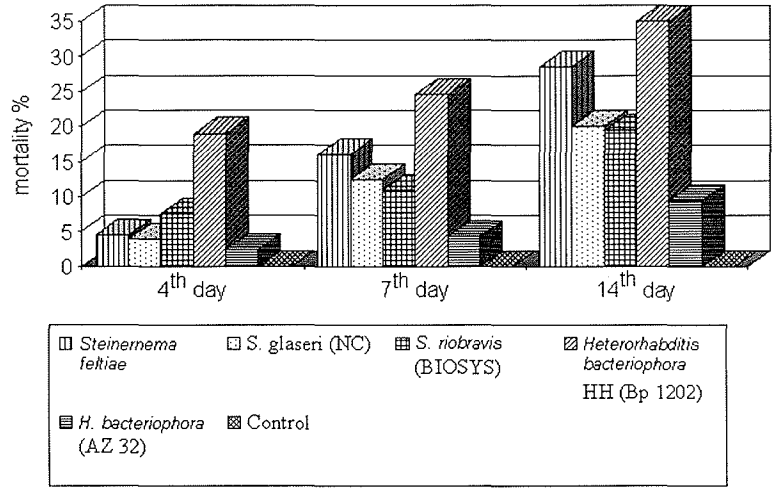


Fig. 3. Effect of entomopathogenic nematodes on L_2 larvae of *Melolontha melolontha* in laboratory

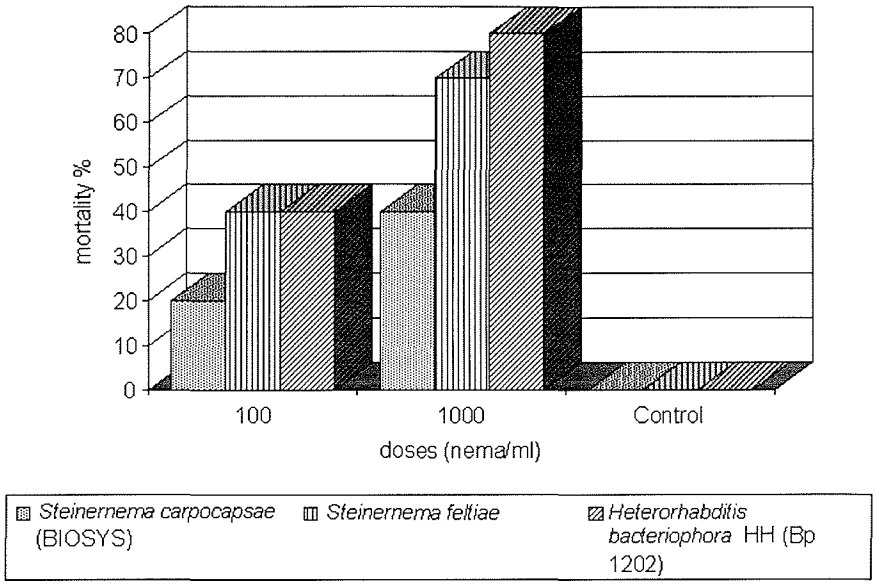


Fig. 4. Effect of entomopathogenic nematodes on larvae of *Scotia segetum* in laboratory

5. As regards their effectivity concerning the larvae the nematode species showed the following order of succession: *Heterorhabditis bacteriophora* HH. (Bp 1202), *Steinernema feltiae, Steinernema glaseri, Steinernema riobravis* and *Heterorhabditis bacteriophora* AZ 32.

6. The success of the experiments greatly depended on the moisture content of the soil. Namely, good result was obtained in those experiments where the soil was regularly watered and its moisture content was abundant (65-70%). In the experiments where watering did not take place the nematodes perished in a couple of days without destroying the larvae.

DISCUSSION

The most important results of the 1996. experiments were the following:

1. Under laboratory conditions, at higher concentrations (1000 nema/ml) the entomopathogenic nematodes can be successfully used against insect pests living in the soil; the mortality of the larvae was nearly 100% in the case of all species examined.

2. In insectary where the area protected and the volume of the soil were larger, any considerable result was attained only with the higher nematode concentration (10,000 nema/ml).

3. The entomopathogenic nematodes do not immediately destroy the larvae living in the soil. This fact must by all means be taken into consideration in the practice, first of all in the course of protection against pests as e.g. *Leptinotarsa decemlineata*, *Athalia rosae* which cause great losses in a short time.

4. From the results it is clear that the HH (Bp 1202) strain of *Heterorhabditis bacteriophora* has proved to be the most efficient nematode, which means that employing in plant protection a nematode accommodated to the domestic ecological conditions may have a greater chance compared with species originating from abroad.

5. The success of control with entomopathogenic nematodes is largely influenced by the moisture content of the soil. The same has been found by the American researchers (POINAR, 1990). Their application is therefore recommended only where the regular watering of the soils is made possible.

SUMMARY

Six strains of five entomopathogenic nematode species against white grubs (*P. fullo, M. melolontha, S. segetum*) were compared in laboratory and field tests. The nematode strains were *H. bacteriophora* HH (Bp 1202) (a new isolate from Hungary), AZ 32 (The Azores, N. Simres), *S. riobravis* and *S. glaseri* NC.

In spite of the effectivity of all species in laboratory tests, in field tests only *H. bacteriophora* HH (Bp 1202), *S. glaseri* were really effective. Surprisingly, *S. feltiae* also caused a significant mortality. The conclusion is that local strains should be preferred when a biocontrol strategy based upon BPN is developed.

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