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Entomopathogenic nematode *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) recorded for the first time in Slovenia

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ABSTRACT

In Slovenia only recently entomopathogenic nematodes were recorded for the first time. In the beginning of 2007, the presence of *Steinernema affine* was confirmed. During the further investigations in the same year *Steinernema feltiae* was recorded on the arable field near Cerknica. In the previous year this field was planted with chicory. In Slovenia, until now the entomopathogenic nematodes had a status of an exotic agents and their use was allowed only in the laboratory experiments. We expect that in Slovenia the use of these biological agents against insect pests will become important alternative to insecticides as it is known in many other countries of the world.

Key words biological control, entomopathogenic nematodes, exotic agents, Slovenia, *Steinernema affine*, *Steinernema feltiae*

IZVLEČEK

ENTOMOPATOGENA OGORČICA *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) PRVIČ UGOTOVLJENA V SLOVENIJI

V Sloveniji so bile entomopatogene ogorčice prvič ugotovljene šele nedavno. V začetku leta 2007 je bila potrjena zastopanost vrste *Steinernema affine*, med nadaljnjimi raziskavami v istem letu pa je bila na njivi v bližini Cerknice najdena tudi ogorčica *Steinernema feltiae*. Na omenjenem zemljišču so leto prej pridelovali radič. Doslej so imele entomopatogene ogorčice v Sloveniji status tujerodnih organizmov, njihova uporaba pa je bila dovoljena le v laboratorijskih poskusih. Pričakujemo, da bo v Sloveniji uporaba omenjenih naravnih sovražnikov škodljivih žuželk postala pomembna alternativa insekticidom, kar je sicer že znano v številnih drugih državah sveta.

Ključne besede biotično varstvo, entomopatogene ogorčice, Slovenija, *Steinernema affine*, *Steinernema feltiae*, tujerodni organizmi,

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1 INTRODUCTION

In Slovenia, the first research on entomopathogenic nematodes (EPNs) was carried out in 2004. Because in Slovenia EPNs still have a status of an exotic agents, all earlier researches were limited merely to laboratory experiments. The aim of previous research was to study the efficacy of the nematodes against foliar pests (Laznik *et al*., 2007).

EPNs from the families Steinernematidae and Heterorhabditidae are important pathogens of insects. These soil organisms are mutually associated with bacteria from genus *Photorhabdus* Boemare, Akhurst and Mourant (genus *Heterorhabditis*) and bacteria from genus *Xenorhabdus* Thomas and Poinar (genus *Steinernema*) (Burnell and Stock, 2000). After infection, the symbiotic bacteria are released into the insect hemocoel, causing septicemia and death of the insect in 24 to 72 hours (Forst and Clarke, 2002).

Because of broad spectrum of target hosts from the class Insecta, their application as a way of biological control of plants against pests is so far very well known (Kaya and Gaugler, 1993). Application of EPNs in biological control was traditionary engaged in controlling soil pests until some years ago (Ishibashi and Choi, 1991). Results from research in the last two decades indicate also their potential against foliar pests, but only under special conditions (Arthurs *et al*., 2004). Poorer efficacy of EPNs in controlling foliar pest is a consequence of unsuitable (too low) moisture (Lello *et al*., 1996), exposure to extreme temperatures (Grewal *et al*., 1994), and ultraviolet radiation (Gaugler and Boush, 1978). These factors are known as crucial for nematodes survival (Kaya, 1990). For this reason the efficacy of foliar pests with EPNs in the open is therefore often worser as expected, although predecessor laboratory tests shows rather better efficacy (Buitenhuis and Shipp, 2005).

Numerous other research showed that at lower concentration EPNs are much more efficient in controlling preadult stages of insects from order Coleoptera (Ansari *et al*., 2003). Similar findings were also confirmed with controlling insects from other orders; Thysanoptera (Premachandra *et al*., 2003), Lepidoptera (Yakir-Ben *et al*., 1998), Diptera (Willmott *et al*., 2002) and some others. In majority of cases it was about the larvae, which life cycle is predominantly linked with soil and easily attacked by EPNs.

2 MATERIALS AND METHODS

In October 2007, we examined 77 soil samples on the occurrence of EPNs in Slovenia. The soil samples were taken in Notranjska region of Slovenia, which is the south central part of the country. We used »Galleria bait method«, which is the most frequently used method of EPNs detection from soil. After the death of wax moth (*Galleria mellonella* [Linnaeus]) larvae, we dried cadavers for 12 days and put them in so called »White trap« (Bedding and Akhurst, 1975) to separate the nematodes from death larvae. With the received suspension we infected artificialy larvae of wax moth again. Following procedure contained the use of centrifuge and 5 % concetration of sodium hypoclorate. The aim of this process was to get infective juveniles from the suspension. We confirmed the presence of nematodes in 9.09 % of samples. Only 1 positive sample, B30 (taken on the chicory arable field near Cerknica (SW Slovenia, 45°48'N, 14°22'E, 572 m alt.) was identified to this time.

3 RESULTS

To confirm the identification of isolated nematodes from larvae of wax moth, a selected sample was analysed by molecular biological approach. Genomic DNA was extracted from individual nematodes and PCR was performed to multiply ITS region using primers TW81 and AB28 after Hominick et al. (1997). PCR product were reisolated from 1% TAE-buffered agarose gel using E.Z.N.A. Gel Extraction Kit (Omega Bio-Tek, USA) (Fig. 1). Reisolated sample was sequenced in the laboratory of Agricultural Biotechnology Centre in Gödöllő, Hungary. Sample DNA sequence was compared to sequences of species *Steinernema* using BLAST search in National Centre for Biotechnology Information (NCBI) web site (www.ncbi.nlm.nih.gov). The sequences producing significant alignments and at least 99% identity were derived from *Steinernema feltiae*: GenBank Accession No. DQ310469 and AF121050 (Nguyen et al., 2001) (Fig. 2).

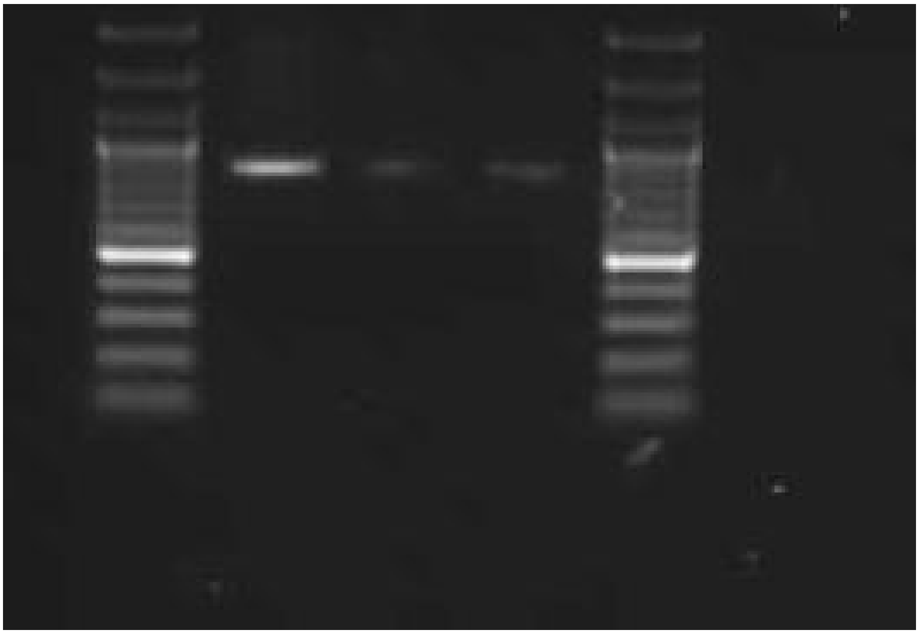

Figure 1: 1% TAE buffered agarose gel, in the 1st and 5th lanes: GeneRuler 100 bp DNA Ladder Plus (Fermentas), in the 2nd lane: PCR product of our sample B30, using the primer pair specified in the text, 3rd lane: PCR product of sample B49, 4th lane: PCR product of sample 3162. The two most strength fragment in the ladder are 500 and 1000 bps length.

4 DISCUSSION

Genetic studies proved that the nematode species is *Steinernema feltiae* Filipjev (1934) (Fig. 3). The ITS1-5.8S-ITS2 region, including the partial 18S and 28S rDNA genes (flanked by above primers) of Slovenian isolate B30 is 742bp long.

BLAST searches (Altschul *et al*., 1990) in GenBank showed that Slovenian isolate B30 has a high similarity (99%) with those sequences available for *S. feltiae* populations (e.g. accession numbers DQ310469 and AF121050). Sequence of other species from *feltiae* group, namely *S. litorale* was obtained from GenBank searches that exhibited a lesser degree of similarity with the Slovenian isolate and other *S. feltiae* populations (e.g. accesion number AB243441) (Fig. 3). The present study constitutes the first report of *S. feltiae* in Slovenia. *S. feltiae* has a wide distribution in temperate regions, being one of the most common species found in Europe, and in many other parts of the world (for a detailed EPN species distribution see Hominick, 2002).

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30381      1    GGCTTA-CCATTT-CTTGGATTCAAATGAATCGAGCTGAAT-TTTCGCTG-
TTCGTTTCA  56
DQ310469  177    .....T......A...........................-........-
.........  233
AF121050  177    .....T......A...........................-........-
.........  233
AB243441  198    .....T......A.........................C.-........A..-
..C...  254

30381      57   AAGCG-TTGT-ATTCTCTCAACTAACGGCTAT-GAATGGTTTCTATAGG-TGT-
CTGGAG  111
DQ310469  234   .....-....-.....................-................-...-
......  288
AF121050  234   .....-....-.....................-................-...-
......  288
AB243441  255   .....-..-A-.....................-................-...-
......  308

30381      112  CAGTTGTATGAGCGTGACTGTGGTGATGGACAT-TTTG--
GTGGCTCCTTAGTCG-GGTC  167
DQ310469  289   .................................-....--
...............-....  344
AF121050  289   .................................-....--
...............-....  344
AB243441  309   ................................--...---.-A.-.T...-..--
--.--  354

30381      168  ACT-AGAATTAAAGAAGTCTGTT-A---TGACTCGCCGTTCTTA-AAAAACT-
TCAATTA  220
DQ310469  345   ...-...................-.---................-.......-
.......  397
AF121050  345   ...-...................-.---................-.......-
.......  397
AB243441  355   ...-...................-.---................-.......-
.......  407

30381      221  ACGTTTGATC-AATTTGACTGCACCAGCC-GT-AGGTGT-ACTT-
AAAGATTTATCAAGT  275
DQ310469  398   ..........-..................-..-......-....-
...............  452
AF121050  398   ..........-..................-..-......-....-
...............  452
AB243441  408   ..........-..................-..-......-G...-
...............  462
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30381     276  CTTGTCGGTGGATCACTCGGTTCGTAGTTCGATGAAAAACGGGGCAAAAA-
CCGTTATTT  334
DQ310469  453  ..................................................-
.........  511
AF121050  453  ..................................................-
.........  511
AB243441  463  ..................................................-
.........  521

30381     335  GGCGTGAATTGCAGACATATTGAACGCTAAAATTTTGAACGCAAATGG-CAC-
TATCAGG  392
DQ310469  512  ................................................-...-
.......  569
AF121050  512  ................................................-...-
.......  569
AB243441  522  ................................................-...-
.......  579

30381     393  TTTATATCTGTTAGTATGTTTGGTTGAGGGTCGATTAATTCGTAACCTGCA-
GTCTGCTG  451
DQ310469  570  ...................................................-
........  628
AF121050  570  ...................................................-
........  628
AB243441  580  ...................................................-
........  638

30381     452  TGACTGTTTTTT-CGATTAGTTATTTG-G-TT--T-TT--TT-A-
TCGAGTACCTTTT-T  500
DQ310469  629  ............-..............-.-..--.-..--..-.-
.............-.  677
AF121050  629  ............-..............-.-..--.-..--..-.-
.............-.  677
AB243441  639  ............-..........C...-A-..--C-.A--..-----
...........-.  684

30381     501  -GGAATGTGAATT--T--GATTGTCTAATTCGTTTCCTAATCG--AAA-
CGAGCTATTTT  552
DQ310469  678  -............--.--.........................--...-
...........  729
AF121050  678  -............--.--.........................--...-
...........  729
AB243441  685  -............A-.T-.........................--...-
.........A.  738

30381     553  TTA-TTTCTGTGCAATGTATTTTTGGTGTTTCGGCGTT-TTTCTTGCCGACTGA-
T-TGG  608
DQ310469  730  ...-..................................-...............-
.-...  785
AF121050  730  ...-..................................-...............-
.-...  785
AB243441  739  C..-..........T......G.....-........C.-...............-
.G..-  793

30381     609  TACAAACTTAACAGT-TCGTATATTTTTCAGAATTT-TTCAGA-GGCCCTTACA-
A-TA-  662
DQ310469  786  ...............-....................-......-..........-
.-..-  839
AF121050  786  ...............-....................-......-..........-
.-..-  839
AB243441  794  ............G..A.-............-....----....-.A..-.-...-
G-..T  842
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30381      663  CATCA-CTT-GACACAACACGTA-T-CGTTTGTCGAG-G--AATTGCGCAAGAA-
AG-AA  713
DQ310469   840  .....-...-.............-.-...........-.--.............-
..-..  890
AF121050   840  .....-...-.............-.-...........-.--.............-
..-..  890
AB243441   843  .-.A.-..C-............-.C.-......T...-A--.............-
..-..  892

30381      714  A-CTTTTCGTT--ACGACCTCAACCCAAGCAA  742
DQ310469   891  .-.........TT...........T.......  921
AF121050   891  .-.........TT...........T.......  921
AB243441   893  .-.........TT...........T.......  923
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Figure 2: Multiple sequence alignment of the ITS rDNA region (including partial fragments of the 18S and 28S rDNA genes) of 4 *Steinernema* species. Code 30381 correspond to the Slovenian isolate of *Steinernema feltiae* (B30). Codes DQ310469 and AF121050 are *Steinernema feltiae* strains from Russia and USA. Code AB243441 correspond to *Steinernema litorale* strain from Japan.

We can place mentioned species into »*feltiae* group« of nematodes from genus *Steinernema* (Nguyen, 2006); for infective juveniles it is known that they are between 1000 and 700 µm long (Fig. 3). This nematode lives in symbiosis with bacterium *Xenorhabdus bovienii* (Poinar, 1988). The nematode was first recorded in 1934, and its appliable value in biological control of insect pests is well known (Ebssa, 2001). Some researchers reported that *S. feltiae, S. intermedium* (or C1) and *S. affine* like to appear on agricultural land (Sturhan, 1996). In Europe, the occurrence of *S. feltiae* was up to now confirmed in Austria, Belgium, Great Britain, Czech Republic, Denmark (original), Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Poland, Slovakia, Spain, Sweden, Switzerland, Netherlands, Norway, Ukraine, Bolgaria and Portugal (Hominick, 2002).

Figure 3: Infective juvenile of *Steinernema feltiae* from sample B30.

Up to the present EPNs in Slovenia had a status of exotic agents and their efficacy against different insect pests was performed merely in laboratory experiments; Colorado potato beetle (*Leptinotarsa decemlineata* [Say]), greenhouse whitefly (*Trialeurodes vaporariorum* [Westwood]), western flower thrips (*Frankliniella occidentalis* [Pergande]) (Perme, 2005), sawtoothed grain beetle (*Oryzaephilus surinamensis* [L.]) and granary weevil (*Sitophilus granarius* [L.]) (Trdan et al., 2006) and flea beetles (*Phyllotreta* spp.) (Trdan et al., 2008). The results of these experiments confirmed already known facts that - in optimal conditions - EPNs represent very effective agents to control insect pests. After the first record of *Steinernema feltiae* in Slovenia, we expect that the use of these biological agents against insect pest will become important alternative to insecticides. These will be especially desired against the pests which is not easy to control with insecticides due to their massive occurrence in the period of harvesting, against the pests which are resistant to insecticides etc. B30 strain of *S. feltiae* will be in the future experiments used against different agricultural pests under laboratory conditions as well as in the experiments taken outside.

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