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INVASIVENESS OF THE ENTOMOPATHOGENIC NEMATODES *Steinernema feltiae* (Filipjev 1934) ISOLATED FROM VARIOUS HABITATS IN POLAND

INWAZYJNOŚĆ NICIENI ENTOMOPATOGENNYCH *Steinernema feltiae* (Filipjev 1934) WYIZOLOWANYCH Z RÓŻNYCH ŚRODOWISK W POLSCE

Abstract: The paper presents results of a study on the effect of environmental lead pollution on the invasiveness of nematodes obtained from the field. The nematode *Steinernema feltiae* and larvae of the last growth stage of the greater wax moth *Galleria mellonella* L. were the study material. Nematodes were isolated from soil under laboratory conditions using Bedding and Akhurst's (1975) method. Physical and chemical analyses of soil samples were made in the Analytical Centre of the Warsaw Agricultural University. Total lead content was analysed with the flame atomic absorption spectrophotometry and soil pH with the potentiometric method acc. to the pB procedure (2 ed. of 21. June 2005). *Steinernema feltiae* were determined based on keys for entomopathogenic nematodes. The invasiveness of nematodes originating from various regions of Poland was evaluated.

Keywords: entomopathogenic nematodes, *Steinernema feltiae*, *Galleria mellonella*, heavy metals, lead ions

Bioinsecticides based on entomopathogenic nematodes are one of the biological plant protection means. Nematodes are characterised by a high reproductive potential, broad food spectrum and an ability to produce dormant stages in a form of invasive larvae. The larvae possess an ability to actively search for host-insect and to penetrate its body [1–5].

These traits are affected by both biotic and abiotic factors like heavy metals in the soil and soil pH.

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In the soils contaminated by heavy metals we may observe changes in the invasiveness and disturbed reproduction [6–9]. Heavy metals (including lead) get to the atmosphere from industrial emissions from various smelters, cement mills, steel works and intense transport. Then the metals fall and accumulate in the upper soil layers where they remain and affect various soil organisms for many years. Lead contamination is the most important threat to light acidic soils [10–12].

Total lead content and soil pH of analysed soil habitats was determined in this study.

Soils in Poland are usually acidic as a result of the presence of carbon dioxide in the air. The gas dissolved in water produces weak carbonic acid which falls onto soil with atmospheric precipitation. Soil pH is the key element decisive for many biological and physicochemical processes that take place in soil. Soil acidification largely affects mobility and bioavailability of heavy metals and ionic organic pollutants. Heavy metal activity increases with the increase of soil habitat acidity. Optimum range of soil pH for metabolic processes of most plant species and soil organisms varies from 5.5 to 7.2 [13]. A decrease in soil pH decreases pathogenicity and survival of entomopathogenic nematodes [14, 15].

New sites and new nematode species are still a matter of studies worldwide [16–19]. Few faunistic studies are carried out in Poland. The sites of entomopathogenic nematodes in the country were studied by Bednarek in the years 1985–1988. Now, results of these studies are outdated since soil properties and composition could alter during several years. Such changes may in turn affect the density and species composition of entomopathogenic nematodes.

This paper presents new sites of entomopathogenic nematodes in Poland. Performed studies were aimed at evaluating the invasiveness of entomopathogenic nematodes from various sites in Poland. At the same time lead content and acidity of these sites were determined.

Materials and methods

To find out the presence and to isolate entomopathogenic nematodes, 50 soil samples were collected from each of the 8 selected study areas representing possibly similar ecosystems in various zoogeographic regions of the country. Samples were collected with the Egner's sampler (diameter 2.5 cm) to the soil depth of 25 cm in an even grid on uniform surfaces. The method guarantees complete and even sampling from a given area which allows to estimating actual spatial distribution of nematode density and lead pollution of the soil habitat.

Soil samples were analysed for the presence of entomopathogenic nematodes.

Nematodes were isolated from soil samples in the laboratory with the Bedding and Akhurst's (1975) method [20]. Well mixed soil samples were placed in plastic boxes of a capacity of 250 cm^3 together with two trap insects (caterpillars of *Galleria mellonella* L.). Samples were placed in Sanyo incubator for 16 days at a temperature of 25 ^oC. Every two days dead insects were removed from boxes to estimate the reason of their death and transferred to the White's traps [21] to obtain invasive larvae. Dead insects were replaced by new live caterpillars.

Nematode larvae obtained in that way were used to infect (at a rate of 50 larvae per insect) next larvae of *Galleria mellonella* (of a mean body mass 0.165 g) to study insects mortality and the extensity and intensity of infection. Soil samples were also analysed for their physical and chemical properties in the Analytical Centre of the Warsaw University of Agriculture. Lead content in soil was estimated with the flame atomic absorption spectrophotometry (FAAS) and soil pH – with potentiometric method (acc. to PB 14 procedure 2nd edition 21. 06.2005) [22]. Analyses were performed at 25 ^{o}C which is the optimum temperature for the growth of *S. feltiae* [23]. All experiments were duplicated.

Results and discussion

Analyses of soil samples (Table 1) showed almost natural lead content (from 5.942 to 34.4 mg/kg) and strongly to slightly acidic reaction (pH from 4.74 to 6.7). Isolated nematodes were estimated with keys for species determination as *Steinernema feltiae*.

Table 1

Results of the analyses of studied soil samples

Site / Habitat	Lubin		Krasnik		Slupsk		Wielun	
	field	meadow	field	meadow	field	meadow	field	meadow
Pb [mg/kg]	17.05	11.25	34.4	22.3	11.88	5.942	7.21	6.86
pH [-]	6.15	4.74	6.64	6.7	6.14	6.64	5.35	5.62
Insect mortality [%]	100	100	100	97	100	100	100	100
Extensity of infection [%]	100	100	100	97	97	100	100	100
Intensity of infection [ind.]	11 (A)	13	13 (B)	10 (C)	22 (A,B)	12	17 (A)	15 (C)

A, B, C – highly significant differences between groups.

Analysis of variance was used to test the significance of differences between sites and habitats. No differences were found in the extensity of infection between particular groups.

Highly significant differences were found in the intensity of infection among the following sites and habitats: Lubin field – Slupsk field – Wielun field, Krasnik field – Slupsk field, Krasnik meadow – Wielun meadow. This result may indicate that populations from various regions of the country may be characterised by different degrees of invasiveness.

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Abstrakt: W pracy przedstawiono wyniki badań nad wpływem zanieczyszczenia środowiska ołowiem na inwazyjność nicieni pozyskanych z terenu. Materiał do badań stanowiły nicienie *Steinernema feltiae* oraz larwy ostatniego stadium barciaka większego (*Galleria mellonella* L.). Nicienie wyizolowano z próbek glebowych warunkach laboratoryjnych, metodą Beddinga i Akhursta (1975). Próbki glebowe zostały zbadane pod względem fizykochemicznym w Centrum Analitycznym SGGW. Oznaczono całkowitą zawartość ołowiu w glebie metodą płomieniowej absorpcyjnej spektrometrii atomowej FAAS oraz odczyn gleby metodą potencjometryczną (wg procedury pB 14, wyd. 2 z 21.06.2005 r.). *Steinernema feltiae* oznaczono na podstawie kluczy do oznaczania gatunku nicieni entomopatogennych. Oceniono inwazyjność nicieni pochodzących z różnych rejonów Polski.

Słowa kluczowe: nicienie entomopatogeniczne, *Steinernema feltiae*, *Galleria mellonella*, metale ciężkie, jony ołowiu