Proceedings of ECOpole
Vol. 5, No. 1 2011

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ASSESSMENT OF THE INVASIVENESS OF ENTOMOPATHOGENIC NEMATODES *Heterorhabditis megidis* FROM SELECTED GRASSLANDS

OCENA INWAZYJNOŚCI NICIENI ENTOMOPATOGENNYCH *Heterorhabditis megidis* POCHODZĄCYCH Z WYBRANYCH UŻYTKÓW ZIELONYCH

Abstract: The use of entomopathogenic nematodes as biological means of plant pest control focussed the attention on relationships between soil contamination with heavy metals and survival of nematodes in the soil habitat. Negative properties of heavy metals (including lead) disturb life functions of entomopathogenic nematodes. The effect of low habitat contamination with lead on the development of subsequent generations of entomopathogenic nematodes *Heterorhabditis megidis* was studied in performed experiments. Study area was situated near Torun, Opole, and Skarzysko-Kamienna. Grain size structure, total lead content and pH were analysed in soil samples. Nematodes from own laboratory culture were used as a control. Nematodes isolated from soil samples with the method of Bedding and Akhurst were determined to species as *H. megidis*. Larvae that left the host's (*Galleria mellonella* L.) body were used to infect the next test insects in the laboratory. Six passages were performed in total. Extensity and intensity of infection by *H. megidis* were determined in subsequent passages.

Keywords: entomopathogenic nematodes, *Heterorhabditis megidis*, *Galleria mellonella*, heavy metals, lead ions

The activity of soil entomopathogenic nematodes depends on many environmental factors. Soil structure, soil temperature and moisture affect nematode biology [1].

Mechanical composition of soil is one of the factors affecting nematode survival and mobility. It was shown [2] that the invasive larvae of entomopathogenic nematodes faster and easier penetrated insect's body in sandy or sandy-loamy substrata and just in such soils larval survival was the highest.

Another equally important factor influencing nematode activity is soil pH. Natural acidification associated with the presence of carbon dioxide in the air prevails in the soils of Poland. This gas when dissolved in rain waters forms weak carbonic acid which falls to the soil with atmospheric precipitation. In agricultural lands soil pH varies between 4.0 and 7.2. The pH value above 8.3 might indicate the presence of antropogenically derived alkalizing substances like eg metal oxides. Optimum pH for biological processes associated with the metabolism of plants and soil organisms is considered to be in range from 5.5. to 7.2 [3]. The mobility, activity and pathogenic properties of entomopathogenic nematodes are higher at pH 6.8 and 8 than at pH 5.5 [4].

Soil pH largely decides upon the bioavailability and mobility of heavy metals. Both features increase with soil acidification [3]. Heavy metals, particularly lead, unfavourably affect nematode invasiveness and reproduction [5-8].

Local populations of nematodes are well fit to changing habitat conditions and may efficiently control insect populations dwelling the same habitat [9].

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Studies carried out on the entomopathogenic nematode-host insect system indicate that multiple infection of insects of a given species results in selection of a specific nematode race which is more pathogenic than the initial race [10, 11]. This may suggest that local populations of entomopathogenic nematodes differentiate under the effect of a characteristic species composition and domination structure of local insect communities [12].

The aim of performed studies was to analyse the invasiveness in subsequent passages through the same insect species of entomopathogenic nematodes *Heterorhabditis megidis* (Poinar, Jackson and Klein 1987) originating from natural habitats and to analyse acidification, grain size structure and lead content in these habitats.

Material and methods

Studies were carried out in the summer seasons of the years 2008-2009. Fifty soil samples were taken with the Egner's sampler from a soil layer 0÷25 cm in three selected study areas (natural meadows). Physical and chemical soil properties were analysed in the Analytical Centre and the Department of Soil Science of the Warsaw University of Life Sciences (SGGW). Grain size structure, total lead content with the flame AAS method and soil pH with the potentiometric method (acc. to the pB procedure, 2nd ed. of 21.06.2005) were determined. Nematodes isolated from soil samples with the method of Bedding and Akhurst [13] were identified as *Heterorhabditis megidis* using keys for species determination.

Experiment was carried out in the Sanyo MIR 253 incubator at 25ºC. Nematode larvae isolated from soil samples were used to infect test insects (*Galleria mellonella* of a mean body weight of 0.160 g) using the invasive dose of 50 nematode larvae per insect on Petri dish. Half of insects dead as a result of nematode infection were dissected to determine the intensity of invasion and the second half were transferred to White's trap [14] to obtain invasive larvae migrating from the host's body. The larvae leaving the host's body were used to infect next test insects (*G. mellonella* L.). Six subsequent passages were made in total. Insects' mortality and the extensity and intensity of invasion of *H. megidis* were determined in every passage. Nematodes bred in the laboratory for years in one host (*G. mellonella*) were used as control in the assessment of subsequent passages. The experiment was repeated twice.

Results and discussion

Physical and chemical properties of analysed soil samples were appropriate for nematode larvae living in soil habitat. Soil samples had natural lead content (from 5.894 to 16.93 mg/kg) and pH from moderately to weakly acidic. Grain size structure of analysed soil samples was typical for loamy sand and weak loamy sand. The highest survival of nematodes was noted in such habitats [2] (Tab. 1).

Table 1

Characteristics of studied sites

Location	Longitude	Latitude	Grain size structure	pH	Pb [mg/kg]
Skarzysko-Kamienna	20,54,49 E	51,07,10 N	Loamy sand	5.4	9.50
Torun	18,20,15 E	53,03,03 N	Weak loamy sand	5.34	5.894
Opole	17,45,45 E	50,41,17 N	Loamy sand	6.07	16.93

Differences in the invasiveness of nematodes isolated from studied soil habitats were observed in subsequent passages. Initially, the invasion of nematodes was slow (insects' death after 72 hours) and lower than in the control sample. Insect mortality, extensity and intensity of invasion were low at that period. In subsequent passages faster killing of test insects was observed (insects' death after 48 hours) and the increase of invasiveness manifesting itself by high insect mortality and the extensity and intensity of invasion. After several passages the invasiveness of nematodes from the field was the same as that of nematodes bred in the laboratory for many years without changing their host (Figs 1-3).

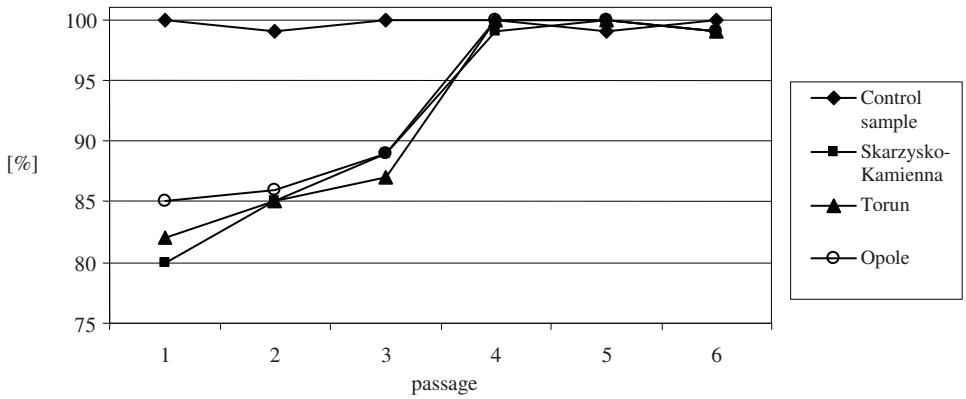

Fig. 1. Mortality [%] of test insects in subsequent passages

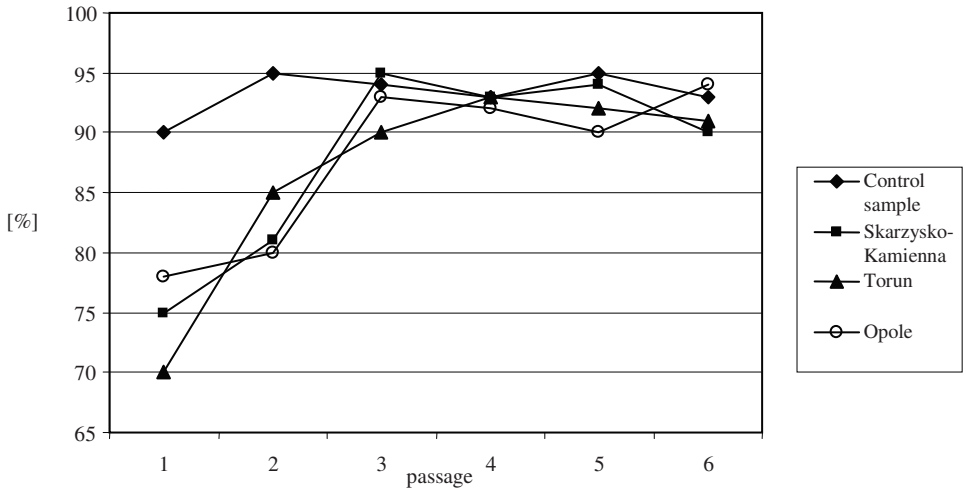

Fig. 2. Extensity [%] of invasion of *H. megidis* in subsequent passages

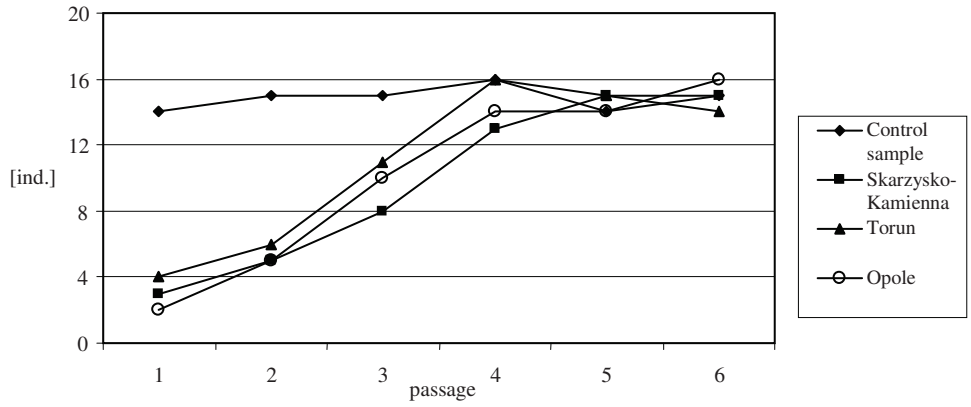

Fig. 3. Intensity [ind.] of invasion of *H. megidis* in subsequent passages

Performed statistical analysis (ANOVA) for the intensity of invasion showed highly significant differences between the control sample and all studied groups of nematodes in passages 1-4. Significant differences were also found in the intensity of invasion in the 3rd and 4th passage between nematodes from all analysed habitats.

A lack of significant differences between the control nematodes and those from natural sites in the 5th and 6th passage may indicate that multiple infections of insects from a given species may lead to selection of such a nematode race which is more pathogenic for this host species than nematodes from the initial race. Results of this study confirm the findings of other authors [10, 11, 15].

Conclusions

Performed studies allow for concluding that multiple passage of entomopathogenic nematodes through the host's organism of a single species increased nematodes' pathogenicity in comparison with initial population. Hence, nematode populations may vary under the effect of species composition and domination structure of local communities of host insects.

Acknowledgements

The paper was financed in the years 2007-2010 as a research project from scientific funds, No. NN304418633.

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OCENA INWAZYJNOŚCI NICIENI ENTOMOPATOGENNYCH *Heterorhabditis megidis* POCHODZĄCYCH Z WYBRANYCH UŻYTKÓW ZIELONYCH

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Abstrakt: Wykorzystanie nicieni entomopatogennych jako biologicznego środka zwalczającego szkodniki roślin spowodowało zwrócenie uwagi na zależności między skażeniem gleb metalami ciężkimi a przeżywalnością larw nicieni w środowisku glebowym. Szkodliwe właściwości metali ciężkich, w tym ołowiu, zaburzają funkcje życiowe nicieni owadobójczych. W przeprowadzonych doświadczeniach zbadano wpływ niskiego skażenia środowiska ołowiem na rozwój kolejnych pokoleń nicieni entomopatogennych *Heterorhabditis megidis*. Tereny badawcze znajdowały się w okolicach miejscowości: Toruń, Opole, Skarżysko-Kamienna. W próbkach glebowych oznaczono skład granulometryczny, całkowitą zawartość ołowiu w glebie oraz odczyn gleby. Kontrolę stanowiły nicienie pochodzące z laboratoryjnej hodowli własnej. Wyizolowane z próbek glebowych metodą Beddinga i Akhursta (1975) nicienie oznaczono na podstawie kluczy do oznaczania gatunku jako *H. megidis*. Larwami, które opuściły ciało żywiciela (*Galleria mellonella* L.), zarażono laboratoryjnie kolejne owady testowe. Wykonano 6 pasaży. Oznaczono ekstensywność i intensywność inwazji *H. megidis* w kolejnych pasażach.

Słowa kluczowe: nicienie entomopatogenne, *Heterorhabditis megidis*, *Galleria mellonella*, metale ciężkie, jony ołowiu