ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 11 2011

Joanna MATUSKA-ŁYŻWA[1] and Marta KAMIONEK[2]

MORPHOMETRIC CHANGES IN *Heterorhabditis megidis* (POINAR, JACKSON AND KLEIN 1987) AFTER DIFFERENT CONTACT WITH LEAD(II) IONS

ZMIANY MORFOMETRYCZNE *Heterorhabditis megidis* (POINAR, JACKSON I KLEIN 1987) PO RÓŻNYM CZASIE KONTAKTU Z JONAMI OŁOWIU(II)

Abstract: Nematodes of the families *Steinernematidae* and *Heterorhabditidae* are a natural factor controlling population density of insects and for many years have been used in biological plant protection. When using entomopathogenic nematodes as biological pest control it was found that heavy metal ions might negatively affect their pathogenic properties.

In laboratory experiments invasive larvae of *H. megidis* were kept for 24 and 120 hours in Petri dishes containing water solutions of lead nitrate(V) at a concentration of 500 ppm Pb(II). Nematodes that survived the contact with lead ions were transferred to pots with soil and test insects (*Galleria mellonella* L.). Nematodes kept in distilled water served as a control. Half of dead insects was dissected two days after their death. Individuals of the first nematode generation were isolated from dead insects and selected body dimensions (body length, body width, length of the pharynx and tail) were measured. Second half of dead insects was intended for reproduction. Larvae obtained from this reproduction were used for further tests aimed at analysing the effect of long term passaging of nematodes through the host. Five passages were made in total. The time of the contact of nematodes with lead ions was found to affect their body dimensions.

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

Environmental pollution is not the only effect of the common use of heavy metals. Sometimes even small amounts of these metals may cause many diseases in man [1], farm animals [2] and free living animals like eg entomopathogenic nematodes [3–9]. Lead is one of the most toxic metals for living organisms. Lead ions in soil habitat

[1] Department of Zoology, Institute of Biology, Faculty of Mathematics and Science, The Jan Kochanowski University of Humanities and Scientes in Kielce, ul. Świętokrzyska 15, 25–406 Kielce, Poland, phone: +48 41 349 63 53, email: joannaujk@op.pl

[2] Division of Zoology, Department of Animal Environment Biology, Faculty of Animal Sciences, Department of Animal and Environmental Biology, Warsaw Agricultural University, ul. J. Ciszewskiego 8, 02–787 Warszawa, Poland, phone: +48 22 593 66 22, email: marta_kamionek@sggw.pl

negatively affect survival, pathogenic properties and reproduction of entomopathogenic nematodes [3–9].

This study was performed to assess the effect of the time of contact of nematodes with lead ions on selected body dimensions of the first generation of *Heterorhabditis megidis*.

Material and methods

Invasive larvae of *H. megidis* and larvae of the last growth stage of *Galleria mellonella* L. (mean body mass 163 mg) were used in experiments. Both entomopathogenic nematodes and insects were taken from the culture of the Department of Zoology, Warsaw Agricultural University.

Invasive larvae of nematodes were kept in Petri dishes for 24 h (sample B) and for 120 h (sample C) in water solutions of lead nitrate(V) at a concentration of 500 ppm Pb(II). Larvae that survived the contact with lead ions were used to infect test insects. Control (sample A) consisted of larvae kept in distilled water. Insects were infected individually: one insect larva and 500 larvae of invasive nematodes were placed in a pot filled with wet sand. Half of dead insects was dissected two days after their death. Mature individuals of the first nematode generation (hermaphroditic individuals) were isolated and measured (body length and width, length of the pharynx and tail) under light microscope. Second half of dead insects was transferred onto individual migration sponges (modified traps for collecting nematode larvae migrating from insects' bodies). So obtained invasive larvae were used to infect next test insects to check the effect of long term passaging of nematodes dealt with lead ions through the same host. Five passages were made in total. Body length and width, length of the pharynx and tail were measured in hermaphroditic individuals whose larvae were subjected to lead ions for different time period.

Experiments were carried out at 25 ^{o}C – an optimum temperature for *H. megidis* at which the species shows the greatest invasiveness [10]. UNIANOVA was used to compare obtained results.

Results and discussion

Results show that adult individuals whose larvae contacted lead ions had smaller body dimensions than those in the control sample A. A slight decrease of body size was noted during consecutive passages.

Individuals isolated from the first passage had larger body dimensions than those isolated during the next four passages. Therefore, results from the first passage were compared with the mean from the next four passages (2–5). This relationship was not observed in one sample (B) and one morphometric feature – body length of nematodes whose larvae had shorter (24 h) contact with lead ions. After the first passage they were markedly shorter (2.52 mm) than individuals which being in the larval stage had longer

(120 h) contact with lead ions (4.64 mm) and than the control individuals (4.8 mm) (Table 1).

Table 1

Body size [mm] of hermaphroditic individuals of *H. megidis*

Samples / Body size [mm]	Sample A (passage 1)	Sample A (mean of passages 2–5)	Sample B (passage1)	Sample B (mean of passages 2–5)	Sample C (passage 1)	Sample C (mean of passages 2–5)
Body length	4.80	4.75	2.52	4.59	4.64	4.51
Body width	0.26	0.25	0.21	0.20	0.23	0.22
Length of the pharynx	0.24	0.23	0.22	0.21	0.23	0.22
Tail length	0.122	0.121	0.103	0.100	0.118	0.115

In consecutive passages the differences diminished ie body size of individuals, which when being in the larval stage contacted lead ions approached those of individuals from samples A and C. Changes in the body dimensions of *H. megidis* in relation to the contact time and passage are presented in Tables 2–5.

Table 2

Body length [mm] of hermaphroditic individuals of *H. megidis*

Samples / Body length [mm]	Sample A	Sample B	Sample C
Passage 1	4.80	2.52	4.64
Passage 2	4.74	4.48	4.55
Passage 3	4.77	4.69	4.49
Passage 4	4.69	4.62	4.47
Passage 5	4.81	4.58	4.53
Mean of passages 2–5	4.75	4.59	4.51

Table 3

Body width [mm] of hermaphroditic individuals of *H. megidis*

Samples / Body width [mm]	Sample A	Sample B	Sample C
Passage 1	0.26	0.21	0.23
Passage 2	0.24	0.21	0.23
Passage 3	0.25	0.20	0.21
Passage 4	0.23	0.19	0.22
Passage 5	0.26	0.21	0.23
Mean of passages 2–5	0.25	0.20	0.22

Table 4

Length of the pharynx [mm] in hermaphroditic individuals of *H. megidis*

Samples / Length of the pharynx [mm]	Sample A	Sample B	Sample C
Passage 1	0.24	0.22	0.23
Passage 2	0.22	0.20	0.23
Passage 3	0.23	0.21	0.19
Passage 4	0.22	0.20	0.24
Passage 5	0.25	0.22	0.22
Mean of passages 2–5	0.23	0.21	0.22

Table 5

Tail length [mm] in hermaphroditic individuals of *H. megidis*

Samples / Tail length [mm]	Sample A	Sample B	Sample C
Passage 1	0.122	0.103	0.118
Passage 2	0.121	0.099	0.117
Passage 3	0.123	0.101	0.104
Passage 4	0.119	0.100	0.118
Passage 5	0.122	0.099	0.122
Mean of passages 2–5	0.121	0.100	0.115

Described above small body length of nematodes from the first generation (after the first passage) which when being in the larval stage had 24 h contact with lead ions was observed in every next experimental repetition.

One factor ANOVA performed for selected body dimensions showed significant differences between studied groups (Table 6). Highly significant differences during the first passage were noted in all body dimensions between control group and nematodes whose former larval generation was kept for 24 h in the solution of lead ions at a concentration of 500 ppm Pb(II). Multiple comparison of the first passage between the control group and the nematodes whose former generation was affected by lead ions showed significant differences in body length and highly significant differences in body width. Comparison of body size of nematodes that survived short contact with lead ions with those that contacted these ions for a longer period revealed no significant differences in the length of pharynx. Other body dimensions between these two groups were highly significant.

Obtained results indicate that body length of hermaphroditic individuals of *H. megidis* was more influenced by shorter (24 h) than longer (120 h) contact period of invasive larvae of the previous generation with lead ions. It seems that the longer contact with lead ions eliminated biologically weaker nematodes from experiment. It was also found that the time of contact with lead ions did not affect internal morphometric feature – length of the pharynx – in studied nematodes.

Table 6

ANOVA for selected body dimensions in nematodes

Body dimensions	Groups	SS	df	MS	F	p
Body length	Between groups	64.827	5	12.965	246.464	.000
	Within groups	11.521	219	.053		
	Total	76.348	224			
Body width	Between groups	.072	5	.014	35.747	.000
	Within groups	.089	219	.000		
	Total	.161	224			
Length of the pharynx	Between groups	.020	5	.004	9.244	.000
	Within groups	.093	219	.000		
	Total	.113	224			
Tail length	Between groups	.018	5	.004	51.449	.000
	Within groups	.015	219	.000		
	Total	.033	224			

The effect of lead ions on nematodes was the topic of many papers. Unfavourable effect of lead(II) ions on the pathogenic properties of entomopathogenic nematodes was demonstrated eg by Jarmuł and Kamionek and by Jaworska et al [5, 6, 8].

When studying biological activity of entomopathogenic nematodes in soil one should pay attention to other factors that might limit or change their population. In urban areas species composition of micro- and mesofauna may vary largely thus affecting animal biodiversity. Environmental contamination by heavy metals might be one of the causative factors in such cases.

Conclusion

Performed studies allow for the conclusion that time of the contact of nematode invasive larvae with lead(II) ions affects morphometric features of the first generation of adult individuals of *H. megidis*. Repetitive passage of nematodes results also in small morphometric changes.

References

[1] Zglinicka A.: Aura 2002, (2), 30–31.
[2] Matras J., Bujanowicz B. and Klebaniuk R.: Aura 2000, (11), 16–17.
[3] Jarmuł J.: Praca doktorska, SGGW, Warszawa 2002, pp. 130.
[4] Jarmuł J. and Kamionek M.: Chem. Inż. Ekol. 2003, **10**(3–4), 281–284.
[5] Jarmuł J. and Kamionek M.: Chem. Inż. Ekol. 2001, **8**(6), 601–607.
[6] Jarmuł J. and Kamionek M.: Chem. Inż. Ekol. 2000, **7**(10), 1023–1029.
[7] Jarmuł J., Pezowicz E. and Kamionek M.: Chem. Inż. Ekol. 2005, **12**(10), 1083–1088.
[8] Jaworska M., Gorczyca A., Sepioł J. and Tomasik P.: Chem. Inż. Ekol. 2000, **7**(4), 313–326.
[9] Jaworska M., Ropek D. and Gorczyca A.: Chem. Inż. Ekol. 1999, **6**(5–6), 469–475.
[10] Trdan S., Valič N., Urek G. and Milevoj L.: Acta Agricult. Sloven. 2005, **85**, 117–124.

ZMIANY MORFOMETRYCZNE *Heterorhabditis megidis* (POINAR, JACKSON I KLEIN 1987) PO RÓŻNYM CZASIE KONTAKTU Z JONAMI OŁOWIU(II)

Zakład Zoologii, Instytut Biologii, Wydział Matematyczno-Przyrodniczy
Uniwersytet Jana Kochanowskiego w Kielcach

Abstrakt: Nicienie z rodziny *Steinernematidae* i *Heterorhabditidae* stanowią naturalny czynnik redukujący liczebność populacji owadów i od wielu lat są wykorzystywane w biologicznej ochronie roślin. W momencie wykorzystania nicieni entomopatogennych jako biologicznego środka w zwalczaniu szkodników upraw, stwierdzono, że jony metali ciężkich mogą w znacznym stopniu niekorzystnie wpłynąć na patogenność nicieni owadobójczych.

W doświadczeniach larwy inwazyjne *H. megidis* przetrzymywano przez 24 godziny i 120 godzin w roztworach wodnych azotanu(V) ołowiu o stężeniu 500 ppm Pb(II) w szalkach Petriego. Nicienie, które przeżyły kontakt z jonami ołowiu, wprowadzano do pojemników z glebą, w których znajdowały się owady testowe (*Galleria mellonella* L.). Kontrolę stanowiły nicienie przetrzymywane w wodzie destylowanej. Połowę martwych owadów poddawano sekcji po dwóch dniach od ich śmierci. Izolowano z nich osobniki pierwszego pokolenia nicieni i oznaczano ich wybrane wymiary ciała (długość ciała, szerokość ciała, długość gardzieli i długość ogona). Drugą połowę martwych owadów przeznaczano na reprodukcję. Otrzymane z reprodukcji larwy wykorzystano do dalszych testów mających na celu analizę wpływu długotrwałego pasażowania nicieni przez jednego żywiciela. Wykonano 5 pasaży. Zaobserwowano, że czas kontaktu nicieni z jonami ołowiu(II) wpływa na wymiary ciała.

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