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THE EFFECT OF LEAD, MANGANESE AND CADMIUM IONS ON ENTOMOPATHOGENIC NEMATODES *STEINERNEMA CARPOCAPSAE* (WEISER 1955)

WPŁYW JONÓW OŁOWIU, MANGANU I KADMU NA NICIENIE ENTOMOPATOGENICZNE *STEINERNEMA CARPOCAPSAE* (WEISER 1955)

Key words: *S. carpocapsae,* heavy metals, lead, cadmium, manganese, soil.
Słowa kluczowe: *S. carpocapsae,* metale ciężkie, ołów, kadm, mangan, gleba.

Summary

The effect of heavy metal ions (Pb, Cd, Mn) on survival and reproduction of entomopathogenic nematodes S. carpocapsae was presented in this paper. The study was also focussed on the influence of these ions on sex structure of nematode population, on survival of invasive larvae and on the infection of Galleria mellonella caterpillars by larvae treated by heavy metal ions. A negative effect of these ions increased with their concentration and the time of their action.

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Streszczenie

W niniejszej pracy przedstawiono wpływ roztworów jonów metali ciężkich (Pb, Cd i Mn) na żywotność oraz rozrodczość nicieni entomopatogenicznych S. carpocapsae. W pracy zwrócono uwagę na wpływ jonów metali ciężkich na strukturę płciową populacji nicieni, śmiertelność larw inwazyjnych oraz porażenie gąsienic owadów G. mellonella przez larwy poddane działaniu triady jonów metali ciężkich.
Efekt negatywnego działania jonów ołowiu, manganu i kadmu wzrastał wraz ze wzrostem stężeń roztworów i długością czasu i ich działania.

1. INTRODUCTION

Nematodes are cosmopolitan organisms present worldwide practically in every habitat (fresh and sea waters, soil, litter, plant and animal tissues). They colonised deserts, polar areas, caves, mountains, hot springs or oceanic abyssal [Hominick et al. 1996, Hazir et al. 2001, Jura 2004]. Due to their high numbers and adaptation to various conditions nematodes play a great role in the cycling of matter. Parasitic species developed many adaptations that enable them to attack plant and animal hosts.

Thanks to the ability to anabiosis and encystment nematodes are able to survive many years (even up to 30) under unfavourable conditions. The eggs of some species are also adapted to such conditions [Jura 2004]. Many nematode species, whose application in biological control of harmful insects in plant production arise much interest and creates new possibilities, belong to the family *Steinernematidae* [Stock et al. 1999, Susurluk 2006].

Biotic and abiotic factors may have a positive or negative effect on soil organisms. Abiotic factors include temperature, soil structure and many others like e.g. heavy metals. The source of heavy metals may be parent rocks, natural dusts, atmospheric precipitation and organic deposition [Jarmuł 2002]. Human activity also increases the content of heavy metals in the natural environment through e.g. the exhausts from motor vehicles or industrial waste waters delivered to surface waters. Many of these pollutants penetrate the soil affecting soil organisms including nematodes [Jaworska et al. 1999, Jarmuł et al. 2001, 2003].

2. MATERIAL AND METHODS

Nematodes *Steinernema carpocapsae* (black strain) and caterpillars of *Galleria mellonella* from the culture of the Department of Zoology SGGW in Warsaw were used in this study. The experiment was carried out with the use of three solutions of manganese, lead and cadmium as nitrate salts ($Mn(NO_3)_2 \cdot 4H_2O$, $Pb(NO_3)_2$ and $Cd(NO_3)_2 \cdot 4H_2O$) in the following concentrations:

Solution I:

- $Pb(NO_3)_2$ – 40mg Pb·dm^{-3},
- $Cd(NO_3)_2 \cdot 4H_2O$ – 1mg Cd·dm^{-3},
- $Mn(NO_3)_2 \cdot 4H_2O$ –200mg Mn·dm^{-3}.

Solution II:

- Pb(NO3)2 – 100mg Pb·dm^{-3},
- Cd(NO3)2·4H2O – 2mg Cd·dm^{-3},
- Mn(NO3)2·4H2O – 500 mg Mn·dm^{-3}.

Solution III:

- Pb(NO3)2 – 500mg Pb·dm^{-3},
- Cd(NO3)2·4H2O – 3mg Cd·dm^{-3},
- Mn(NO3)2·4H2O – 1000mg Mn·dm^{-3},
- Water solutions were made with distilled water.

Experiments, each in three repetitions, were carried out at 25°C which is optimum for *S. carpocapsae*. Observations were made every second day. Dead caterpillars infected by nematodes were dissected to count the number and estimate the sex of nematodes. Nematode mortality was determined based on the number of dead invasive larvae. The effect of heavy metals on pathogenicity of *S. carpocapsae* was checked with the test insect *G. mellonella*.

Three double pots were used in the experiment; one with the bottom made of a net was placed inside the other. Internal pots were filled with 100 cm^3 of dry roasted sand and 100 cm^3 of prepared solutions of heavy metal mixture. Five thousand nematodes were placed in each pot. Three caterpillars of *G. mellonella* were placed in the external pot – immediately after nematode application to the internal pot in the first repetition, 7 days later in the second and 14 days later in the third repetition. Control was performed in the same conditions, for the same time period but without heavy metal solutions. Obtained results were processed using the SPPS 14 statistical software.

3. RESULTS

Tests of the effect of heavy metals on nematode population and mortality of the infected caterpillars of *G. mellonella* showed different rates of nematode movement in sand between particular repetitions.

At simultaneous introduction of nematodes and the host insect the former started migration 6 days after the set up of experiment (Fig. 1).

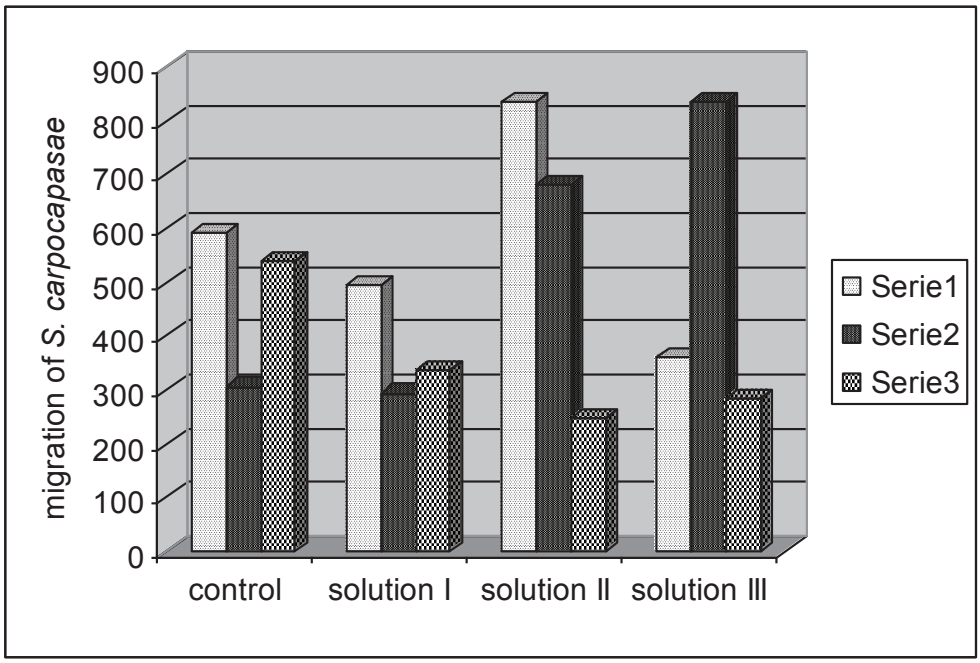

Fig. 1. The effect of heavy metals on the migration of nematodes *S. carpocapsae*
Rys. 1. Wpływ jonów metali ciężkich na migracje nicieni *S. carpocapsae*

Higher concentration of heavy metals in solution II markedly stimulated nematodes to migration through sand. The number of migrating nematodes was over 40% higher than in the control. Similar effect had the time period spent by nematodes in contaminated sand without host. In solution III the introduction of host insects 14 days after the placement of nematodes resulted in the highest migration of nematodes to insect's body (Fig. 1).

Results of performed experiment demonstrate an important effect of heavy metals on sex structure in nematode populations (Tab. 1).

Table 1. Sex structure of *S. carpocapsae* infecting the caterpillars of *G. mellonella*
Tabela 1. Struktura płciowa nicieni *S. carpocapasae* porażających gąsienice *G. mellonella*

Repeat	Control		Solution I		Solution II		Solution III	
	female	male	female	male	female	male	female	male
1	277	119	345	251	746	202	276	136
2	168	121	341	22	455	363	764	389
3	396	146	169	155	194	99	247	85

The number of females in solution I was lower by 31.5% than in the control. Greater contamination and prolonged time of the contact with polluted environment had the most unfavourable effect on nematodes (Tab. 1). Test insects were reached by only half of released nematodes in this solution. The time of their migration was also prolonged.

First, both males and females migrated in all repetitions, later on – only females. Reproduction increased when females dominated over males in numbers as compared with the situation of the equal number of females and males.

In all cases the time of exposition under given environmental conditions correlated with the number of migrating individuals and the number of invasive larvae. Appropriate regressions showed that the time of exposition of *S. carpocapsae* invasive larvae to heavy metal ions had an effect on migration and invasiveness of nematodes and on the sex ratio of invasive larvae found in caterpillars.

4. DISCUSSION

Studies on practical application of nematodes in controlling insect pests have been carried out since the finding of entomopathogenic properties of some nematodes. The efficiency of entomopathogenic nematodes against these pests decreased the use of plant protection chemicals. Nematodes of the family Steinernematidae found particularly wide application in pest control. Studies on the effect of heavy metals on nematodes have been carried in the recent years because of the systematic increase of their concentrations in soils. Many authors were of the opinion that manganese at low concentrations stimulated the increase of nematode pathogenicity [Ishibashi et al. 1987, Mracek et al. 2005]. Increased pathogenicity was observed in this study when two solutions were applied:

200 ppm Mn + 40 ppm Pb + 1 ppm Cd and 500 ppm Mn + 100 ppm Pb + 2 ppm Cd.

The effect of lead on entomopathogenic nematodes has also been studied. The metal was found to limit migration and viability of nematodes. It also affected the survival of nematodes in soil habitat. Lead ions used in the present experiment had similar effects [Jaworska et al. 1999, Jarmuł et al. 2001, 2003]. Already at low concentrations the changes in sex structure were observed. Ropek [2005] analysed the pathogenicity and mortality of invasive larvae of *S. feltiae* kept for a long time in solutions of lead salts. Apart from the effect of concentration, he found the role of the exposition time of nematodes.

Cadmium ions had the most pronounced effect on nematodes. Already at low concentrations they worsened the reproductive abilities of nematodes.

Based on literature data and own studies one may conclude that the groups of metals exert an effect on entomopathogenic nematodes of the family *Steinernema*. Performed experiments showed that a mixture of heavy metals negatively affected sex structure and migration ability. At low concentrations of Pb and Cd manganese ions may play a protec-

tive role. Manganese had a positive effect by stimulating nematodes' migration. However, at high concentrations of lead and cadmium such effect was not observed [Jaworska et al. 1999, Jarmuł et al. 2005, Pezowicz et al. 2005].

5. CONCLUSION

1. Females of nematodes showed a higher resistance and tolerance to unfavourable effects of heavy metal ions.
2. Under the stronger stress (solutions II and III) nematodes accelerated their migration through sand.
3. Fewer males than females create better conditions for the reproduction of nematodes.

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