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The Effect of Metal Ions on Mortality, Pathogenicity and Reproduction of Entomopathogenic Nematodes *Steinernema feltiae* Filipjev *{Rhabditida, Steinernematidae)*

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Abstract

The effect of cadmium, copper, lead, zinc, manganese and magnesium on entomopathogenic nematodes *S. feltiae* was tested under laboratory conditions. The mortality of nematode invasive larvae, their pathogenicity expressed as test insect *G. mellonella* mortality and reproduction from the test insect bodies were investigated. It was revealed that cadmium, copper, lead and zinc affected an increase of *S. feltiae* nematode invasive larvae mortality, decreased their pathogenicity for test insects and reproduction. Manganese in 400 mgdm^3 Mn (II) concentration positively affected entomopahogenic nematode *S.feltiae* during their storage causing decreased mortality, improved pathogenicity and reproduction from insect bodies in comparison to distilled water.

Keywords: metal ions, entomopathogenic nematodes, *S.feltiae,* mortality, pathogenicity, reproduction.

Introduction

Invasive larvae of entomopathogenic nematodes actively or passively penetrate into the bodies of insects through natural apertures or cuticles and thus are considered one of the most efficient microorganisms used for biological crop protection [1]. Representatives of several nematode families are used for insect pest control [2, 3]. Species from *Steinernematidae* and *Heterorhabditidae (Nematoda: Rhabditida)* families are most important. The species most frequent in the natural environment in Poland are *Steinernema carpocapsae, S. feltiae* and *Heterorhabditis bacteriophora.* Advantages of nematodes as biopreparations include the wide range of their host insects, ability for fast insect killing, fast breeding, no immunization of pests and no hazard for the environment or higher animals, as well as a possibility of storing material used for infection and its easy application. Entomopathogenic nematode pathogenicity is conditioned by such factors as temperature, soil moisture, soil structure and oxygen content. This investigation aimed at determining the effect of selected heavy metal ions and manganese and magnesium on vitality, pathogenicity and reproduction of S. *feltiae* entomopathogenic nematodes.

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Experimental Procedures

S. feltiae, as the most common species in the soils of Poland, was selected for the experiment. The strain came from the collection of the Institute of Plant Protection in Poznan, it was earlier acquired in the Wielkopolska region and marked with PL II symbol. It was passaged on *G. mellonella* L. caterpillars using commonly accepted methods and stored in cold storage in 0.01% formaldehyde solution until the outset of the experiment. During the experiment toxicity of individual heavy metal salts was tested separately and then jointly with manganese or magnesium salts. The following salts were used: 75 $mg \cdot dm^{-3}$ Cd(II) of cadmium chloride $CdCl_2$, 7.5 $mg \cdot dm^{-3}$ Cu(II) of copper sulphate $CuSO_4 \cdot 5H_2O$, 200 $mg \cdot dm^{-3}$ Pb(II) of lead nitrate $Pb(NO_3)_2$, 200 $mg \cdot dm^{-3}$ Zn(II) of zinc chloride $ZnCl_2$, 400 $mg \cdot dm^{-3}$ Mn(II) of manganese sulphate $MnSO_4 \cdot 5H_2O$ and 160 $mg \cdot dm^{-3}$ Mg(II) of magnesium sulphate $MgSO_4 \cdot 7\ H_2O$.1 ml of water solutions of individual salts or a mixture of cadmium, copper, lead and zinc with manganese or magnesium salt were pipetted into 2 ml sterile vessels placed in Petri dishes. Distilled water was used in the control object. About 50 specimens of 5. *feltiae* invasive larvae were introduced into the vessels. The experiment included 5 replications. The nematodes were kept at 20-22°C for 96 hours. Afterwards mortality rate of invasive larvae was assessed by counting dead specimens in the experimental objects. Metal ion effect on *S. feltiae* nematode pathogenicity was tested on *G. mellonella* caterpillars. The dishes were incubated at 20-22°C for 4 days and then mortality rate of the test insects was estimated. Dead insects were put on watch glasses placed in Petri dishes filled with distilled water in order to determine *S. feltiae* nematode progeny production from the test insects. Live *S. feltiae* nematode invasive larvae migrating into the water were decanted and their total number was counted under binocular magnifying glass. The obtained results were then subjected to statistical evaluation using single factor analysis of variance and means were differentiated by Duncan test at the significance level p = 0.05.

Results

Mortality, pathogenicity and reproduction of *S. feltiae* nematodes observed in the experiment after 96-hour contact with selected metal ions and their mixtures with manganese or magnesium are presented in Table 1. Only in 400 $mg\text{-}dm^{-3}$ manganese salt solution was visibly lower nematode mortality was observed in comparison with mortality of nematodes kept in distilled water. Mixtures of Cu+Mn and Pb+Mg had a similar, i.e. neutral effect on the nematodes as distilled water itself (control). The other solutions used in the experiment caused increased nematode mortality rates in comparison with the control in water (Figure 1), so they revealed toxic effects, the most apparent for lead and Cd+Mn and Cu+Mg mixtures. The influence of selected metal ions on *S. feltiae* nematode pathogenicity for the tested caterpillars in relation to control is shown in Figure 2. *S. feltiae* nematodes from cadmium, copper, lead and zinc mixtures with manganese or magnesium and after their contact with lead had decreased abilities to infect the test insects, even if the activity of the mentioned metals did not previously cause any marked nematode mortality in comparison with the control. After contact with the other single metal ions (Cd, Cu, Mg, Zn) *S. feltiae* nematodes infected the test insects similarly as those from distilled water, whereas manganese improved pathogenic abilities of invasive *S. feltiae* nematode larvae as compared with the control. Entomopathological nematode progeny production from the test insects compared to control is shown in Figure 3. Manganese slightly stimulated *S. feltiae* reproduction in comparison with the control. 5. *feltiae* nematodes reproduced worst after contact with copper and zinc, and also following their contact with zinc and magnesium mixture. The number of invasive larvae obtained from 1 mg of test insect body for the combination was approximate.

Table 1. Effect of heavy metals and heavy metal mixtures with manganese and magnesium on mortality and pathogenicity expressed as test insect mortality, and on reproduction of *S. feltiae* entomopathogenic nematodes.

Metal	*S. feltiae* mortality %	Pathogenicity (test insect mortality) %	Nematode reproduction mean/mg test insect body items
Cd	7.5 bcdef*	68 e	329 c
Cu	9.6 cdef	66 e	222 ab
Mn	0.8 a	91 f	412 d
Mg	6.5 bcdef	42 c	351 cd
Pb	13.6 f	24 a	233 b
Zn	6.9 bcdef	41 c	213 a
Cd+Mn	13.1 ef	51 d	238 b
Cd+Mg	7.8 bcdef	21 a	337 c
Cu+Mn	2.9 ab	34 ab	287 b
Cu+Mg	10.9 def	21 a	229 b
Pb+Mn	6.2 bcde	29 a	312 bc
Pb+Mg	4.1 abc	25 a	336 c
Zn+Mn	4.7 bcd	20 a	218 a
Zn+Mg	6.8 bcdef	36 b	289 b
Control	2.6 ab	66 e	408 d

* Means marked by the same letters in columns do not differ statistically at the significance level p = 0.05

Discussion of Results

For the tested *S. feltiae* nematodes metal ions of lead, cadmium and copper proved toxic. In the lead salt solution even five times higher nematode mortality was noticed in comparison with the control. Contact with lead also considerably decreased the nematode pathogenicity for the tested insects and nematode reproduction in the

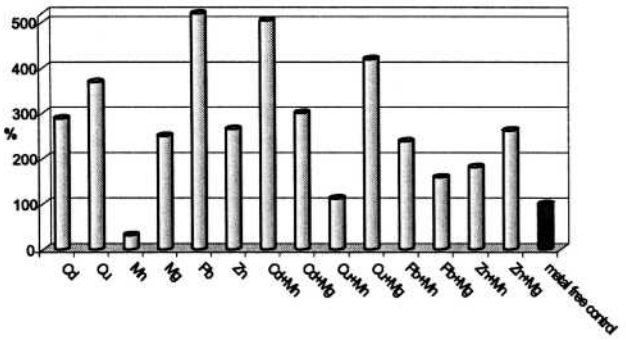

Fig. 1. Effect of heavy metal ions and their mixtures with manganese or magnesium on S. feltiae mortality as compared to the control (100%).

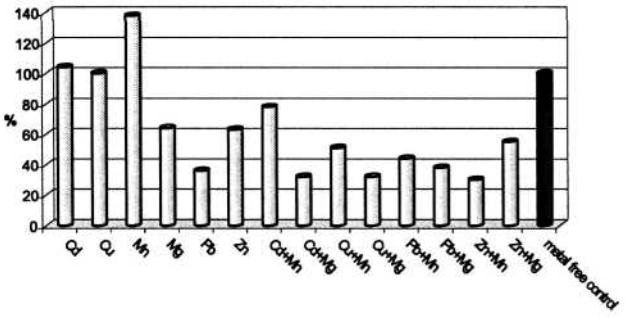

Fig. 2. Effect of heavy metal ions and their mixtures with manganese or magnesium on S. feltiae pathogenicity in % of test insect mortality as compared to the control (100%).

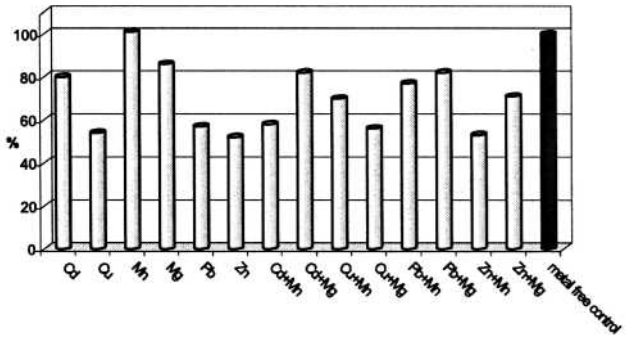

Fig. 3. Effect of heavy metal ions and teir mixtures with manganese or magnesium on S. feltiae reproduction as compared to the control (100%).

host body. While testing the effect of lead ions on *S. feltiae* and *Heterorhabditis megidis* entomopathogenic nematodes Jarmul and Kamionek [4] observed high mortality and decreased invasiveness in relation to the test insects. During their research on the effect of metal ions on *Steinemema carpocapsae* Jaworska et al. [5] found that lead, zinc and copper ions were toxic for them. In the presented study manganese revealed stimulating effect on *S. feltiae*. Jaworska and Gorczyca [6] observed a similarly positive effect on other entomopathogenic nematodes. In the presented research both manganese and magnesium neutralized a negative effect of lead on *S. feltiae* mortality. However, they did not neutralize the adverse lead effect on these nematodes pathogenicity. Jaworska and Ropek [7] observed much more positive reaction of *S. carpocapsae* and *Heterorhabditis bacteriophora* to heavy metal ion interactions with manganese or magnesium. In the experiment discussed in the paper manganese revealed protective activity towards copper. Manganese and cadmium interactions had a toxic effect and influenced 5. *feltiae* mortality, their pathogenicity and progeny production. Magnesium did not neutralize the toxic effect of cadmium on 5. *feltiae,* either.

In conclusion it may be stated that *in vitro* tests carried out in a laboratory revealed that cadmium, copper, lead and zinc affect an increase in mortality of *S. feltiae* invasive larvae, decrease their pathogenicity for the test insects and reproduction. In 400 $mg \cdot dm^{-3}$ concentration manganese Mn(II) positively affected *S. feltiae* entomopathogenic nematodes during their storage, caused a decrease in their mortality rate, improved pathogenicity and reproduction rate from the insect bodies in comparison with those kept in distilled water.

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