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The effect of silver nanoparticles on mortality and pathogenicity of entomopathogenic nematodes *Heterorhabditis bacteriophora* (Poinar, 1976) from Nematop biopreparation

Abstract

The effect of silver nanoparticles on mortality of entomopathogenic nematodes *Heterorhabditis bacteriophora* from Nematop biopreparation was studied. It was found that the mortality depends on nano-Ag concentrations and duration of larval (IJs) contact with them. In this study the effect of different concentrations of nano-Ag on pathogenicity of entomopathogenic nematodes was also studied.

Keywords and phrases: EPN, entomopathogenic nematodes, *Heterorhabditis bacteriophora*, Nematop, silver nanoparticles, nano-Ag

Introduction

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Entomopathogenic nematodes occur naturally in soil environment and contribute to harmless insect populations control [1]. Preparations made from entomopathogenic nematodes are the safest means of pest control. They are used in agriculture (crop fields, greenhouses, forest nurseries) but also in grain stores and broiler houses [2]. Insects infected by the nematodes die within several hours [3]. Application of biological insecticides may be used to control a lesser mealworm – *Alphitobius diaperinus* (Panzer, 1797). It is a dangerous pest and a vector of many diseases (e.g. Mareka, bird influenza). Most threatened group of animals are bred birds which have the contact with insects brought to farm buildings [2].

Nanotechnology has a great impact on biological sciences and more and more nanomaterials are used in medicine, pharmacy and agriculture [4, 5]. In the ionic form silver might be toxic for organisms but silver nanoparticles have a broad spectrum of biological properties even at low concentrations [6].

Material and methods

The effect of silver nanoparticles on the mortality and pathogenic properties of entomopathogenic nematodes *Heterorhabditis bacteriophora* (Poinar, 1976) was studied in experimental conditions. Colloidal silver nanoparticules was from the firm Nano-tech Polska Sp. zo.o. Silver nanoparticles suspended in deionised water in concentrations of 5 ppm and 0.5 ppm were used in the experiments. *H. bacteriophora* originated from biopreparation Nematop of the German firm E-nema.

Experiment was carried out during 5 days under laboratory conditions at a temperature of 25°C. Larvae of the 3rd invasive growth stage (IJs) were placed in water solutions containing appropriate concentration of nano-Ag. The control group consisted of larvae kept in distilled water. Samples of solution were taken and nematodes mortality was estimated every day. Tests were performed in 5 repetitions. After 5 days the nematodes that survived the contact with nano-Ag were separated by sedimentation. Nematodes *H. bacteriophora* obtained from nano-Ag solution of 5 ppm were neglected since their number was insufficient for further experiments. Live nematodes obtained in that way were used to infect various growth stages of *Alphitobius diaperinus* (four week larvae, pupae and adults).

Experiments were performed in Petri dishes of a diameter of 9 cm lined with filter paper in which 10 insects from particular growth stages were placed. Each dish received 500 IJs. Tests were made in 3 repetitions. Mortality was checked for 7 days. Dead insects were dissected to check whether nematodes were the reason of their death. The control consisted of insects in respective growth stage infected with nematodes which did not contact nano-Ag. Mortality, the extensiveness and intensity of infection of insects by *H. bacteriophora* were analysed.

The obtained results were statistically processed (chi square, ANOVA) with the SPSS 15 software. Statistical significance was tested at $p<0.05$.

Results and discussion

The mortality of entomopathogenic nematodes increased with increasing concentration of nano-Ag (Fig. 1). The highest concentration of nanoparticles (5 ppm) caused 99% mortality in *H. bacteriophora*. Lower concentration caused much lower mortality 4%. Nematodes mortality measured on the last day of experiment in the control was also 4%. In the nearest future, studies on nano-Ag accumulation in nematodes bodies are planned.

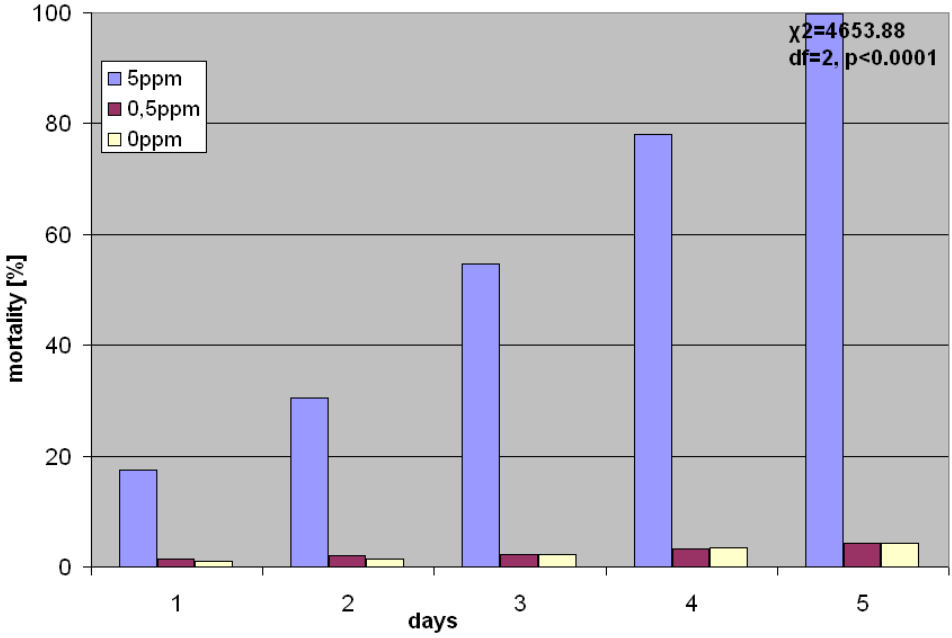

Fig. 1. The effect of nano-Ag on the mortality of the IJs of *Heterorhabditis bacteriophora* (test Chi² refers to the last day of experiment).

Entomopathogenic nematodes that contacted with different concentrations of nano-Ag solutions did not differ in their ability to kill the host *A. diaperinus* which can show that nematodes' symbiotic bacteria are immune to nano-Ag (Figs 2, 3, 4). The mortality of insects infected by *H. bacteriophora* that survived 5 days' long contact with 0.5 ppm nano-Ag was 83, 23 and 20% in larvae, pupae and adult insects respectively. The extensiveness of insect infection finally achieved 70, 10 and 13% in larvae, pupae and adult insects respectively and 97, 27 and 13% respectively in the control.

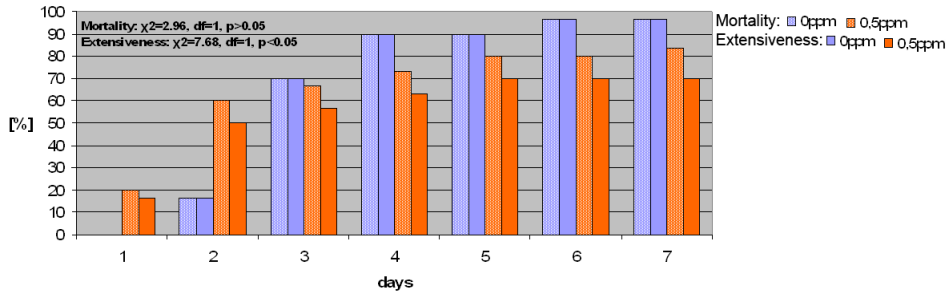

Fig. 2. The effect of nano-Ag on pathogenic properties of the nematode *Heterorhabditis bacteriophora* exposed for 5 days to solutions of various concentrations (the test of mortality percentage and extensiveness of infection of *Alphitobius diaperinus* larvae) (test Chi²).

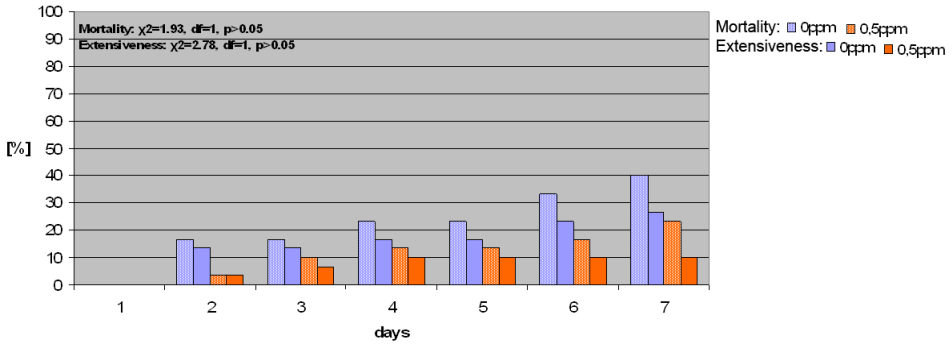

Fig. 3. The effect of nano-Ag on pathogenic properties of the nematode *Heterorhabditis bacteriophora* exposed for 5 days to solutions of various concentrations (the test of mortality percentage and extensiveness of infection of *Alphitobius diaperinus* pupae) (test Chi²).

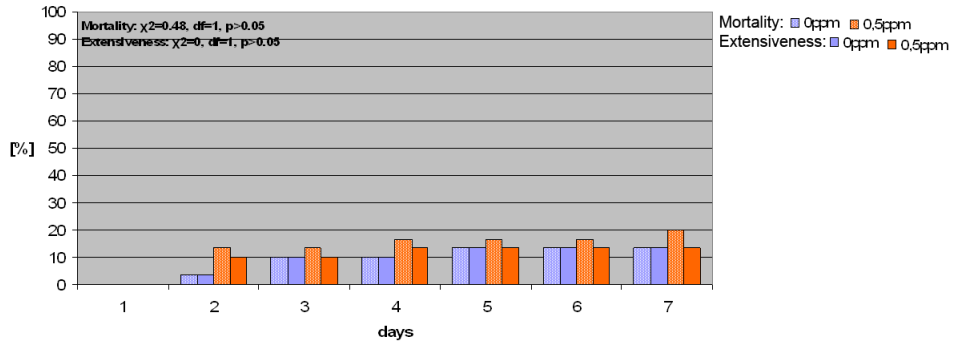

Fig. 4. The effect of nano-Ag on pathogenic properties of the nematode *Heterorhabditis bacteriophora* exposed for 5 days to solutions of various concentrations (the test of mortality percentage and extensiveness of infection of *Alphitobius diaperinus* imagines) (test Chi²).

The intensity of infection is the mean number of invasive larvae of nematodes that entered the insect and developed into the L4 form and hermaphroditic individuals (Tab. 1). The intensity of infection was 5.8, 1.13 and 1.83 in larvae, pupae and adult insects respectively at a concentration of 0.5 ppm and from 10.23 to 0.97 respectively in the control. Contribution of particular growth stages to the population structure of the parasitic generation is shown in table 2. Hermaphrodites dominated in larvae and pupae but L4 dominated in adult insects. In the control dominated hermaphrodites in adult insects.

Tab. 1. The effect of nano-Ag on the intensity of infection of *Alphitobius diaperinus* by *Heterorhabditis bacteriophora* (ANOVA).

Nematode species	Concentrations of nano-Ag	Intensity of infection (Means)			ANOVA
		larva	pupa	imago	
Heterorhabditis bacteriophora (Nematop)	5ppm	-	-	-	-
	0,5ppm	5.8	1.13	1.83	F=22.07 p<0.05
	0ppm	10.23	2.2	0.97	

Tab. 2. The effect of nano-Ag on the population structure of the parasitic generation of *Heterorhabditis bacteriophora* in *Alphitobius diaperinus*.

Nematode species	Concentrations of nano-Ag	Population structure of parasitic generation (Means)					
		larva		pupa		imago	
		Hermaphrodite	L4	Hermaphrodite	L4	Hermaphrodite	L4
Heterorhabditis bacteriophora (Nematop)	5ppm	-	-	-	-	-	-
	0,5ppm	5.67	0.13	1.13	0	0.2	1.63
	0ppm	10.23	0	2.17	0.03	0.53	0.44

Conclusions

1. The mortality of invasive larvae of *H. bacteriophora* exposed to nano-Ag depended on the concentration of nanoparticles and the time of exposure.

2. Mortality and extensiveness of infection of *A. diaperinus* were different for nematodes that contacted with nano-Ag and those from the control.

3. The intensity of infection was higher in larvae.

4. Hermaphrodites dominated in the population structure of the parasitic generation in nematodes from the larvae and pupae.

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