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Pathogenicity of entomopathogenic nematodes *Steinernema feltiae* (Filipjev, 1934) after their contact with silver nanoparticles

Abstract

The effect of silver nanoparticles on IJs of *Steinernema feltiae* from Owinema biopreparation was studied under laboratory conditions. It was found that the mortality depends on nano-Ag concentrations and duration of larval contact with them. Different concentrations of nano-Ag (0.5 and 5ppm) were toxic to the nematodes, but IJs, which survived contact with nanoparticles, were able to infect insects (*Alphitobius diaperinus*).

Keywords and phrases: EPN, entomopathogenic nematodes, *Steinernema feltiae*, Owinema, silver nanoparticles, nano-Ag

Introduction

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Entomopathogenic nematodes are important pest control agents, used in biological control [1]. They are used e.g. in farm houses (henhouses, cowsheds and pigpens) [2, 3]. Third stage (IJs) are free-living in soil. Insects infected by the IJs die within several hours [4]. Biopreparations may control a lesser mealworm – *Alphitobius diaperinus* (Panzer, 1797). It is a pest and a vector of many diseases (e.g. Gumboro, Newcastle). The most threatened group of animals are bred birds [2, 3].

Nanomaterials have become an integral component of many products. Silver nanoparticles are used in pharmacy and agriculture [5]. Nano-Ag have a broad spectrum of biological properties e.g. antibacterial [6].

Material and methods

The effect of silver nanoparticles on the mortality and pathogenic properties of entomopathogenic nematodes *Steinernema feltiae* (Filipjev, 1934) was studied in experimental conditions. Colloidal silver nanoparticules (Nano-tech Polska Sp. zo.o.) in concentrations of 5 ppm and 0.5 ppm were used in the experiments. *S. feltiae* originated from biopreparation Owinema made by OWIPLANT in Owińska near Poznań.

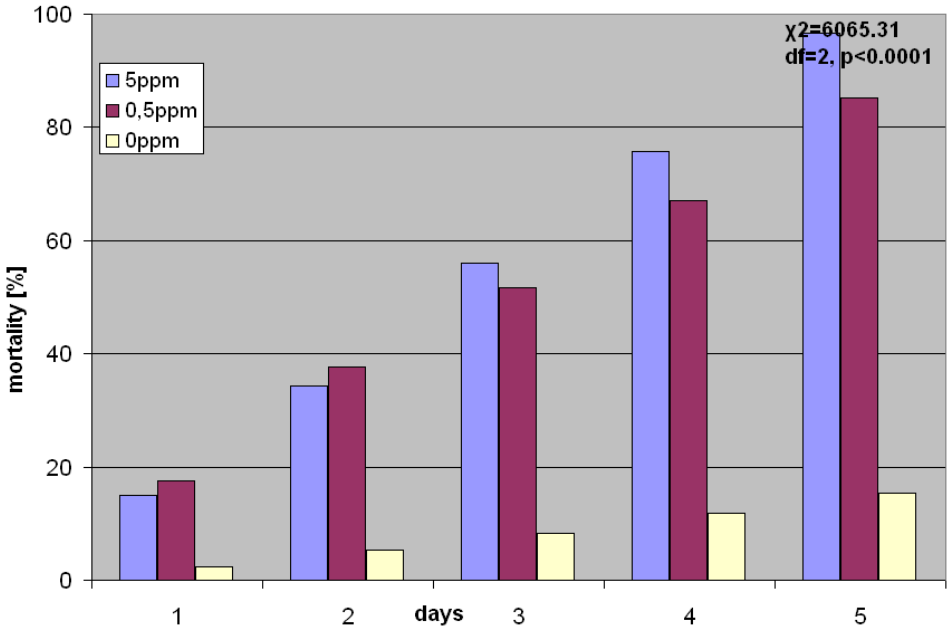
Experiment was carried out during 5 days under laboratory conditions at a temperature of 25°C. IJs were placed in water solutions containing appropriate concentration of nano-Ag. The control group consisted of larvae in distilled water. Samples of solution were taken and nematodes mortality was estimated 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 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 (diameter of 9 cm) 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 *S. feltiae* were analysed.

The obtained results were statistically processed (chi square, ANOVA). 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 97% mortality in *S. feltiae*. Lower concentration caused lower mortality (85%). Nematodes mortality meas-



ured on the last day of experiment in the control was 15%. 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 *Steinernema feltiae* (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 *S. feltiae* that survived 5 days' long contact with nano-Ag was 87, 37, 30% (for 0.5ppm) and 57, 47, 33 (for 5ppm) in larvae, pupae and adult insects respectively. The extensiveness of insect infection finally achieved 70, 13, 30% (for 0.5ppm) and 20, 33, 20% (for 5ppm) in larvae, pupae and adult insects respectively and 63, 73, 47% respectively in the control.

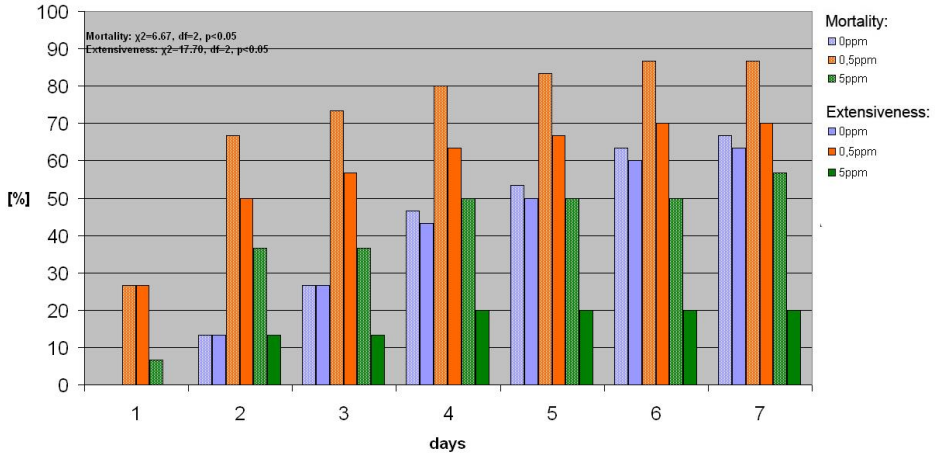


Fig. 2. The effect of nano-Ag on pathogenic properties of the nematode *Steinernema feltiae* exposed for 5 days to solutions of various concentrations (the test of mortality percentage and extensiveness of infection of *Alphitobius diaperinus* larvae) (test Chi²).

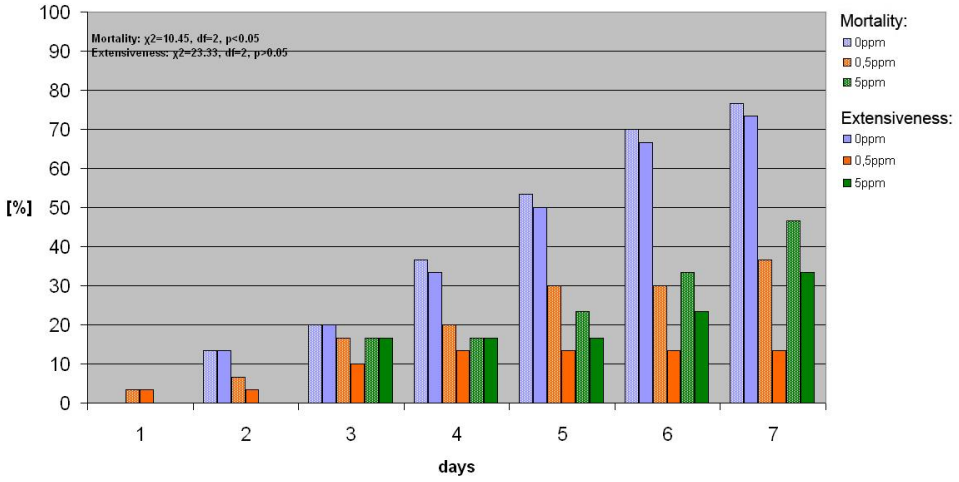


Fig. 3. The effect of nano-Ag on pathogenic properties of the nematode *Steinernema feltiae* exposed for 5 days to solutions of various concentrations (the test of mortality percentage and extensiveness of infection of *Alphitobius diaperinus* pupae) (test Chi²).

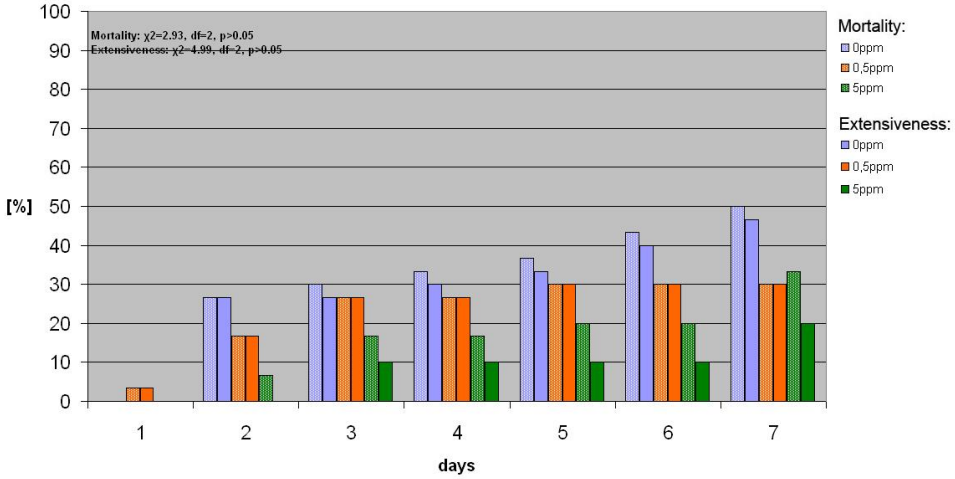


Fig. 4. The effect of nano-Ag on pathogenic properties of the nematode *Steinernema feltiae* exposed for 5 days to solutions of various concentrations (the test of mortality percentage and extensiveness of infection of *Alphitobius diaperinus* imagines) (test Chi²).

The intensity of infection is the mean number of invasive larvae of nematodes that entered the insect and developed into the L4 form, females and males individuals (Tab. 1). The intensity of infection was 4.63, 1.03, 3.43 (for 0.5ppm) and 2.13, 2.77, 1.63 (for 5ppm) in larvae, pupae and adult insects respectively. Contribution of particular growth stages to the population structure of the parasitic generation is shown in table 2. Females dominated in larvae and pupae but L4 dominated in adult insects. Males dominated only in larvae (for 5ppm).

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

Nematode species	Concentrations of nano-Ag	Intensity of infection (Means)			ANOVA
		larva	pupa	imago	
Steinernema feltiae (Owinema)	5ppm	2.13	2.77	1.63	F=23.60, p<0.05
	0,5ppm	4.63	1.03	3.43	
	0ppm	12.7	16.37	16.87	

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

Nematode species	Concentrations of nano-Ag	Population structure of parasitic generation (Means)								
		larva			pupa			imago		
		Female	Male	L4	Female	Male	L4	Female	Male	L4
Steinernema feltiae (Owinema)	5ppm	0.7	1.23	0.2	1.37	1.17	0.23	0.07	0.1	1.46
	0,5ppm	2.36	2.27	0	0.6	0.43	0	0.33	0.3	2.8
	0ppm	7.27	5.43	0	8.63	3.67	4.07	2.77	0.83	13.27

Conclusions

1. The mortality of invasive larvae of *S. feltiae* 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 the highest in the all control groups.

4. Females dominated in the population structure of the parasitic generation in nematodes from the larvae and pupae, L4 dominated in adult insects.

References

[1] G. O. Poinar, *Nematode for biological control of insects*, CRC Press, Hic., Boca Raton, Florida 1979.

[2] E. Pezowicz, *Nicienie owadobójcze jako czynnik zmniejszający liczebność populacji pleśniakowca lśniącego (Alphitobius diaperinus Panzer) w brojlerniach*, Rozprawy Naukowe i Monografie, Wydaw. SGGW, Warszawa, 2005.

[3] M. Szczepanik, *Temperature effects on the efficacy of nematodes against the lesser mealworm Alphitobius diaperinus (Coleoptera: Tenebrionidae)*, Polish Journal of Entomology 69, 2000, 483-490.

[4] R. Georgis, R. Gaugler, *Predictability in biological control using entomopathogenic nematodes*, J. Econ. Entomol. 84, 1991, 713-720.

[5] A. Myczko, *Zastosowanie nanotechnologii w praktyce rolniczej*, Inż. Rol. 2, 2006, 45-50.

[6] D. Williams, *Medical technology: How small we can go?*, Med Device Tech 4, 2002, 7-9.