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EFFECT OF SILVER NANOPARTICLES ON THE MORTALITY AND PATHOGENICITY OF ENTOMOPATHOGENIC NEMATODES

WPŁYW NANOCZĄSTEK SREBRA NA ŚMIERTELNOŚĆ I WŁAŚCIWOŚCI PATOGENNE NICIENI ENTOMOPATOGENNYCH

Abstract: The effect of silver nanoparticles on the mortality of entomopathogenic nematodes *Heterorhabditis bacteriophora* from Nematop biopreparation and *Steinernema feltiae* from Owinema biopreparation was researched. It was found that mortality depends on nano-Ag concentrations and on the time of larval contact with them. In this study the effect of different concentrations of nano-Ag on pathogenic properties of entomopathogenic nematodes was also studied. No significant differences were observed.

Keywords: entomopathogenic nematodes, *Heterorhabditis bacteriophora*, *Steinernema feltiae*, Nematop, Owinema, silver nanoparticles, nano-Ag

Entomopathogenic nematodes are the natural component of soil mesofauna and an important factor limiting insect density [1]. Steinernematidae and Heterorhabditidae are associated with mutualistic bacteria *Xenorhabdus* and *Photorhabdus*, respectively [2]. Preparations made from entomopathogenic nematodes are the safest means of pest control. Nematodes have many advantages including simple and cheap productive cultures, a wide range of hosts and safety for the environment and higher organisms [3].

The development of nanotechnologies is now being observed worldwide. Nanotechnology is a discipline dealing with particles of 1 to 100 nm (1 nm = 1×10^{-9} m) which are named nanoparticles. Nanotechnology has a great impact on biological sciences and more and more nanomaterials are used in medicine, pharmacy and agriculture [4, 5]. Silver is a noble metal whose antibacterial properties have been known since the ancient times. In the ionic form silver might be toxic for organisms but

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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) and *Steinernema feltiae* (Filipjev 1934) was studied in experimental conditions. Colloidal silver nanoparticles came 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 and *S. feltiae* came from Owinema made by OWIPLANT in Owinska near Poznan.

Experiment 1 was carried out during 5 days under laboratory conditions at a temperature of 25 ^{o}C. Larvae of the 3rd invasive growth stage (IJs) were placed in water solutions containing the 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 the caterpillars of *Galleria mellonella* of a mean weight of 140–160 mg.

Experiment 2 was performed in Petri dishes of a diameter of 9 cm lined with filter paper in which 10 insects were placed. Five hundred invasive larvae (IJs) of the appropriate nematode species were added to each dish, which made 50 IJs/insect. Tests were made in 5 repetitions. Mortality was controlled during 5 days. Dead insects were transferred to empty dishes and placed in the incubation chamber for 48 h. Then the insects were dissected to check whether nematodes were the cause of their death. The experiment was performed at 25 ^{o}C and 90 % relative humidity of the substratum. The control consisted of insects in the respective growth stage infected by nematodes which had no contact with nano-Ag. The mortality, the extensiveness and intensity of infection of *G. mellonella* larvae by two species of entomopathogenic nematodes were analyzed.

The obtained results were statistically processed with the SPSS 15.0 programme (multifactor ANOVA, Chi^2 test, Tukey test). Statistical significance was tested at $p < 0.05$.

Results and discussion

Nematodes mortality in solutions of silver nanoparticles (5 ppm, 0.5 ppm) was analysed every day during 5 days. The mortality of entomopathogenic nematodes increased with increasing concentration of nano-Ag (Figs. 1 and 2). The highest concentration of nanoparticles (5 ppm) caused 99 and 96 % mortality in *H. bacteriophora* and *S. feltiae*, respectively on the fifth day of experiment. Lower concentration

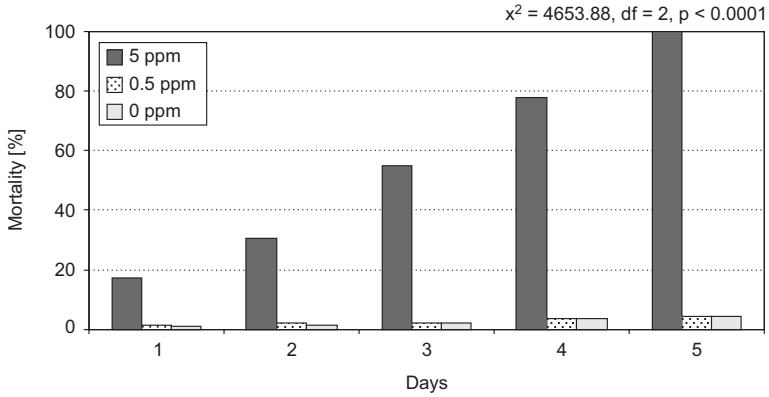

Fig. 1. The effect of nano-Ag on the mortality of the IJs of *Heterorhabditis bacteriophora* (test Chi^2 refers to the last day of experiment)

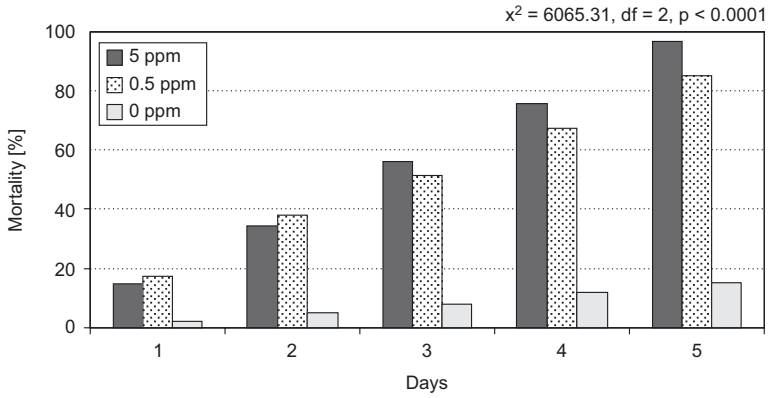

Fig. 2. The effect of nano-Ag on the mortality of the IJs of *Steinernema feltiae* (test Chi^2 refers to the last day of experiment)

caused much lower mortality of 4 % in *H. bacteriophora* but 85 % mortality in *S. feltiae*. Nematodes mortality measured on the last day of experiment in the control was 4 % in *H. bacteriophora* and 15 % in *S. feltiae*. In the nearest future, studies on nano-Ag accumulation in nematodes bodies are planned.

Entomopathogenic nematodes that contacted different concentrations of nano-Ag (5 ppm, 0.5 ppm) solutions did not differ in their ability to kill the host *G. mellonella* which can show that nematodes' symbiotic bacteria are immune to nano-Ag (Table 1). In all cases mortality and the extensiveness of infection after the contact of nematodes with nano-Ag were similar to those in the control when measured on the last day of the experiment. The mortality of insects infected by *H. bacteriophora* that survived 5 days' long contact with 0.5 ppm nano-Ag was 100 % while that in the control was 98 %. On consecutive days insects mortality grew, however, faster with Ag-treated nematodes than in the control.

Table 1

The effect of nano-Ag on pathogenic properties of the nematodes *Heterorhabditis bacteriophora* and *Steinernema feltiae* exposed for 5 days to nano-Ag solutions (test of mortality and extensiveness of infection [%] of the *Galleria mellonella* larvae) (test Chi^2)

Nematode species	Nano-Ag concentration						Chi-square test	
	5 ppm		0.5 ppm		0 ppm			
	Mortality	Extensiveness	Mortality	Extensiveness	Mortality	Extensiveness	Mortality	Extensiveness
H. bacteriophora (Nematop)								
1st day	—	—	0	0	6	2	$x^2 = 3.09$; $p > 0.05$	$x^2 = 1.010$; $p > 0.05$
2nd day	—	—	86	52	50	24	$x^2 = 19.385$; $p < 0.05$	$x^2 = 9.653$; $p < 0.05$
3rd day	—	—	96	56	90	40	$x^2 = 12.000$; $p < 0.05$	$x^2 = 4.000$; $p < 0.05$
4th day	—	—	98	56	92	42	$x^2 = 0.000$; $p > 0.05$	$x^2 = 1.010$; $p > 0.05$
5th day	—	—	100	56	98	46	$x^2 = 1.042$; $p > 0.05$	$x^2 = 2.041$; $p > 0.05$
S. feltiae (Owinema)								
1st day	7	7	7	7	13	13	$x^2 = 1.098$; $p > 0.05$	$x^2 = 1.098$; $p > 0.05$
2nd day	97	97	97	93	100	100	$x^2 = 0.225$; $p > 0.05$	$x^2 = 0.207$; $p > 0.05$
3rd day	97	97	100	97	100	100	$x^2 = 2.022$; $p > 0.05$	$x^2 = 2.022$; $p > 0.05$
4th day	100	100	100	97	100	100	$x^2 = 2.022$; $p > 0.05$	$x^2 = 2.022$; $p > 0.05$
5th day	100	100	100	97	100	100	—	—

The extensiveness of insect infection finally achieved 56 % in the experiment and 46 % in the control. The mortality of *G. mellonella* after contact with *S. feltiae* was very high and attained 100 % on the last day in all cases. Similar results were obtained for the extensiveness of infection by *S. feltiae* which contacted 5 ppm solution of nano-Ag and in the control. Slightly lower extensiveness was noted for nematodes originating from 0.5 ppm solution.

The intensity of infection is the mean number of invasive larvae of nematodes that have entered the insect and developed into the L4 form, females, males and hermaphroditic individuals in the case of Heterorhabditidae. In *H. bacteriophora* (Table 2) the intensity of infection was 2.96 at a concentration of 0.5 ppm and 1.58 in the control. Contribution of particular growth stages to the population structure of the parasitic generation is shown in table 2. Hermaphrodites dominated among the studied populations.

Table 2

The effect of nano-Ag on the intensity of infection of *Galleria mellonella* and on the population structure of the parasitic generation (*Heterorhabditis bacteriophora* and *Steinernema feltiae*) (different letters mean statistically significant differences at $p < 0.05$, Tukey test and ANOVA).

Nematode species	Concentrations of nano-Ag	Intensity of infection (Means)	ANOVA	Population structure of the parasitic generation (Means)		
				Female or herm-aphrodite	Male	L4
Heterorhabditis bacteriophora	5 ppm	—	—	—	—	—
	0.5 ppm	2.96	$F_{1.96} = 4.33$, $p < 0.05$	2.60	0	0.35
	0 ppm	1.58		1.28	0	0.30
Steinernema feltiae	5 ppm	12.27 A	$F_{2.87} = 19.44$, $p > 0.05$	8.03	4.17	0.07
	0.5 ppm	9.13 A		6.47	2.57	0.10
	0 ppm	23.70 B		15.90	7.63	0.27

The intensity of infection by *S. feltiae* was 12.27, 9.13 and 23.70 for 5 ppm, 0.5 ppm and 0 ppm nano-Ag, respectively (Table 2). Contribution of particular growth stages to the population structure of the parasitic generation is presented in Table 2. Females were the main component of the studied nematode populations.

Conclusions

1. The mortality of invasive larvae of *H. bacteriophora* and *S. feltiae* exposed to nano-Ag depended on the concentration of nanoparticles and the time of exposure.
2. Mortality and extensiveness of infection of *G. mellonellla* larvae were similar for nematodes that contacted with nano-Ag and those from the control.
3. The intensity of infection was higher in *S. feltiae*.

4. Hermaphrodites dominated in the population structure of the parasitic generation in nematodes from the family Heterorhabditidae and females dominated in nematodes from the family Steinernematidae.

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Abstrakt: Badano wpływ nanocząstek srebra na śmiertelność nicieni entomopatogennych *Heterorhabditis bacteriophora* pochodzących z biopreparatu Nematop oraz *Steinernema feltiae* pochodzących z biopreparatu Owinema. Stwierdzono, że śmiertelność ich zależy od stężenia nanocząstek srebra oraz czasu kontaktu larw z tymi roztworami. Zbadano również wpływ różnych stężeń nano-Ag na patogenność nicieni. Nie stwierdzono różnic istotnych statystycznie.

Słowa kluczowe: nicienie entomopatogenne, *Heterorhabditis bacteriophora*, *Steinernema feltiae*, Nematop, Owinema, nanocząstki srebra, nano-Ag