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# The effect of gold nanoparticles on the mortality and pathogenicity of entomopathogenic nematodes from Owinema biopreparation

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**Abstract:** The effect of gold nanoparticles on the mortality of entomopathogenic nematodes *Steinernema feltiae* from Owinema biopreparation was studied. The effect of different concentrations of nano-Au on pathogenic properties of entomopathogenic nematodes was also studied. It was found that mortality depended on nano-Au concentrations and on the time of larval contact with them.

**Key words***:* entomopathogenic nematodes, *Steinernema feltiae*, Owinema, gold nanoparticles, nano-Au.

## Introduction

Biopreparations based on entomopathogenic nematodes are considered safe plant protection measures against pests. They are used e.g. in broiler houses to control the lesser mealworm (*Alphitobius diaperinus* Panzer 1797) – a beetle of the family Tenebrionidae. It is a dangerous pest and a vector of many diseases (Geden et al., 1987, Pezowicz 2005). Two families of nematodes: Steinernematidae and Heterorhabditidae play a key role in the control of pest insects. Their invasive stage possesses many features which determine commercial value of nematodes as a biological control (Kowalska 2006).

Nanotechnology is a discipline that deals with production and application of structures whose size is expressed in nanometres. Nanomaterials have some biological properties. The main aim of nanotechnology is to use these properties in various scientific disciplines and in everyday life (Myczko 2006). Already in the ancient times people believed in miraculous power and therapeutic properties of gold. Now, gold nanoparticles are used e.g. in medicine, cosmetology and agriculture (www.nano-tech.pl).

## Material and methods

The effect of gold nanoparticles (firm Nano-tech Polska Sp. zo.o.) on the mortality and pathogenic properties of entomopathogenic nematodes *Steinernema feltiae* (Filipjev 1934) (Owinema made by the firm OWIPLANT in Owińska) was studied in experimental conditions. Gold nanoparticles suspended in deionised water in concentrations of 5 ppm, 2 ppm and 0.5 ppm were used in experiments.
The experiment 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-Au. 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-Au were separated by sedimentation. Live nematodes obtained in that way were used to infect the four week old larvae and adult insects of *Alphitobius diaperinus*.
Next experiment 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) were added to each dish, which made 50 IJs/insect. Tests were made in 3 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 control consisted of insects in the respective growth stage infected by nematodes which had no contact with nano-Au. The mortality and the extensiveness of infection of *A. diaperinus* were analysed.
The obtained results were statistically processed with the SPSS 15.0 software ($Chi^2$ test). Statistical significance was tested at $p<0.05$.

## Results and discussion

Mortality of entomopathogenic nematodes was observed to increase with the increase of nano-Au concentrations (Fig. 1). The highest concentration of nanoparticles (5 ppm) caused 78% mortality in *S. feltiae* on the fifth day of experiment. Lower concentrations (0.5 ppm) caused much lower mortality of only 9%. Mortality in the control on the last day of experiment was 15%.

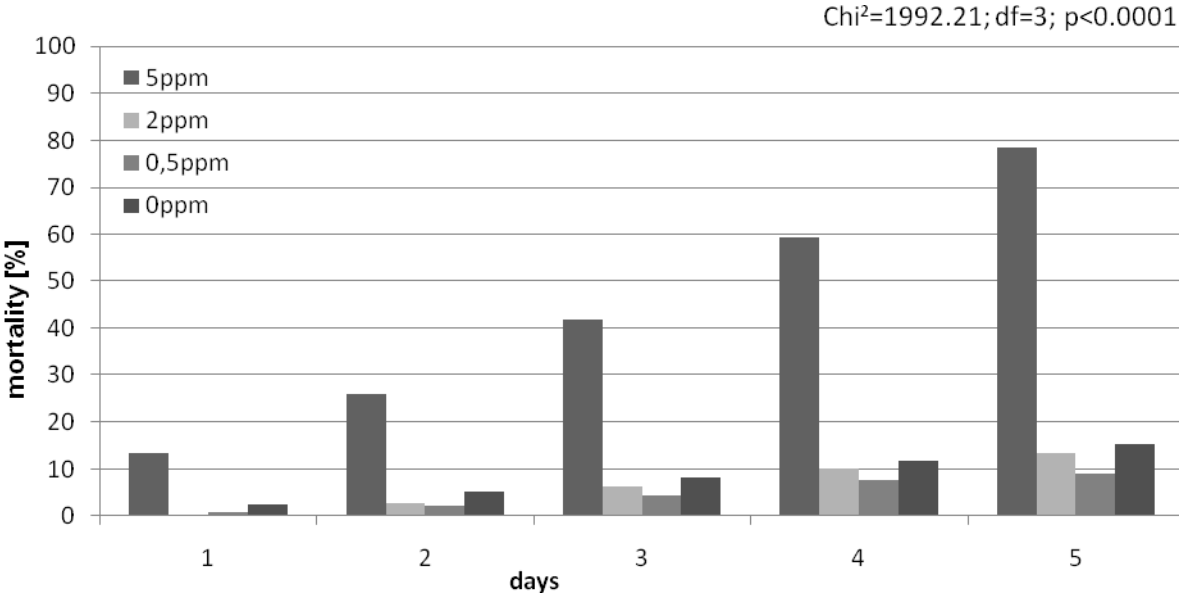


Figure 1. The effect of nano-Au on mortality of the IJs of *Steinernema feltiae* (test $Chi^2$ refers to the last day of experiment).

Various concentrations of nano-Au (5 ppm, 2 ppm, 0.5 ppm) to which the IJs of entomopathogenic nematodes were subjected did not significantly affect their ability to kill *A. diaperinus* larvae (Fig. 2). Mortality and extensity of infection after the contact of nematodes with nano-Au (5 ppm) on the last day of experiment were 93% and 17%, respectively. Lower

mortality was noted at lower concentrations of nanoparticles and in the control while the extensity of infection was highest in the control (50%) and similar (40%) at concentrations of 2 ppm and 0.5 ppm.

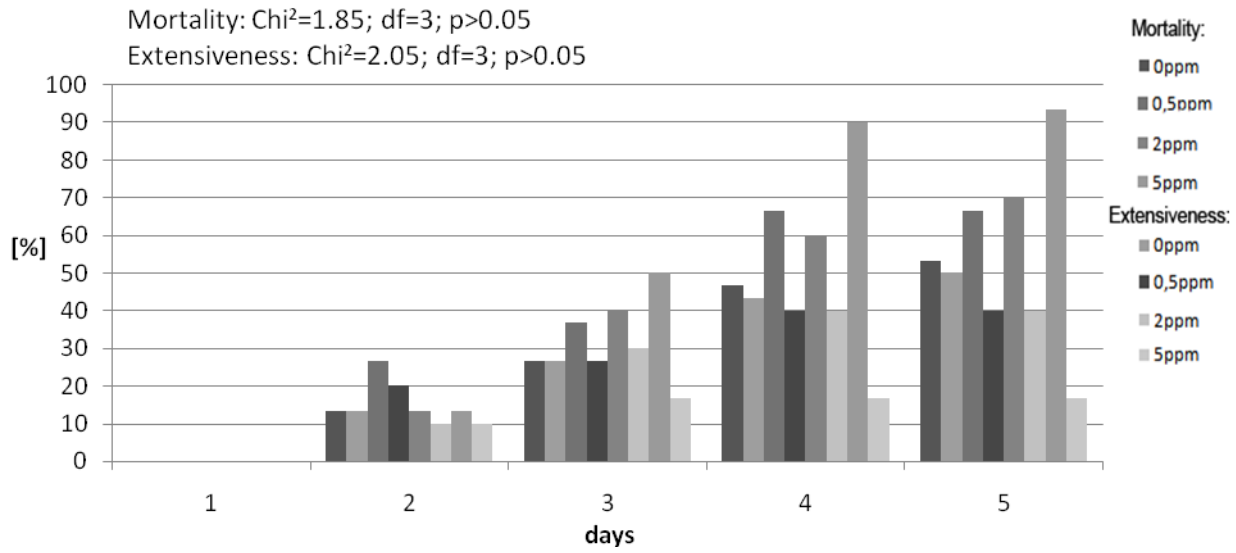


Figure 2. The effect of nanoparticles on pathogenic properties of the nematodes *Steinernema feltiae* exposed for 5 days to nano-Au solutions (test of mortality and extensiveness of infection (%) of the *Alphitobius diaperinus* larvae) (test $Chi^2$).

Negative effect was found of analysed solutions of nano-Au on pathogenic properties of *S. feltiae* to imagines of *A. diaperinus*. Mortality and extensity of infection was 0% in this case.

## Conclusions

1. Mortality of invasive larvae of *S. feltiae* depends on concentrations of nano-Au and on the time of exposition.
2. Mortality of *A. diaperinus* after the contact of nematodes with nano-Au is higher than in the control.
3. The extensity of infection of *A. diaperinus* larvae after the contact of nematodes with nano-Au is highest in the control group (50%) and lowest at a concentration of 5 ppm (16%).
4. Invasive larvae of nematodes treated with various concentrations of nano-Au do not kill adult beetles.

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