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Nanoparticles of copper and entomopathogenic nematodes *Steinernema feltiae* (Filipjev, 1934) in reducing the number of the lesser mealworm beetle *Alphitobius diaperinus* (Panzer, 1797)

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Abstract: *Nanoparticles of copper and entomopathogenic nematodes Steinernema feltiae (Filipjev, 1934) in reducing the number of the lesser mealworm beetle Alphitobius diaperinus (Panzer, 1797).* Antibacterial properties of metal nanoparticles are well documented and known, but its potential use in agriculture as an anti pest agents – not. Possible negative or positive reactions with popular integrated pest management (IPM) methods need to be checked and verified. The effect of copper nanoparticles on the mortality of entomopathogenic nematodes (EPNs) *Steinernema feltiae* from Owinema biopreparation was tested. This biopreparation is being used against serious agricultural pests. It was found that mortality of nematodes depends on nano-Cu concentrations and on the length of contact of *Steinernema* larvae with nano-Cu solution. In this study the effect of different concentrations of nanoparticles on pathogenic properties of entomopathogenic nematodes – mortality of pest beetle *Alphitobius diaperinus* (Panzer, 1797) infected by EPNs and extensiveness of infection, was also studied. It showed that the high concentrations of Cu may decrease abilities of EPNs to enter, grow and proliferate inside the host body.

Key words: entomopathogenic nematodes, *Steinernema feltiae*, Owinema, *Alphitobius diaperinus*, lesser mealworm beetle, copper nanoparticles

INTRODUCTION

Entomopathogenic nematodes (EPNs) are different nematode species, that during their development display pathogenicity against insects (Brzeski and Sandner 1974). Two nematode families (Steinernematidae and Heterorhabditidae) are being used as biological control agents on a commercial scale (Kowalska 2006). Invasive larvae of EPNs live together, in mutualistic relationship, with bacteria from the Enterobacteriacae family. These microorganisms use nematodes as vectors, that inject them into the insect bodies. Finally, bacteria kill the new host (Martens et al. 2003). EPNs are used in poultry houses to control the lesser mealworm beetle *Alphitobius diaperinus* (Tenebrionidae). It is a pest of bred birds and a vector of many diseases, example Marek's, Gumboro and Newcastle diseases (Lancaster and Simco 1967, De la Casas et al. 1972, De la Casas et al. 1976, Geden et al. 1987,

Avancini and Ueta 1990, Despins et al. 1994, Goodwin and Waltman 1996, Steelman 1996, Pezowicz 2005, Ignatowicz 2008, Ignatowicz 2009, Chernaki-Leffer et al. 2010, Walldorf et al. 2012).

History of nanotechnology starts in 1950s. With use of nanoparticle materials it is possible to create, for example, new biopreparations with different biochemical properties. "Nano" scale in connection with huge reactive surface of nanoparticles causes that they are characterized by high biochemical reactivity, even at low concentrations (Myczko 2006). Nanocolloidal copper is being used in cosmetics, household, industry, medicine and in agriculture (www.nanotech.pl).

MATERIAL AND METHODS

The effect of copper nanoparticles (firm Nano-tech Polska sp. z o.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. Copper nanoparticles suspended in deionized water in concentrations of 5, 2 and 0.5 ppm were used in the experiments.

The experiment was carried out during 5 days under laboratory conditions at a temperature of 25 ±1°C. Larvae of the 3rd invasive growth stage (IJs) were placed in water solutions containing the appropriate concentration of nano-Cu. 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-Cu were separated by sedimentation. The sedimentation did not, however, allow for complete removing of chemical compounds from the sample. Live nematodes obtained in that way and the residues of chemical substances were used to infect the four-week larvae and the adult insects of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae).

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-Cu. The mortality and the extensiveness of infection of *A. diaperinus* were analyzed.

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

With increasing concentrations of nano-Cu, higher mortality of entomopathogenic nematodes was observed (Fig. 1). The highest concentration of nanoparticles (5 ppm) caused 48% mortality in *S. feltiae* in the fifth day of experiment. The lowest concentration (0.5 ppm) caused much lower death rate – 10%. Mortality in the control group, in the last day of experiment, averaged 15%. Wang et al. (2009) showed that the nano-Al_2O_3, nano-ZnO i nano-TiO_2 affected reduction of body length of *C. elegans* and the number of eggs produced by nematodes. In contrast, Oberdörster et al. (2005), Jones et al. (2008) and Ma et al. (2011) found high toxicity of nano-ZnO and nano-TiO_2. The above-mentioned high concentration of nanoparticles and hydroxylated fullerenes also negatively affect nematode survival (Wang et al. 2009, Cha et al. 2012). In Poland, the impact of nanocolloidal silver and copper on EPNs was studied by Kuźniar (2009). He found that nano-Ag in concentrations ranging from 5 to 50 mg/dm^3 were highly toxic to nematodes *S. feltiae* from biopreparations: Owinema, Nemasys, Nemaplus. After 2 h of direct contact of IJs with different concentrations of nano-Ag he reported almost 100% mortality. A different situation was related to nano-Cu, in nematodes from biopreparations Owinema and Nemasys. In these nematodes 100% survival was observed even at the highest concentration of compound (50 mg/dm^3). However, in the case of nematodes from Nemaplus biopreparа-

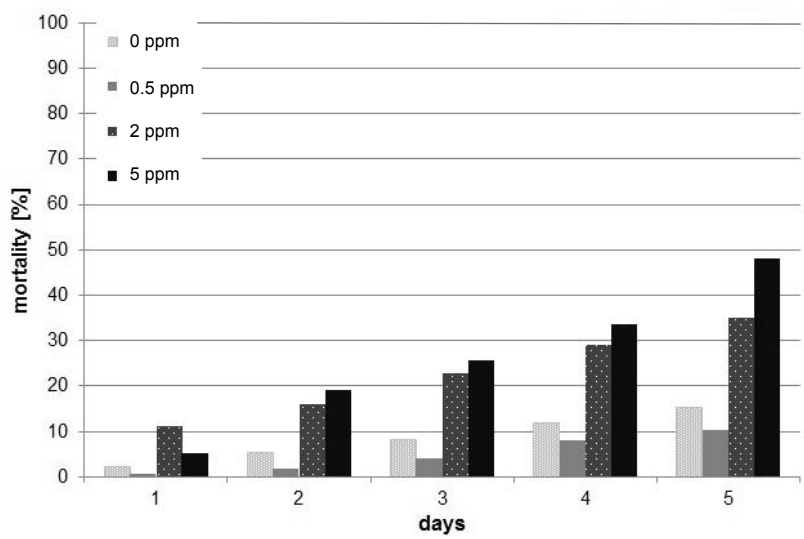

FIGURE1. The effect of nano-Cu on the mortality of the IJs of *Steinernema feltiae* (test Chi^2 refers to the last day of experiment – Ch^2 = 2002.2, df = 3, p < 0.001)

tion, at the highest concentration, larval mortality was approximately at 29%.

Different concentrations of nano-Cu solutions (5, 2, 0.5 ppm), with which IJs had contact, did not influence their abilities to kill larvae of *A. diaperinus*. This may give evidence that nematode mutualistic bacteria are not vulnerable to nano-Cu (Fig. 2). Mortality and extensity of infection, after the contact of EPNs with nano-Cu (2 ppm) in the last day of experiment, averaged 90 and 56% respectively. Lower death rate was observed in lower concentrations of the solution (0.5 ppm) and in a control group. In both cases it reached 53%. The extensity (13%) was lower after the contact of EPNs with nano-Cu. Mortality and extensity of infection of adult beetles was the highest in control group (36 and 33% respectively). After the contact of EPNs with nanoparticles, death rate averaged 3 and 10% and extensity of infection – 0 and 3% (Fig. 3). Kuźniar (2009) found that nematodes *S. feltiae* from Owinema biopreparation, that have been exposed to solutions of nano-Cu concentrations ranging from 1 to 50 mg/dm^3, caused mortality of larvae *Tenebrio molitor* ranging from 40 to 80%.

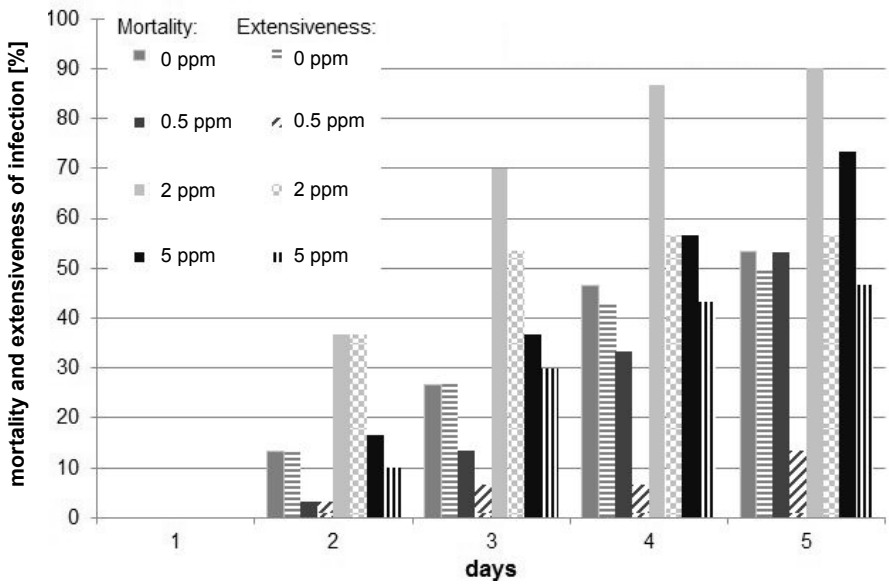

FIGURE 2. The effect of nanoparticles on pathogenic properties of the nematodes *Steinernema feltiae* exposed for 5 days to nano-Cu solutions. Tests Chi^2 of mortality ($Ch^2 = 1.3$, $df = 3$, $p > 0.05$) and extensiveness of infection ($Ch^2 = 6.1$, $df = 3$, $p > 0.05$) of the *Alphitobius diaperinus* larvae

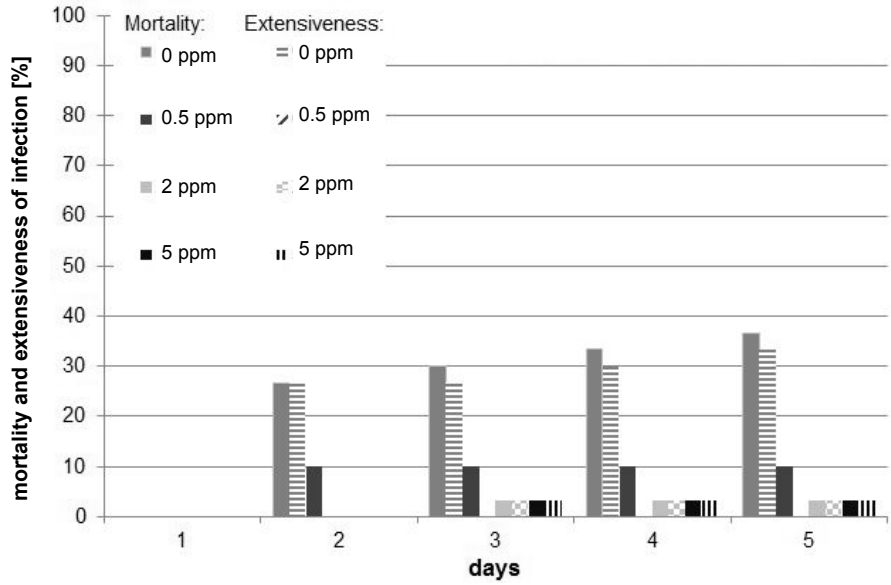

FIGURE 3. The effect of nanoparticles on pathogenic properties of the nematodes *Steinernema feltiae* exposed for 5 days to nano-Cu solutions. Tests Chi^2 of mortality (Ch^2 = 14.03, df = 3, $p < 0.01$) and extensiveness of infection (Ch^2 = 12.22, df = 3, $p < 0.01$) of the *Alphitobius diaperinus* imagines

CONCLUSIONS

1. Mortality of invasive larvae of *Steinernema feltiae* depends on concentrations of nano-Cu and on the time of exposition.
2. Mortality of *Alphitobius diaperinus* larvae after the contact of nematodes with nano-Cu is higher than in the control.
3. The extensity of infection of *A. diaperinus* larvae after the contact of nematodes with nano-Cu is the highest at 2 ppm (56%), and the lowest at a concentration of 0.5 ppm (13%).
4. Invasive larvae of nematodes treated with various concentrations of nano-Cu cause low mortality (3 and 10%) and extensity of infection (0 and 3%) in adult beetles. In the control group mortality and extensiveness of infection are 37 and 33%.

REFERENCES

AVANCINI R.M.P, UETA M.T., 1990: Manure breeding insects (Diptera and Coleoptera) responsible for cestoidosis in caged layer hens. J. Appl. Entomol. 110, 307–312.

BRZESKI M., SANDNER H., 1974: Zarys nematologii. PWN, Warszawa.

CHA Y.J., LEE J., CHOI S.S., 2012: Apoptosis – mediated in vivo toxicity of hydroxylated fullerene nanoparticles in soil nematode *Caenorhabditis elegans*. Chemosphere. 87, 49–54.

CHERNAKI-LEFFER A.M., KUTTEL J., MARTINS L.M., PEDROSO A.C., ASTOLFI--FERREIRA C.S., FERREIRA F., FERREIRA A.J., 2010: Vectorial competence of

larvae and adults of *Alphitobius diaperinus* in the transmission of *Salmonella* Enteritidis in poultry. Vector Borne and Zoonotic Diseases 10 (5), 481–487.

De La CASAS E., HAREIN P.K., DESHMUKH D.R., POMEROY B.S., 1976: Relationship between the lesser mealworm, fowl pox, and Newcastle disease virus in poultry. J. Econ. Entomol. 69 (6), 775–779.

De La CASAS., POMEROY B.S., HAREIN P.K., 1972: Infection and quantitative recovery of *Salmonella typhimurium* and *Escherichia coli* from within the lesser mealworm, *Alphitobius diaperinus* (Panzer). Poult. Sci. 47 (6), 1871–1875.

DESPINS J.L., AXTELL R.C., RIVES D.V., GUY J.S., FICKEN M.D., 1994: Transmission of enteric pathogens of turkeys by darkling beetle larvae (*Alphitobius diaperinus*). J. Appl. Poultry Res. 3, 61–65.

GEDEN C.J., ARENDS J.J., AXTELL R.C., 1987: Field trials of *Steinernema feltiae* (Nematoda: Steinernematidae) for control of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in commercial broiler and turkey houses. J. Econ. Entomol. 80 (1), 136–141.

GOODWIN M.A., WALTMAN W.D., 1996: Transmission of *Eimeria*, viruses, and bacteria to chicks: Darkling beetles (*Alphitobius diaperinus*) as vector of pathogens. J. Appl. Poult. Res. 5, 51–55.

IGNATOWICZ S., 2008: Pleśniakowiec lśniący w fermach drobiarskich i jego zwalczanie. Polskie Drobiarstwo 12, 42–44.

IGNATOWICZ S., 2009: Pleśniakowiec lśniący jego szkodliwość i zwalczanie. Bydło 6, 78–81.

JONES N., RAY B., RANJIT K.T., MANNA A.C., 2008: Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiol. Lett. 279, 71–76.

KOWALSKA J., 2006: Wzajemne powiązania pomiędzy nicieniami owadobójczymi, owadami i bakteriami oraz ich wykorzystanie w praktyce. Wiadomości Parazytologiczne 52 (2), 93–98.

KUŹNIAR T. 2009: Wpływ nanocząsteczkowej miedzi i srebra na żywotność i patogeniczność owadobójczych nicieni. Monografia 2009: Wielokierunkowość badań w rolnictwie i leśnictwie. Wyd. Uniwersytetu Rolniczego w Krakowie 1, 347–354.

LANCASTER J.L., SIMCO J.S., 1967: Biology of the lesser mealworm, a suspected reservoir of avian leucosis. Report series (University of Arkansas (Fayetteville campus). Agricultural Experiment Station). Fayetteville.

MA H., KABENGI N.J., BERTSCH P.M., UNRINE J.M., GLENN T.C., WILLIAMS P.L., 2011: Comparative phototoxicity of nanoparticulate and bulk ZnO to a free – living nematode *Caenorhabditis elegans*: The importance of illumination mode and primary particle size. Environ. Pollut. 159, 1473–1480.

MARTENS E.C., HEUNGENS K., GOODRICH-BLAIR H., 2003: Early colonization events in the mutualistic association between *Steinernema carpocapsae* nematodes and *Xenorhabdus namatophila* bacteria. J. Bacteriol. 185, 3147–3154.

MYCZKO A., 2006: Zastosowanie nanotechnologii w praktyce rolniczej. Inż. Rol. 2, 45–50.

OBERDÖRSTER G., OBERDÖRSTER E., OBERDÖRSTER J., 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect. 113 (7), 823–839.

PEZOWICZ E., 2005: Nicienie owadobójcze jako czynnik zmniejszający liczebność populacji pleśniakowca lśniącego (*Alphitobius diaperinus* Panzer) w brojlerniach. Rozprawy Naukowe i Monografie. Wyd. SGGW w Warszawie, Warszawa, 91.

STEELMAN D., 1996: Darkling beetles are costly pests. Poul. Digest. 55, 22–23.

WALLDORF V., MEHLHORN H., Al-QURAISHY S., Al-RASHEID K.A.S., ABDEL-GHAFFAR F., MEHLHORN J., 2012: Treatment with a neem seed extract (MiteStop®) of beetle larvae parasitizing the plumage of poultry. Parasitol. Res. 110, 623–627.

WANG H., WICK R. L., XING B., 2009: Toxicity of nanoparticulate and bulk ZnO, Al2O3 and TiO2 to the nematode *Caenorhabditis elegans*. Environ. Pollut. 157 (4), 1171–1177.

Streszczenie: *Nanocząstki miedzi i nicienie entomopatogeniczne Steinernema feltiae (Filipjev, 1934) w ograniczaniu liczebności pleśniakowca lśniącego Alphitobius diaperinus (Panzer, 1797).* Właściwości antybakteryjne nanocząsteczek metali są dość dobrze znane, jednak ich możliwe wykorzystanie w rolnictwie, do zwalczania szkodliwych bezkręgowców, już nie. Pozytywne i negatywne interakcje z popularnymi, zintegrowanymi metodami zwalczania szkodników (IPM) powinny zostać sprawdzone i zweryfikowane. Zbadano wpływ nanocząsteczek miedzi na śmiertelność nicieni entomopatogenicznych *Steinernema feltiae* pochodzących z biopreparatu Owinema. Stwierdzono, że śmiertelność ich zależy od stężenia nanocząsteczek miedzi oraz czasu kontaktu larw z tymi roztworami. Zbadano również wpływ różnych stężeń nano-Cu na patogeniczność nicieni – śmiertelność zainfekowanych chrząszczy *Alphitobius diaperinus* (Panzer, 1797), ekstensywność infekcji. Wykazano, że w wyższych stężeniach Cu nicienie mogą mieć mniejszą zdolność do penetrowania, wzrostu i rozmnażania się w ciele gospodarza.

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