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Control of the lesser mealworm *Alphitobius diaperinus* using entomopathogenic nematodes (EPNs) combined with nanoparticles

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Abstract: *Control of the lesser mealworm* Alphitobius diaperinus *using entomopathogenic nematodes (EPNs) combined with nanoparticles.* We examined the efficacy of entomopathogenic nematodes (EPNs), which were in contact with nanoparticles, in the control of *A. diaperinus*. Treatments were performed in laboratory conditions and consisted of one of the four species and strains of EPNs *Steinernema feltiae* and *Heterorhabditis bacteriophora*, which earlier were exposed to Ag, Au or Cu nanoparticles. All three development stages of the beetle were exposed to different EPNs. The mortality, the extensity, the intensity of infection of beetles were studied for 7 days. Most of nematodes, that survived contact with nanoparticles, developed in *A. diaperinus* larvae, pupae and adults. Significant differences were found in the sensitivity and susceptibility to penetration by parasites to various growth stages of the host. The most studied nematodes and nanoparticles caused a high mortality and the extensity of infection in host larvae, from 12 to 100% and from 8 to 83%, respectively. A negative effect of gold nanoparticles on the mortality was observed in adult insects infected by *S. feltiae* (Owinema). Despite this, in many cases, the addition of nanoparticles may increase efficiency of EPNs, used in the integrated pest control.

Key words: *Steinernema*, *Heterorhabditis*, pest, poultry houses, Ag-NPs, Au-NPs, Cu-NPs

INTRODUCTION

The lesser mealworm (*Alphitobius diaperinus* Panzer, 1797) is a beetle of the family Tenebrionidae. It is a vector of many disease factors like fungi (*Aspergillus* sp., *Fusarium roseum*), viruses causing Mareka, Gumboro and Newcastle disease, bird flu and enteritis, bacteria (mainly of the genera *Escherichia*, *Salmonella*, *Bacillus*, *Streptococcus*), protozoans (*Eimeria* sp.) and tapeworm larvae (*Raillietina* sp., *Choanotaenia* sp.) (De la Casas et al. 1976, Chernaki-Leffer et al. 2010). Pathogen transmission takes place when chickens eat infected insects. Lesser mealworm beetles have shown resistance to many insecticides, for example with pyrethroids (Lambkin and Rice 2006). Increased disease incidence and the mortality and the loss of body weight in chickens is observed during mass appearance of pests in broiler houses. Insects eat their way into the tissues of weakened, ill or dead animals. Chickens are in constant move, do not rest and are exhausted. Adult insects

found in apartments pose a direct risk to humans. The lesser mealworm as a pest of grain stores eats food products and contaminates them with exuvia, dead individuals, excreta and faeces, bacteria and fungi. Feeding beetles affect the quality of food products by making them wet, mouldy, unpleasant in smell and taste (De la Casas et al. 1976, Chernaki-Leffer et al. 2010).

Entomopathogenic nematodes (EPNs) are one of the most promising biological control methods to fight *A. diaperinus*. Infective juveniles (IJs) of the nematodes penetrate body of the insect. Inside, they release mutualistic bacteria that kill the host and after that, they develop and reproduce, giving one to three generations. New IJs return to the soil to find another host (Laznik and Trdan 2013).

More and more nanomaterials are being used in medicine, pharmacy and agriculture. Nanotechnology is a promising discipline which may have a broad application in the pest control. "Nano" dimensions in combination with large active surface area, neutral valence, make nanoparticles biologically active already at very low concentrations. Using nanoparticles one may produce preparations of various biochemical properties. Nanocolloidal silver, gold and copper are used in cosmetics, household, industry, medicine and agriculture (Myczko 2006). They can act as antibacterial and antifungal agents and they are being used in production of plant growth stimulants (Karimi et al. 2010, Kim et al. 2012). Still it is not known if nanoparticles have any synergistic or antagonistic influence on popular biological control agents, such as EPNs (Kucharska et al. 2011).

Although tests have been performed with EPNs on *A. diaperinus* in laboratory conditions (Szalanski et al. 2004, Pezowicz 2005), there are no reports showing influence of both, EPNs and nanoparticles on pests. Our hypothesis was that nanoparticles, used for disinfection in broiler houses, may affect entomopathogenic properties of EPNs.

MATERIAL AND METHODS

The effect of silver, gold and copper nanoparticles on pathogenic properties of entomopathogenic nematodes *Heterorhabditis bacteriophora* (Poinar, 1976) and *Steinernema feltiae* (Filipjev, 1934) were studied in experimental conditions. Analyzed parameters for *A. diaperinus* were: the mortality – percent of dead insects in the last day of experiment, the extensity of infection – percent of dead insects with nematodes discovered after dissection and the intensity of infection – number of nematodes found in dead host.

Colloidal solutions of nanoparticles were from Nano-Tech Polska Sp. z o.o. (Poland). Solutions of nanoparticles suspended in deionised water at concentrations of 0.5, 2 and 5 ppm were used in experiments. *Heterorhabditis bacteriophora* originated from biopreparations: Nematop (E-nema, Germany) and Larvanem (Koppert, The Netherlands), while *Steinernema feltiae* was from biopreparations: Owinema (OWIPLANT,

Poland) and Entonem (Koppert, The Netherlands).

Larvae of EPNs were placed in water solutions containing the respective concentrations of nanosilver, nanogold and nanocopper. Control group consisted of IJs kept in distilled water. After 7 days the nematodes that survived contact with nanocolloids were separated by sedimentation. Multiple sedimentation did not, however, allow for complete removing of chemical compounds from the sample. Particular growth stages of *A. diaperinus* were exposed to the residues of chemical substances and to EPNs that survived seven-day contact with these substances. Experiments involved the analysis of pathogenic properties of IJs whose mortality after the contact with nanoparticles (at concentrations of 0.5, 2 and 5 ppm) was above 90%. So obtained alive nematodes were used to infect three growth stages of the lesser mealworm: four-week larvae, pupae and adult beetles. The experiment was carried out on Petri dishes 9 cm in diameter lined with filter paper where 10 insects in appropriate growth stage were placed. Excluding control group, 900 insects were used in experiment for one insect growth stage. Three repetitions of every variant were made, including: three different nanoparticles (Ag, Au, Cu) in three different concentration (0.5, 2, 5 ppm), four nematode strains. Five hundred IJs of appropriate nematode species were introduced onto Petri dish (50 IJs/1 insect). Because of 100% mortality or low survival (above 90%) of IJs (Nematop, Owinema) after the contact with nano-Ag (2 and 5 ppm), studying their effect on the mortality, the extensity and the intensity of infection of all growth stages of the lesser mealworm was omitted. For the same reason the effect of IJs (Larvanem, Entonem) on *A. diaperinus* after the contact with silver nanoparticles at a concentration of 5 ppm was also not analysed. The mortality was controlled every 24 h for 7 days. Dead insects were transferred to empty dishes and placed in the incubation chamber for 48 h. Later, the insects were sectioned to check whether nematodes and associated mutualistic bacteria were the cause of their death. Experiments were performed at 25°C and 85–90% relative moisture of the substratum. The control consisted of insects of respective growth stage infected by IJs deprived of the contact with nanoparticles. The mortality, the extensity and the intensity of infection of *A. diaperinus* larvae, pupae and adult beetles were studied.

Obtained results were statistically processed with SPSS 15.0 and SAS 9.2 software. ANOVA was used to estimate the significance of differences in: the mortality, the extensity and the intensity of infection of *A. diaperinus*. Statistical significance was tested at $P < 0.05$. Analysis of variance was followed by Tukey post-hoc test to compare the differences between means.

RESULTS DISCUSSION

Based on experiments presented in this paper it was found that nanoparticles of silver, gold and copper in different concentrations may positively increase nematode pathogenicity. The mortality of the lesser mealworm larvae after the contact with EPNs from Owinema preparation and at concentrations of nano-Ag 0.5 ppm, nano-Au 5 ppm and nano-Cu 2 ppm was 83, 100 and 97%, respectively (Table 1). The extensity of infection of insects was lower or equal to the mortality. Only in one case, after using nematodes from the Owinema preparation and nano-Cu at a concentration of 0.5 ppm, the extensity of adults infection was 0% while the mortality was much higher (Table 3). This may be associated with a possibility of killing the host by bacteria released from alimentary tract of only one IJ or with releasing microorganisms and limiting further growth of nematodes. All above mentioned situations made finding the presence of EPNs during the insect dissection impossible. Nematodes of the Owinema biopreparation (at all concentrations of nano-Au and at 2 ppm of nano-Cu) were not pathogenic to adults. Insect mortality was 0% (Table 3). Significant differences were found in the sensitivity and susceptibility to penetration by IJs to various growth stages of the lesser mealworm. The mortality, the extensity and the intensity of infection of larvae (Table 1) and pupae (Table 2) of *A. diaperinus* by pathogenic nematodes *S. feltiae* from Owinema and Entonem previously treated with nano-Au at a concentration of 0.5 ppm may serve as an example. Most studied nematodes and nanoparticles (at all concentrations) caused a high mortality and the extensity of infection in mealworm larvae. At simultaneous application of EPNs and the remains of nano-Ag, nano-Au and nano-Cu, larval mortality was 12–93, 44–100 and 67–100%, respectively, and the extensity of infection was 8–73, 30–69 and 30–83%, respectively (Table 1). A negative effect of nanoparticles on the intensity of infection was observed in adults of *A. diaperinus* infected by *S. feltiae* (Owinema, nano-Cu, 5 ppm). Mean number of nematodes in an adult was 0.1 (Table 3).

Most nematodes were found to preserve their invasive abilities when IJs that survived seven-day contact with various nanoparticles were placed on filter paper in Petri dish with insects. One cannot, however, unambiguously state whether the studied IJs would be able to find a host in the natural environment. This was impossible in view of a direct contact of IJs with insects in a Petri dish. However, despite the action of various chemicals used in broiler houses for disinfection, mutualistic bacteria associated with nematodes survived in their alimentary tract. These microorganisms are food source for EPNs and stimulate their development. This information is very important since it determines the application of EPNs as enemies of *A. diaperinus* in broiler houses. Literature data presents the results of experiments exploring the possibility of this pest control in houses for bird produc-

TABLE 1. Pathogenic properties of entomopathogenic nematodes (percentage mortality, extensity and intensity of infection of *A. diaperinus* larvae) which contacted nanoparticles of silver, gold and copper in various concentrations (n = 30 in every variant of experiment)

Trade name	Active substance	Nematode species	Permanent contact of entomopathogenic nematodes with nanoparticles														
			A. diaperinus larvae														
			mortality (%) ±*SD*					extensity of infection (%) ±*SD*					intensity of infection (nematodes per host) ±*SD*				
			K	A	B	C	ANOVA	K	A	B	C	ANOVA	K	A	B	C	ANOVA
Silver Water	nano-Ag (Ag-NPs)	*H. bacteriophora* (Nematop)	97 ±6	93 ±6			$p = 0.52$	97^{b} ±6	73^{a} ±6			$p = 0.008$	11.0 ±8.9	7.7 ±9.4			$p = 0.16$
		H. bacteriophora (Larvanem)	80 ±35	70 ±44	12 ±11		$p = 0.09$	65 ±42	65 ±49	8 ±11		$p = 0.16$	2.3^{a} ±2.5	8.7^{b} ±7.9	1.2^{a} ±4.9		$p < 0.0001$
		S. feltiae (Owinema)	70 ±26	83±15			$p = 0.49$	67 ±21	67 ±11			$p = 1.00$	13.4^{b} ±18.4	4.2^{a} ±5.3			$p = 0.01$
		S. feltiae (Entonem)	66 ±23	66 ±31	22 ±11		$p = 0.10$	60 ±35	51 ±44	11 ±10		$p = 0.22$	5.3 ±5.9	3.3 ±4.4	2.1 ±6.7		$p = 0.10$
Gold Water	nano-Au (Au-NPs)	*H. bacteriophora* (Nematop)	97 ±6	80 ±10	57 ±30	57 ±15	$p = 0.07$	97^{b} ±6	60^{ab} ±17	43^{a} ±21	37^{a} ±23	$p = 0.01$	11.0^{b} ±8.9	7.5^{ab} ±9.4	4.6^{a} ±8.4	4.6^{a} ±8.7	$p = 0.02$
		H. bacteriophora (Larvanem)	80 ±35	67 ±30	44 ±15	52 ±21	$p = 0.40$	65 ±42	55 ±45	37 ±21	31 ±26	$p = 0.57$	2.3^{a} ±2.5	7.6^{b} ±7.9	4.9^{ab} ±7.6	3.1^{a} ±5.5	$p = 0.007$
		S .feltiae (Owinema)	70 ±26	90 ±10	90 ±0	100 ±0	$p = 0.14$	67 ±21	53 ±6	50 ±10	30 ±20	$p = 0.10$	13.4^{b} ±18.4	6.9^{ab} ±9.2	6.2^{ab} ±9.3	3.9^{a} ±9.4	$p = 0.02$
		S. feltiae (Entonem)	66 ±23	77 ±40	81 ±20	47 ±21	$p = 0.48$	60 ±35	65 ±35	69 ±17	32 ±15	$p = 0.34$	5.3^{a} ±5.9	9.0^{b} ±8.1	4.9^{a} ±4.4	3.8^{a} ±6.3	$p = 0.01$
Copper Water	nano-Cu (Cu-NPs)	*H. bacteriophora* (Nematop)	97 ±6	87 ±6	100 ±0	90 ±10	$p = 0.12$	97^{b} ±6	67^{ab} ±21	83^{ab} ±11	57^{a} ±6	$p = 0.02$	11.0^{b} ±8.9	7.3^{ab} ±7.5	5.8^{a} ±5.6	3.1^{a} ±4.0	$p = 0.0002$
		H. bacteriophora (Larvanem)	80 ±35	92 ±11	77 ±32	80 ±35	$p = 0.90$	65 ±42	68 ±30	67 ±42	35 ±25	$p = 0.67$	2.3 ±2.5	5.3 ±6.0	5.8 ±6.3	3.7 ±6.0	$p = 0.06$
		S. feltiae (Owinema)	70 ±26	80 ±10	97 ±6	90 ±0	$p = 0.20$	67 ±21	30 ±20	70 ±17	70 ±0	$p = 0.05$	13.4^{b} ±18.4	1.6^{a} ±3.4	12.2^{b} ±18.0	5.6^{ab} ±6.5	$p = 0.002$
		S. feltiae (Entonem)	66 ±23	76 ±40	70 ±36	67 ±15	$p = 0.97$	60 ±35	44 ±25	62 ±35	62 ±21	$p = 0.81$	5.3^{a} ±5.9	2.4^{a} ±3.8	11.2^{b} ±11.6	7.2^{ab} ±6.6	$p = 0.0002$

Different small letters denote significant differences at $p < 0.05$ within each nematode species and at various concentrations of nanoparticles – Tukey test. K – control (nematodes from distilled water without nanoparticles). Concentrations of nanoparticles: A – 0.5 ppm, B – 2 ppm, C – 5 ppm. For some concentrations of nano-Ag, study of the mortality, the extensity and the intensity of infection was impossible, due to almost 100% mortality of nematodes.

TABLE 2. Pathogenic properties of entomopathogenic nematodes (percentage mortality, extensity and intensity of infection of *A. diaperinus* pupae) which contacted nanoparticles of silver, gold and copper in various concentrations (n = 30 in every variant of experiment)

Trade name	Active substance	Nematode species	Permanent contact of entomopathogenic nematodes with nanoparticles														
			A. diaperinus pupae														
			mortality (%) ±*SD*					extensity of infection (%) ±*SD*					intensity of infection (nematodes per host) ±*SD*				
			K	A	B	C	ANOVA	K	A	B	C	ANOVA	K	A	B	C	ANOVA
Silver Water	nano-Ag (Ag-NPs)	*H.bacteriophora* (Nematop)	47 ±11	27 ±11			$p = 0.10$	33 ±15	17 ±6			$p = 0.15$	2.7 ±5.0	2.2 ±5.6			$p = 0.70$
		H.bacteriophora (Larvanem)	32 ±25	33 ±15	14 ±15		$p = 0.39$	22 ±15	12 ±11	9 ±10		$p = 0.44$	2.1 ±4.4	1.8 ±4.9	0.7^{a} ±2.3		$p = 0.37$
		S.feltiae (Owinema)	83 ±6	43 ±25			$p = 0.05$	73^{b} ±15	17^{a} ±15			$p = 0.01$	16.4^{b} ±19.7	2.7^{a} ±7.9			$p = 0.0008$
		S. feltiae (Entonem)	67^{B} ±25	30^{A} ±10	11^{A} ±10		$p = 0.01$	56 ±35	11 ±10	10 ±10		$p = 0.06$	14.9^{b} ±16.1	2.7^{a} ±9.2	3.1^{a} ±10.1		$p = 0.0002$
Gold Water	nano-Au (Au-NPs)	*H.bacteriophora* (Nematop)	47^{B} ±11	43^{B} ±6	20^{A} ±10	17^{A} ±6	$p = 0.005$	33^{b} ±15	27^{ab} ±6	13^{ab} ±6	7^{a} ±6	$p = 0.03$	2.7 ±5.0	4.7 ±11.4	1.8 ±6.0	0.2 ±0.6	$p = 0.09$
		H.bacteriophora (Larvanem)	32 ±25	39 ±10	11 ±10	12 ±6	$p = 0.10$	22 ±15	12 ±6	7 ±11	10 ±10	$p = 0.36$	2.1 ±4.4	4.0 ±11.0	1.9 ±7.4	0.7 ±2.1	$p = 0.34$
		S.feltiae (Owinema)	83 ±6	47 ±25	47 ±11	60 ±30	$p = 0.18$	73^{b} ±15	3^{a} ±6	7^{a} ±11	23^{a} ±11	$p = 0.0003$	16.4^{b} ±19.7	0.5^{a} ±2.7	0.3^{a} ±1.0	0.9^{a} ±1.9	$p < 0.0001$
		S. feltiae (Entonem)	67^{B} ±25	33^{AB} ±15	10^{A} ±17	16^{A} ±11	$p = 0.02$	56^{b} ±35	8^{a} ±6	4^{a} ±6	6^{a} ±6	$p = 0.02$	14.9^{b} ±16.1	3.0^{a} ±11.6	3.6^{a} ±19.7	1.2^{a} ±4.7	$p = 0.001$
Copper Water	nano-Cu (Cu-NPs)	*H.bacteriophora* (Nematop)	47 ±11	50 ±10	50 ±26	33 ±15	$p = 0.60$	33 ±15	10 ±10	10 ±0	10 ±10	$p = 0.06$	2.7^{b} ±5.0	0.7^{ab} ±2.2	0.8^{ab} ±3.0	0.4^{a} ±1.2	$p = 0.02$
		H.bacteriophora (Larvanem)	32 ±25	23 ±6	14 ±6	15 ±15	$p = 0.44$	22 ±15	12 ±6	11 ±10	11 ±10	$p = 0.43$	2.1 ±4.4	0.7 ±2.0	0.7 ±2.1	1.9 ±5.9	$p = 0.36$
		S.feltiae (Owinema)	83^{B} ±6	27^{A} ±15	20^{A} ±10	20^{A} ±10	$p = 0.0002$	73^{b} ±15	17^{a} ±11	3^{a} ±6	3^{a} ±6	$p < 0.0001$	16.4^{b} ±19.7	1.4^{a} ±4.1	1.7^{a} ±9.5	0.1^{a} ±0.5	$p < 0.0001$
		S. feltiae (Entonem)	67^{B} ±25	22^{A} ±21	16^{A} ±15	9^{A} ±17	$p = 0.03$	56^{b} ±35	16^{ab} ±15	12^{ab} ±11	4^{a} ±6	$p = 0.05$	14.9^{b} ±16.1	1.9^{a} ±4.9	1.8^{a} ±4.7	1.9^{a} ±10.4	$p < 0.0001$

Different small letters denote significant differences at $p < 0.05$ within each nematode species and at various concentrations of nanoparticles – Tukey test. K – control (nematodes from distilled water without nanoparticles). Concentrations of nanoparticles: A – 0.5 ppm, B – 2 ppm, C – 5 ppm. For some concentrations of nano-Ag, study of the mortality, the extensity and the intensity of infection was impossible, due to almost 100% mortality of nematodes.

TABLE 3. Pathogenic properties of entomopathogenic nematodes (percentage mortality, extensity and intensity of infection of *A. diaperinus* adults) which contacted nanoparticles of silver, gold and copper in various concentrations ($n = 30$ in every variant of experiment)

Trade name	Active substance	Nematode species	Permanent contact of entomopathogenic nematodes with nanoparticles														
			A. diaperinus adults														
			mortality (%) ±*SD*					extensity of infection (%) ±*SD*					intensity of infection (nematodes per host) ±*SD*				
			K	A	B	C	ANOVA	K	A	B	C	ANOVA	K	A	B	C	ANOVA
Silver Water	nano-Ag (Ag-NPs)	*H.bacteriophora* (Nematop)	13 ±6	13 ±15			$p = 1.00$	13 ±6	10 ±10			$p = 0.64$	1.1 ±3.0	1.7 ±6.7			$p = 0.64$
		H.bacteriophora (Larvanem)	11 ±10	10 ±10	5 ±6		$p = 0.87$	10 ±10	9 ±10	3 ±6		$p = 0.59$	1.1 ±3.8	1.9 ±6.0	1.4 ±7.7		$p = 0.87$
		S.feltiae (Owinema)	50^{B} ±10	30^{A} ±0			$p = 0.03$	43 ±15	27 ±6			$p = 0.15$	16.3^{b} ±32.0	3.4^{a} ±8.2			$p = 0.04$
		S. feltiae (Entonem)	45^{B} ±15	9^{A} ±17	12^{A} ±11		$p = 0.04$	9 ±0	7 ±11	8 ±6		$p = 0.82$	14.2 ±46.2	3.4 ±13.0	2.6 ±9.9		$p = 0.21$
Gold Water	nano-Au (Au-NPs)	*H.bacteriophora* (Nematop)	13 ±6	73 ±46	73 ±46	63 ±40	$p = 0.25$	13 ±6	57 ±40	53 ±45	57 ±42	$p = 0.44$	1.1^{a} ±3.0	2.1^{a} ±2.4	4.5^{ab} ±7.5	11.2^{b} ±20.3	$p = 0.002$
		H.bacteriophora (Larvanem)	11^{A} ±10	67^{B} ±6	64^{B} ±25	65^{B} ±35	$p = 0.04$	10^{a} ±10	48^{b} ±15	38^{ab} ±6	54^{b} ±21	$p = 0.02$	1.1^{a} ±3.8	2.4^{a} ±3.0	5.0^{ab} ±7.0	10.9^{b} ±14.9	$p < 0.0001$
		S.feltiae (Owinema)	50 ±10	0 ±0	0 ±0	0 ±0	$p < 0.0001$	43 ±15	–	–	–	–	16.3 ±32.0	–	–	–	–
		S. feltiae (Entonem)	45 ±15	56 ±15	56 ±6	66 ±11	$p = 0.35$	9 ±0	27 ±25	44 ±11	47 ±15	$p = 0.07$	14.2 ±46.2	7.0 ±12.8	8.0 ±11.6	11.1 ±14.2	$p = 0.48$
Copper Water	nano-Cu (Cu-NPs)	*H.bacteriophora* (Nematop)	13^{A} ±6	57^{AB} ±30	100^{B} ±0	17^{A} ±11	$p = 0.0006$	13^{ab} ±6	43^{b} ±21	87^{c} ±11	7^{a} ±6	$p = 0.0002$	1.1^{a} ±3.0	3.1^{a} ±4.6	6.1^{b} ±6.7	0.4^{a} ±1.7	$p < 0.0001$
		H.bacteriophora (Larvanem)	11^{A} ±10	65^{AB} ±21	89^{B} ±17	16^{A} ±11	$p = 0.0006$	10^{a} ±10	48^{b} ±21	67^{b} ±21	9^{a} ±0	$p = 0.005$	1.1^{a} ±3.8	4.4^{a} ±5.6	6.1^{b} ±5.7	1.1^{a} ±3.4	$p < 0.0001$
		S.feltiae (Owinema)	50^{B} ±10	13^{A} ±15	0^{A} ±0	10^{A} ±17	$p = 0.006$	43^{b} ±15	0^{a} ±0	–	7^{a} ±11	$p < 0.001$	16.3^{b} ±32.0	0^{a} ±0	–	0.1^{a} ±0.6	$p < 0.0001$
		S. feltiae (Entonem)	45^{AB} ±15	14^{A} ±6	90^{B} ±17	11^{A} ±10	$p = 0.0002$	9^{a} ±0	10^{a} ±0	88^{b} ±15	8^{a} ±6	$p = 0.001$	14.2 ±46.2	7.7 ±24.7	9.3 ±6.4	3.3 ±12.7	$p = 0.48$

Different small letters denote significant differences at $p < 0.05$ within each nematode species and at various concentrations of nanoparticles – Tukey test. K – control (nematodes from distilled water without nanoparticles). Concentrations of nanoparticles: A – 0.5 ppm, B – 2 ppm, C – 5 ppm. For some concentrations of nano-Ag, study of the mortality, the extensity and the intensity of infection was impossible, due to almost 100% mortality of nematodes. In some cases, caused by zero mortality of insects, extensity and intensity of infection could not be calculated. They were marked with dash sign.

tion and breeding. Fungi and nematodes were used alone as a method of biological control (Gindin et al. 2009). Insect mortality ranged from 63 to 87% and parasites remained in the substratum for ca 7 weeks (Geden et al. 1987). The number of offspring IJs obtained from 16 adults per 1 m^2 decreased the population density of beetles by even 50% (Szalanski et al. 2004). Pezowicz (2005) found a high efficiency of the lesser mealworm control by using nematodes and entomopathogenic fungi simultaneously. The effect was two times more efficient compared with single invasions.

Entomopathogenic nematodes that contacted with various chemical substances developed in all studied growth stages of insects. As those isolated from areas of various industrial pollution, they showed biological activity (Pezowicz et al. 2008). The latter means an ability of IJs to infect an insect (the intensity of infection), its population (the extensity of infection) and to reproduce in the host's body. The activity is affected by many environmental and ecological factors: natural resistance and physiological status of insects, physical, chemical and biotic conditions, and population density of the host. Probably, chemical substances described above may stimulate surviving EPNs which was reflected in high values of studied parameters. Koppenhöfer et al. (2000) also noted the increased number of IJs attached to grubs treated with neonicotinoids preparations. Results of other studies on IJs of *S. carpocapsae* (Weiser, 1955) from areas heavily polluted with SO_2 and NO_x showed that the nematodes caused over 90% mortality in *G. mellonella* (L.) caterpillars on the fifth day of experiment and *H. megidis* (Poinar, Jackson, Klein 1987) was the reason of over 60% mortality in the same insects. Both parasite species developed in the caterpillar's body, the intensity of infection was 4.2 and 6.4, respectively (Pezowicz et al. 2008). Various strains of *S. feltiae* from Polish areas of different lead pollution showed a high mortality and the extensity of infection (from 97 to 100%) of *G. mellonella* caterpillars while the intensity of infection was from 10 to 22 (Matuska and Kamionek 2011).

Species of different insect orders are prone to the infection by EPNs in various ways. The same is true for species that belong to the same family. Most susceptible are the caterpillars of Lepidoptera. Lepidopteran pupae and dipteran maggots are less susceptible to infection. Only 30% of *Ostrinia nubilalis* (Hubner, 1796) pupae were infected by *S. carpocapsae* compared with 100% infection of larvae and adults (Lewis and Raun 1978). Various sheaths and thecae are mechanical barriers hampering penetration of IJs to the host's body. Relatively resistant to infection are also adult beetles. It was found that adult beetles of *A. diaperinus* were not infected by *H. heliothidis* (Poinar, 1979) whereas their larvae and pupae were susceptible (LD_{50} equal 26 and 36, respectively). *Steinernema glaseri* (Steiner, 1929), however, infected only the adult lesser mealworms but all growth stages were susceptible to infection by *S. carpocapsae* (LD_{50} equal 9–56) (Geden et al.

1985). Adult beetles of *Curculio caryae* (Horn, 1873) were more prone to the infection by various strains of *S. carpocapsae* as compared with larvae (Shapiro-Ilan et al. 2003). Ramos-Rodriguez et al. (2006) found that larvae of various species of beetles – pests in corn stores – were more susceptible to infection by *S. feltiae* than adult individuals. Similar observations were made by Kakouli-Duarte et al. (1997) and Rumbos and Athanassiou (2012) in insects *Sphenophorus* spp. (Pallas, 1776), *Otiorhynchus sulcatus* (Fabricius, 1775), *Hylobius abietis* L. and *Tribolium confusum* Jacquelin du Val., 1863. The sensitivity of insects to nematode infection decreased with age, an effect associated with well-developed chitin external skeleton which was a mechanical barrier hampering penetration of EPNs (Rumbos and Athanassiou 2012). Kuźniar (2009) found that nematodes from biopreparations Owinema, Nemasys and Nemaplus which contacted with nano-Cu caused a high mortality in *Tenebrio molitor* L. larvae – from 40% (Owinema, concentration 1 mg/dm^3) to 95% (Nemaplus, concentrations 1 and 10 mg/dm^3). The contact of EPNs (Owinema, Nemasys, Nemaplus and Nematop) with multi-walled carbon nanotubes did not deprive nematodes of their pathogenic properties. The mortality of the yellow mealworm larvae caused by the mentioned above IJs species exceeded 80% (Kuźniar et al. 2011). The extensity of infection of the larvae may be determined by the size and availability of natural body openings like e.g. stigmas, by mobility and the amount of kairomones released to the external environment (Pezowicz 2005).

Despite being used in low doses (50 IJs per insect), the IJs caused the mortality/the extensity of infection at various levels. Pezowicz (2005) did not find mortality in *A. diaperinus* at so low dose of nematodes. The author had to increase the number of IJs from 50 to 2,000 individuals per insect. The extensity and the intensity of infection depend on the defence mechanisms in hosts. Moreover, the intensity of infection is determined by control mechanisms operating within parasite population. The number of nematodes developing in haemocoel determines the number of IJs recovered from an insect. Low intensity of infection may also contribute to a low number of IJs leaving the host's body (Laznik et al. 2010, Laznik and Trdan 2015).

CONCLUSIONS

Use of nanoparticles in agriculture, as antibacterial and antifungal agents, does not affect negatively EPNs pathogenicity under laboratory conditions. Instead of that, addition of nano particles of Ag, Au or Cu may slightly increase the efficacy of nematodes. Further experiments are needed in field trials to verify the observed effects.

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Streszczenie: *Ograniczanie liczebności pleśniakowca lśniącego* Alphitobius diaperinus *nanocząstkami i nicieniami entomopatogenicznymi.* Trzy stadia rozwojowe *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) zostały zarażone czterema gatunkami i szczepami nicieni entomopatogenicznych *Steinernema feltiae* i *Heterorhabditis bacteriophora*, które wcześniej miały kontakt z nanocząstkami srebra, złota i miedzi. Przez 7 dni badano śmiertelność, ekstensywność i intensywność zarażenia chrząszczy. Większość badanych EPNs i nanocząstek powodowała wysoki poziom śmiertelności i dużą ekstensywność zarażenia larw gospodarza, odpowiednio od 12 do 100% oraz od 8 do 83%. Zaobserwowano również negatywny wpływ nicieni *S. feltiae* (Owinema) i nanocząstek złota na śmierelność dorosłych owadów. Mimo to, w wielu przypadkach dodatek nanocząstek może zwiększać skuteczność działania biopreparatów na bazie EPNs. Nanocząstki mogę być również stosowane w integrowanym zwalczaniu szkodników.

Słowa kluczowe: *Steinernema*, *Heterorhabditis*, szkodnik, brojlernia, nanosrebro, nanozłoto, nanomiedź

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