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Compatybility of entomopathogenic nematodes with active substances of popular biocidal products, in controlling of the lesser mealworm beetle – *Alphitobius diaperinus* (Panzer, 1797)

KORNELIA KUCHARSKA, BARBARA ZAJDEL, ELŻBIETA PEZOWICZ, JOANNA JARMUŁ-PIETRASZCZYK
Faculty of Animal Sciences, Warsaw University of Life Sciences – SGGW

Abstract: *Compatybility of entomopathogenic nematodes with active substances of popular biocidal products, in controlling of the lesser mealworm beetle* – Alphitobius diaperinus *(Panzer, 1797)*. Different species of entomopathogenic nematodes (*Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema affine*, *S. carpocapsae and S. carpocapsae* DD-136) and insecticides (chitin synthesis inhibitors – Baycidal WP 25 and pyrethroid – Solfac 10 WP) were used against larvae of *Alphitobius diaperinus* (Panzer, 1797) in a series of laboratory experiments. Four week larvae of the lesser mealworm were exposed to various species of EPNs (entomopathogenic nematodes), which survived contact with insecticides and to the residues of chemical substances. Mortality of *A. diaperinus* and the extensity of infection was assessed weekly. This study indicates that entomopathogenic nematodes and insecticides could serve as a more environmentally friendly integrated pest management method against harmful pests like *A. diaperinus* compared with conventional methods. The results of this study can be used as a basic information for the application of IPM (integrated pest management) in broiler houses.

Key words: IPM, integrated pest management, *Alphitobius diaperinus*, EPNs, entomopathogenic nematodes, insecticides

INTRODUCTION

The needs of modern farming and livestock production make environmental protection and the production of organic food the priority tasks. Chemical methods to combat harmful organisms increasingly give way to biological and integrated pest control methods (known as integrated pest management – IPM).

Alphitobius diaperinus (Panzer, 1797) (Coleoptera: Tenebrionidae) is an economically important pest in broiler houses and other livestock housing, warehouses and storages of food products and in the cellars. Increased mortality of birds is noted during the mass occurrence of pests in broiler houses. *Alphitobius diaperinus* is a vector of many pathogens like viruses (causing Mareka, Gumboro and Newcastle diseases, bird flu and enteritis), fungi (*Aspergillus* sp., *Fusarium roseum*), bacteria (*Escherichia* sp., *Salmonella* sp., *Bacillus* sp., *Streptococcus* sp.), protozoans (*Eimeria* sp.) and tapeworm larvae (*Raillietina* sp., *Choanotaenia* sp.) (De la Casas et al. 1976, Chernaki-Leffer et al. 2010). In addition, the larvae of lesser mealworm beetle feed on the eggs and larvae of advantageous beetle (*Carcinops pumilio*), which significantly reduces the population of *Musca domestica* in broiler houses (Watson et al. 2001). The larvae of *A. diaperinus* burrow tunnels in the insulating material of buildings and destroy

wood and foamed polystyrene elements reducing thermal insulation properties of buildings by up to 30% (Chernaki-Leffer et al. 2010).

Currently, organophosphorus insecticides, synthetic pyrethroids and insect growth regulators are mainly used to control *A. diaperinus*. However, the insecticides do not totally eliminate insect populations because they do not reach into all the gaps, in which the pests can hide. Moreover, individuals resistant to chemicals appear in isolated populations (Lambkin 2005). Therefore, the development and demonstration of alternative integrated pest management methods are needed. Several non-chemical control methods have been developed against lesser mealworm beetle, but all of them have limitations (Pezowicz 2005).

Entomopathogenic nematodes of the Steinernematidae and Heterorhabditidae (Rhabditida) families are lethal parasites associated with symbiotic bacteria in the *Enterobacteriacae*. Steinernematids are associated with *Xenorhabdus* spp. and heterorhabditids with *Photorhabdus* spp. They are characterized by a high tolerance for insecticides and other chemicals. Studies confirmed the possibility of parallel application of biological and chemical means, which increases the severity of disease processes and accelerates the death of pests. Thus, entomopathogenic nematodes offer an environmentally safe and IPM compatible alternative. Selection based on two factors also prevent the development of resistance in the population of insects treated with IPM methods (Koppenhöfer et al. 2000b).

In the simultaneous infection of *A. diaperinus* by nematodes *Steinernema feltiae* (Owinema) (Filipjev) and entomopathogenic fungi *Beauveria bassiana* (Naturalis – L) (Vuill.) or *Metarhizium anisopliae* (Metsch.) it was found that the mortality of beetles depended on various factors (the species of entomopathogenic organism, dose of bioinsecticides, developmental stage of an insect, the type of substrate). The mortality of larvae on straw after the simultaneous contact with nematodes and fungi *B. bassiana* or *M. anisopliae* was about 70 or 20%, but in the case of imagines it was 50 or 48%, respectively (Pezowicz 2005).

In this research, we studied the combined effect of five species of entomopathogenic nematodes *Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema affine*, *S. carpocapsae* and *S. carpocapsae* DD-136) with three doses of two insecticides (chitin synthesis inhibitors – Baycidal WP 25, and pyrethroid – Solfac 10 WP). The hypothesis was that EPNs (entomopathogenic nematodes which survived contact with insecticides) and the residues of insecticides act as a stressors and cause high mortality of larvae of *A. diaperinus*. A stressor is defined as any stimulus that disrupts the homeostasis of an organism. Our objectives were: to determine the susceptibility of IJs (infective juveniles) to different doses of insecticides (the recommended dose, dose 10 times higher than recommended and dose 10 times lower than recommended); to determine the susceptibility of larvae of *A. diaperinus* to strains of entomopathogenic nematodes and the residues of insecticides.

MATERIAL AND METHODS

Insect and nematodes

Alphitobius diaperinus originated from a broiler farm located near Warsaw, Poland. Lesser mealworm beetles were cultured in 10 five-litre plastic boxes that contained 1 kg of artificial medium (Sante wheat bran, Dr Oetker yeast and apple halves). Wheat mediums were the most common culture diets in published lesser mealworm rearing systems, particularly wheat enriched with yeasts. Each box contained pupation substrate (cotton wool), in which the final instar larvae could burrow and pupate. The boxes had a plastic lid with a 10 × 5 mm vent and organdy cloth sealed between the lid and box, which closed the vent while allowing passage of air. The insects were stored under laboratory conditions at temperature 30°C, humidity 70%. The optimum temperature and humidity for lesser mealworm is 32°C and 70% RH, respectively (Steinkraus et al. 1991). Four-week larvae of the lesser mealworm beetle were used in experiments.

Heterorhabditis bacteriophora (origin: Warsaw University of Life Sciences – SGGW), *H. megidis* (origin: Warsaw University of Life Sciences – SGGW), *Steinernema affine* (origin: University of Kiel), *S. carpocapsae* (origin: Warsaw University of Life Sciences – SGGW) and *S. carpocapsae* strain DD-136 (origin: University of Kiel) were cultured at 25°C in last – instar larvae of the greater wax moth *Galleria mellonella* (L.) according to procedures described by Kaya and Stock (1997). Infective juveniles emerging within the first 2–6 days were collected from White traps and stored at 4°C for 2 weeks. Before use in the laboratory experiments, nematodes were acclimatized to room temperature for at least 2 h.

Insecticides

Triflumuron, 2-chloro-N-[[4 (trifluoromethoxy)phenyl]carbamoyl]benzamide (chitin synthesis inhibitors) was obtained as a wettable powder with 25% active ingredient (Baycidal WP 25) and 75% inert ingredients.

Cyfluthrin, 3-(2,2-dichlorovinyl)--2,2-dimethylcyclopropanecarboxylate--α-cyano-4-fluoro-3-phenoxybenzyl (pyrethroid) was obtained as a wettable powder with 10% active ingredient (Solfac 10 WP) and 90% inert ingredients.

Laboratory experiments

The effect of Baycidal WP 25 in three doses (0.013 g – recommended one, 0.13 g and 0.0013 g) dissolved in 10 ml of distilled water and the effect of Solfac 10 WP in three doses (0.0013 g – recommended dose, 0.013 g and 0.00013 g) on mortality of entomopathogenic nematodes (*Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema affine*, *S. carpocapsae and S. carpocapsae* strain DD--136) were studied in experimental conditions at a temperature of 20 ±1°C. Each Petri dish, 9 cm in diameter (surface area: 63.6 cm^2) lined with filter paper contained about 1,600 IJs and appropriate dose of insecticide. During 7 days the number of dead and alive nematodes in 1 ml of suspension was determined daily. Tests were made in five repetitions. Infective juveniles from control group were kept in distilled water. Percentage IJs mortality was calculated on every Petri dish. Means and standard deviations were calculated for

each five repetitions of every treatment. After 7 days the nematodes that survived contact with insecticides were separated by sedimentation. The sedimentation did not allow for complete removal of chemical compounds from the sample. Thus, four-week larvae of *A. diaperinus* were exposed to the residues of chemical substances and to entomopathogenic nematodes that survived seven-day contact with these substances.

Next experiments were initiated by transferring 10 larvae of *A. diaperinus* to Petri dishes (9 cm in diameter) lined with filter paper. For treatments with entomopathogenic nematodes, the filter paper was moistened with the suspension of one species of IJs (500 IJs per Petri dish) and the residues of chemical insecticides (Baycidal WP 25 or Solfac 10 WP). In all experiments, each treatment was replicated six times with 10 larvae of *A. diaperinus* per replicate. Insects from control group were infected by IJs deprived of the contact with insecticides. Experiments were conducted at 25 ±1°C and 85–90% relative moisture of the substratum. The mortality and the extensiveness of infection of insects were assessed daily through 7 days. Dead insects were transferred to empty dishes and placed in the incubation chamber for 48 h. Later, the larvae were sectioned to check whether nematodes and associated mutualistic bacteria were the cause of their death. Percentage larvae mortality and extensiveness of infection was calculated for every Petri dish. Means and standard deviations were calculated for six repetitions of each treatment.

Statistics

Data were subjected to analysis with SPSS 15.0 and SAS 9.2 software. The differences between groups, the percentage EPNs mortality for each treatment, insects mortality, their extensiveness and intensity of infection were analyzed using one-way ANOVA. For each nematode species differences between concentrations of the active substance were analyzed. When ANOVA showed the significance of difference, means were compared by using Tukey's post hoc test. Results of this test are marked with letters (a, b, c, d) in tables. Different letters indicate significant differences at $P < 0.05$.

RESULTS AND DISCUSSION

Many scientists studying the effect of various insecticides on nematodes focuses on the toxicity of these compounds to IJs. The harmfulness of insecticides manifests itself in, for example, disturbances in movement and inability or limited ability to kill the host (Li et al. 2012). Different species of the same genus (*Steinernema* and *Heterorhabditis*) are characterized by diverse sensitivity to biocidal products and their different concentrations. For example, beta-cyfluthrin (Turbo biocidal product) and triflumuron (Certero biocidal product) are more toxic to *S. carpocapsae* (nematode mortality was respectively 50 and 25%) but less toxic to *S. glaseri* (nematode mortality was respectively 18.8 and 7.8%) (Negrisoli et al. 2010b). Solfac 10 WP of the group of pyrethroids caused higher mortality in *H. bacteriophora* and *H. megidis* (all doses) than Baycidal WP 25 (Table 1).

Cuticle of EPNs is composed of several layers: the cortex, elementary and fibre, which are a barrier against adverse

TABLE 1. Effect of various doses of Baycidal WP 25 and Solfac 10 WP on mortality of invasive larvae (IJs), various species and strains of entomopathogenic nematodes, after 7 days of contact with insecticides

Trade name	Active substance	Nematode species	Permanent contact of entomopathogenic nematodes with insecticides				
			Invasive larvae of entomopathogenic nematodes				
			Mortality (%) ±*SD*				
			K	A	B	C	ANOVA
Baycidal WP 25	Triflumuron	*S. carpocapsae*	22^{b} ±6	3^{c} ±1	10^{c} ±2	57^{a} ±4	$P < 0.0001$
		S. carpocapsae (DD-136)	20^{b} ±3	5^{c} ±3	9^{c} ±2	44^{a} ±3	$P < 0.0001$
		S. affine	17^{b} ±4	2^{c} ±1	8^{c} ±3	63^{a} ±4	$P < 0.0001$
		H. bacteriophora	1^{b} ±1	1^{b} ±0	1^{b} ±0	3^{a} ±1	$P < 0.0005$
		H. megidis	1^{b} ±1	4^{a} ±1	4^{a} ±1	4^{a} ±1	$P < 0.0001$
Solfac 10 WP	Cyfluthrin	*S. carpocapsae*	22^{a} ±6	2^{c} ±1	3^{c} ±1	10^{b} ±1	$P < 0.0001$
		S. carpocapsae (DD-136)	20^{a} ±3	7^{b} ±2	11^{b} ±3	9^{b} ±2	$P < 0.0001$
		S. affine	17^{a} ±4	$0{,}3^{b}$ ±0	2^{b} ±2	2^{b} ±1	$P < 0.0001$
		H. bacteriophora	1^{d} ±1	11^{c} ±1	14^{b} ±2	18^{a} ±2	$P < 0.0001$
		H. megidis	1^{d} ±1	10^{c} ±2	14^{b} ±2	18^{a} ±2	$P < 0.0001$

SD – standard deviation, different small letters denote significant differences at $P < 0.05$ within each nematode species and at various concentrations of insecticides – Tukey test. K – control (nematodes from distilled water without insecticides). Concentrations of insecticides: A – 0.0013 g Baycidal WP 25 or 0.00013 g Solfac 10 WP, B – 0.013 g Baycidal WP 25 or 0.0013 g Solfac 10 WP, C – 0.13 g Baycidal WP 25 or 0.013 g Solfac 10 WP.

external factors. Infective juveniles have also obstructed intestinal tract, and in addition, they are covered by moult of earlier developmental stage L2. Their contact with the external environment is significantly limited, which largely determines the survival in many unfavourable conditions, and total or partial preservation of the ability to infect hosts. In all EPN species tested after treatment with insecticides Baycidal WP 25 and Solfac 10 WP, not 100% mortality was observed (Table 1). Baycidal WP 25 containing triflumuron, probably did not cause microdamages in the cuticle of IJs, because their cuticle does not contain chitin. Negrisoli et al. (2010a) also reported such observations. Mortality of *S. carpocapsae*, which had contacted the insecticide Certero (triflumuron) was 25%, and those that survived caused 70.2% mortality of *G. mellonella*. In turn, mortality of *Spodoptera frugiperda* larvae 2 and 4 days after application of the pesticide Certero and nematodes amounted to 26 and 72%, respectively (Negrisoli et al. 2010b). This shows that entomopathogenic nematodes are able to adapt to changing environmental conditions (Gondek and Ropek 2007). Infective juveniles after contact with different chemicals preserved the pathogenic properties against larvae of *A. diaperinus*. Many chemicals are not toxic to nematodes or their toxicity is minimal (pyrethroids, chlorinated hydrocarbons, preparations uracil and triazine). For example, triazine compounds (metribuzin, prometryn) caused a mortality of *S. feltiae* not exceeding 30% (Kamionek 1992). According to Kamionek (1992), the high concentrations of herbicides, even 10 times higher than those used in field treatments, also had no significant impact on the mortality of *S. carpocapsae*. Only a limited reproduction of EPNs in the host's body was observed. In other cases, the active substances, e.g. oxamyl (nematicide) and other pesticides stimulate nematode movement (sinusoidal movement, vibration, and turns). Such stimulation increased the efficiency of infection of insects by nematodes (Fedorko et al. 1977). The mortality of *S. affine* after exposure to insecticides Baycidal WP 25 and Solfac 10 WP (10 times lower and recommended doses), were lower than in controls. In all of these variants the differences were statistically significant in relation to the control groups (Table 1). This species was also characterized by usually higher than in the control pathogenicity (mortality, extensiveness of infection) with respect to larvae (Table 2).

Some compounds may, however, reduce the pathogenic properties of organisms, which in turn affects the development of the next generations of nematodes. Pesticides, belonging to carbamates, urea compounds and organophosphates limited pathogenicity and proper development of nematodes, causing paralysis and death. For example, carbamates and preparations of urea completely killed all *S. feltiae* nematodes after 72 h (Hara and Kaya 1983, Kamionek 1992). Witkowski (1979) and Hara and Kaya (1983) found that chemical substances (carbofuran, DDT, lindane and oxamyl) negatively affected entomopathogenic nematodes. Their numbers, after using these substances, decreased by about 50%. Jaworska et al. (2002) also found the high sensitivity of *Xenorhabdus* bacteria colonizing

TABLE 2. Pathogenic properties of entomopathogenic nematodes (percentage mortality, extensity and intensity of infection of *A. diaperinus* larvae) which contacted insecticides of Baycidal WP 25 and Solfac 10 WP in various concentrations (n = 60 in every variant of experiment)

Trade name	Active substance	Nematode species	Permanent contact of entomopathogenic nematodes with insecticides														
			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
Baycidal WP 25	Triflumuron	*S. carpocapsae*	100 ±0	90 ±10	87 ±15	100 ±0	$P = 0.03$	98^{a} ±4	87^{a} ±15	83^{b} ±12	100^{a} ±0	$P = 0.017$	6.9^{b} ±4.1	12.7^{a} ±12.3	12.8^{a} ±16.1	12.7^{a} ±7.4	$P = 0.002$
		S. carpocapsae (DD-136)	100 ±0	100 ±0	100 ±0	97 ±5	$P = 0.24$	97 ±5	90 ±10	93 ±6	87 ±8	$P = 0.16$	8.4^{b} ±5.2	35.6^{a} ±23	36.1^{a} ±26.2	7.4^{b} ±6.8	$P < 0.0001$
		S. affine	78 ±16	100 ±0	90 ±10	85 ±10	$P = 0.12$	35^{b} ±14	87^{a} ±6	67^{a} ±6	0^{c} ±0	$P < 0.0001$	1.7^{c} ±3.3	22.4^{a} ±19.6	10.2^{b} ±14.3	0^{c} ±0	$P < 0.0001$
		H. bacteriophora	93^{A} ±12	60^{B} ±10	83^{AB} ±12	100^{A} ±0	$P = 0.004$	70 ±20	50 ±17	57 ±6	67 ±23	$P = 0.53$	7.2^{a} ±11.3	1.5^{b} ±2.3	1.6^{b} ±2	3^{b} ±3.2	$P = 0.001$
		H. megidis	53 ±23	80 ±17	93 ±12	80 ±26	$P = 0.18$	7^{b} ±12	53^{a} ±29	30^{ab} ±10	7^{b} ±6	$P = 0.025$	0.2^{b} ±0.8	3.8^{a} ±5.6	3.7^{a} ±8.2	0.6^{ab} ±2.3	$P = 0.006$
Solfac 10 WP	Cyfluthrin	*S. carpocapsae*	100 ±0	100 ±0	100 ±0	100 ±0	–	98^{a} ±4	93^{a} ±6	93^{a} ±6	73^{b} ±15	$P = 0.007$	6.9^{b} ±4.1	15.7^{a} ±13.6	19.4^{a} ±18.5	13^{ab} ±14.3	$P < 0.0001$
		S. carpocapsae (DD-136)	100 ±0	100 ±0	100 ±0	100 ±0	–	97 ±5	90 ±10	100 ±0	100 ±0	$P = 0.15$	8.4^{b} ±5.2	24.3^{a} ±15	25.2^{a} ±21.9	33.8^{a} ±18.1	$P < 0.0001$
		S. affine	78 ±16	100 ±0	100 ±0	100 ±0	$P = 0.022$	35^{b} ±14	100^{a} ±0	97^{a} ±6	97^{a} ±6	$P < 0.0001$	1.7^{c} ±3.3	15.2^{b} ±9.8	16.4^{ab} ±11.5	21.8^{a} ±13.4	$P < 0.0001$
		H. bacteriophora	93 ±12	100 ±0	100 ±0	100 ±0	$P = 0.44$	70^{a} ±20	37^{ab} ±12	27^{b} ±12	53^{ab} ±12	$P = 0.025$	7.2^{a} ±11.3	1.8^{b} ±3.3	1.4^{b} ±3.5	2.8^{ab} ±3.8	$P = 0.003$
		H. megidis	53^{B} ±23	100^{A} ±0	100^{A} ±0	100^{A} ±0	$P = 0.002$	7^{b} ±12	77^{a} ±6	67^{a} ±23	70^{a} ±20	$P = 0.003$	0.2^{b} ±0.8	9.8^{a} ±16	5.6^{ab} ±8	5.7^{ab} ±7.1	$P = 0.003$

SD – standard deviation, different small letters denote significant differences at $P < 0.05$ within each nematode species and at various concentrations of insecticides – Tukey test. K – control (nematodes from distilled water without insecticides). Concentrations of insecticides: A – 0.0013 g Baycidal WP 25 or 0.00013 g Solfac 10 WP, B – 0.013 g Baycidal WP 25 or 0.0013 g Solfac 10 WP, C – 0.13 g Baycidal WP 25 or 0.013 g Solfac 10 WP.

the digestive tract of Steinernematidae to heavy metal ions (Cu, Mn, Ni). Heavy metals negatively affected the growth rate of bacterial colonies. High mortality of *S. carpocapsae* strain DD-136 (44%), *S. carpocapsae* (57%) and *S. affine* (63%) was caused by the highest dose of Baycidal WP 25 (Table 1). In relation to the controls all the differences were statistically significant. In most cases, these nematodes did not lose pathogenicity and contributed to the relatively high mortality and extensiveness of infection of *A. diaperinus* (Table 2). Baycidal WP 25 and Solfac 10 WP are soluble in water, and not long overdue in the soil. However, the use of excess doses of these agents in relation to the recommended doses could have a negative impact on beneficial organisms, for example entomopathogenic nematodes.

In experiments with invasive larvae contacting insecticides, Solfac 10 WP caused no more than 18% mortality (*H. bacteriophora* and *H. megidis* at the highest dose), whereas the contact of IJs with Baycidal WP 25 caused of up to 63% mortality in *S. affine* at a dose 10 times higher than recommended (Table 1). In all cases, experimental results significantly differed from the control. The diverse impact of chemicals on the IJs could be affected by various defensive and/or compensative mechanisms induced by these substances in the body. In the natural environment such direct and continuous contact of these animals with insecticides is not possible and results in limited mortality of entomopathogenic nematodes.

In studies concerning the occurrence of nematodes in forests contaminated in different degree by industrial pollutants Kamionek et al. (1995) found that invasive larvae are often resistant to a variety of chemicals, which results in their low mortality. The highest number of *S. carpocapsae* and *S. feltiae* (32,378 individuals per m^2) was found in areas with the highest concentration of industrial pollution (southern Poland). In areas with low and medium contamination, much smaller numbers of nematodes were noted. Measured abundances were, respectively, 9,548 individuals per m^2 and 7,145 individuals per m^2. The presence of heavy metals in the environment also positively influenced the pathogenicity of *H. megidis*. Reproduction of this species was possible even in very contaminated area (Jarmuł and Kamionek 2004). Pezowicz (2002) noted the presence of invasive larvae of entomopathogenic nematodes in soil with a high content of lead (200–300 mg Pb/kg), their density was 714 individuals per m^2. In the present study, mortality of IJs was low after treatment with high doses of insecticides. For most species and strains of entomopathogenic nematodes, their mortality did not exceed 18% for the highest dose of Baycidal WP 25 or Solfac 10 WP. The exception was the nematodes *S. carpocapsae*, *S. carpocapsae* DD-136 and *S. affine*. However, they also retained the pathogenic properties. The lowest doses of chemicals, to which IJs were exposed caused lower mortality, usually not exceeding 10% (Table 1). This shows that the entomopathogenic nematodes can be used in integrated control of *A. diaperinus*.

Most insecticides successfully controlled larvae of *A. diaperinus*. However, long term use of chemicals resulted in the emergence of biotypes of

A. diaperinus resistant to pesticides. Their number has increased in recent years (Lambkin 2005). Control of *A. diaperinus* through the use of the active substance – cyfluthrin and triflumuron was effective. The observed decline in the number of *A. diaperinus* in broiler houses reached 86%. The insecticides, however, did not penetrate all the places where pests could hide. Some individuals survived aerial spraying and after 2 months the pests appeared in the poultry house again. Insect resistance was also associated with their lowered mobility. Occasionally, immobile stages (pupae) appeared. To prevent the occurrence of insect resistance to insecticides, rotation of pesticides and methods of integrated pest management should be used. Integrated pest management counteract the formation of immune insects and can increase the effectiveness of infection by nematodes. Friedel et al. (1988) found that cyromazine (chitin biosynthesis inhibitor) damages the cuticle and the gut of insects and causes muscular dystrophy. Cracked cuticle helps EPNs to penetrate the host, which in turn improves the effectiveness of the biological agent. Reduced doses of chemicals, in combination with biological agents are very effective. Contact with a toxic substance decreases the immunity of beetles. Furthermore, the chemicals, such as fertilizers (NPK, Mikrovit) and calcium, have also impact on the average time required to kill the hosts. Gondek and Ropek (2007) noted slightly shorter time (2.25 days compared to the control of 2.52 days) required to kill wax moth caterpillars by *S. feltiae*, which were exposed to the fertilized soil. Also, the percentage of infected insects by EPNs was the highest in the experiment with mineral fertilizers and liming. It reached the value of 95%. Synergism was also observed in the case of extensity of infection of *A. diaperinus* by nematodes. The extensity of larvae infected by EPNs, which were in contact with Baycidal WP 25 (the dose 10 times lower than recommended) reached a value of 87% for *S. affine*, 53% for *H. megidis*. An analogous phenomenon in variants with Solfac 10 WP (the dose 10 times lower than recommended) was noted for the extensity of larvae (100% for *S. affine* and 77% for *H. megidis*) – Table 2. All the differences between experimental groups and controls were statistically significant. Koppenhöfer and Fuzy (2008) studied the effect of integrated pest management on beetle grubs (Scarabaeidae) using an insecticide containing chlorantraniliprole and *H. bacteriophora*. In the greenhouse and field experiments, synergistic effect in the control of *Popillia japonica*, *Cyclocephala borealis* and *Anomala orientalis* was observed in 64% of combinations. For example, mortality of *C. borealis* after application of invasive larvae (109 IJs/ha) and insecticide (0.3 kg ai/ha) approached 80%, while after the contact with nematodes or pesticide alone, the mortality did not exceed 20%. Also *S. glaseri* or *H. bacteriophora* in combination with an insecticide (imidacloprid) showed a synergistic effect in the control of grubs. Beetle larvae mortality was significantly higher after the use of combination of the insecticide and *H. bacteriophora* nematodes (63–90%) than after the application of imidacloprid alone (1–20%) or just *H. bacteriophora* (30%) (Koppenhöfer et al. 2000a, b). The increased infection of

insect larvae was determined by reduction of the preening intensity (Koppenhöfer et al. 2000a, b). Combined control of *Hoplia philanthus* larvae by spores of *M. anisopliae* fungus with the nematodes *H. megidis* or *S. glaseri* increased insect mortality. *Metarhizium anisopliae* was a stressor, which increased the susceptibility of insects to infection with EPNs. The high value of synergistic effect was observed when *H. philanthus* larvae were infected with EPNs after at least 3 or 4 weeks earlier exposition to the fungus spores (Ansari et al. 2004). Irigaray et al. (2003) concluded that biopreparation Naturalis – L containing the spores of *B. bassiana* can be used in the integrated pest management of *Tetranychus urticae* in combination with the active substance – triflumuron. Chitin synthesis inhibitor, did not inhibit the germination of fungal spores but inhibited the proper conduct of melanization and damaged cuticle of spider mites. Such activity resulted in an increase in the susceptibility of *T. urticae* to *B. bassiana* (mortality was 38%). In other studies, *H. megidis* (density of 1,035 mg/ml), that survived contact with the colloidal silica solution, showed high pathogenicity against larvae of *T. molitor* (Wilson and Ivanova 2004).

Pezowicz (2005) also observed that the mortality of *A. diaperinus* larvae in one week old litter with a mixed infection was lower (30%) from the mortality of insect infected just by *S. feltiae* nematodes (45%). In our experiments, similar effect was also observed when comparing the use of nematodes and chemicals with the effect of *H. bacteriophora* alone. The mortality of *A. diaperinus* larvae (60%) was lower after the application of nematodes and residues of Baycidal WP 25 (the lowest dose), than after the application of nematodes, which have had no contact with the insecticide (93%) – Table 2. Statistically significant differences from controls were noted.

Mortality of insects, including *A. diaperinus*, is influenced by the nematode species/strain, by contact or a lack of contact with chemicals, by virulence of symbiotic bacteria and stage of insect development.

Contact of IJs with different doses of insecticides did not cause the loss of their invasive abilities. This allows to use them in IPM methods in broiler houses. Geden et al. (1987) also confirmed the possibility of using EPNs indoor in farms in the system of biological control methods. Entomopathogenic nematodes caused up to 87% mortality of *A. diaperinus*. Extensiveness of infection of beetles was lower than or equal to mortality. Only in a few cases the extensiveness of infection equal 0% was found (*S. affine* against larvae, Baycidal WP 25, the highest dose – Table 2). Symbiotic bacteria that colonize the digestive tract of nematodes, after the release, can kill the host and inhibit the development of nematodes. This case makes determining the presence of EPNs during the autopsy of insects impossible (Pezowicz 2005).

All entomopathogenic nematodes used in these experiments have the ability to infect *A. diaperinus*. An interesting observation was that *H. megidis* IJs which survived contact with the highest dose of Solfac 10 WP (mortality rate of 18%) (Table 1) contributed to the higher mortality (100%) and the extensiveness of infection (70%) of *A. diaperinus* larvae, compared to the control (Table 2).

Chemical insecticides can stimulate EPNs to increased activity associated

with finding new host, which results in an increased mortality and the extensiveness of infection of pests. The literature data confirm that the use of neonicotinoid substances results in an increased number of IJs attached to grubs (Koppenhöfer et al. 2000a, b).

Many studies have shown that the effectiveness of Heterorhabditidae is lower than that of Steinernematidae. Sandner and Pezowicz (1986) and Renn et al. (1985) found above-mentioned relationship by examining the impact of nematodes from both families on larvae of *Barathra brassicae* and *Musca domestica*. A similar relationship was observed for the extensiveness of infection of *A. diaperinus* larvae by *H. bacteriophora* and *H. megidis* (Baycidal WP 25 and Solfac 10 WP, all doses). In comparison with the majority of nematodes of the genus *Steinernema*, the value of extensiveness of infection caused by the *Heterorhabditis* EPNs were from tens to dozen percent lower (Table 2). Differentiated pathogenicity also characterized two *Heterorhabditis* species. Values of extensiveness of infection in larvae after application of *H. megidis* at all Solfac 10 WP doses, were typically a several dozen percent higher than after the application of *H. bacteriophora* (Solfac 10 WP). In the control groups the extensiveness was 7% for *H. megidis* and 70% for *H. bacteriophora* (Table 2). Perhaps increased pathogenicity of *H. megidis* nematodes was caused by contact with the insecticide. Studies of Kaya and Stock (1997) confirmed that some biologically active compounds can stimulate the movement of parasites, which increases the efficiency of finding and infecting the host. This may also be caused by diverse degree of penetration of the host's body.

Increased demand for livestock production requires the use of chemicals to control pest and disease – causing pathogens. Therefore, more or less negative impact of the environment (including the organisms inhabiting the soil) cannot be avoided. As many as 99% of soil mesofauna are nematodes from different trophic groups, including species associated with bacteria. Effect of different chemicals may reduce populations of beneficial EPNs and affect their pathogenicity to insects. It is therefore necessary to limit the use of chemicals that are toxic to the environment. It is proposed to apply integrated pest control methods (for example EPNs and reduced doses of insecticides). From 1 January 2014 the introduction of protection by IPM is obligatory in the European Union (Article 14 of Directive 2009/128/EC and Regulation EC 1107/2009). It is necessary to: use biopreparations based on different living organisms or their spores, use chemicals least harmful for the beneficial organisms and the environment, reduce insecticide doses and use chemicals from a variety of groups and with different mechanisms of action (Olejarski and Ignatowicz 2011).

CONCLUSIONS

Use of insecticides in agriculture does not affect negatively EPNs pathogenicity under laboratory conditions. Instead of that, addition of triflumuron and cyfluthrin may slightly increase the efficacy of nematodes. Further experiments are needed in field trials to verify the observed effects.

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Streszczenie: *Możliwości stosowania nicieni entomopatogenicznych i substancji czynnych zawartych w popularnych produktach biobójczych, do ograniczania liczebności pleśniakowca lśniącego* Alphitobius diaperinus *(Panzer, 1797).* Larwy pleśniakowca lśniącego zostały zarażone pięcioma gatunkami i szczepami nicieni entomopatogenicznych: *Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema affine*, *S. carpocapsae* i *S. carpocapsae* DD-136. Wcześniej nicienie miały kontakt z insektycydami Baycidal WP 25 oraz Solfac 10 WP. Przez 7 dni badano śmiertelność, ekstensywność i intensywność zarażenia larw. Prawie wszystkie badane EPN i pozostałości insektycydów powodowały dużą śmiertelność i znaczną ekstensywność zarażenia larw gospodarza, odpowiednio od 60 do 100% oraz od 0 do 100%. Zaobserwowano negatywny wpływ nicieni *S. affine* i Baycidal WP 25 na ekstensywność zarażenia larw. W wielu przypadkach kontakt nicieni z insektycydami nie hamował działania biopreparatów. Zmniejszone dawki insektycydów mogą być z powodzeniem stosowane w integrowanym zwalczaniu szkodników.

Słowa kluczowe: IPM, integrowane metody zwalczania szkodników, *Alphitobius diaperinus*, EPN, nicienie entomopatogeniczne, insektycydy

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Authors' address:
Kornelia Kucharska
Zakład Zoologii
Katedra Biologii Środowiska Zwierząt
Wydział Nauk o Zwierzetach
Szkoła Główna Gospodarstwa Wiejskiego
w Warszawie
ul. Ciszewskiego 8, 02-787 Warszawa
Poland
e-mail: kornelia.kucharska@op.pl