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**MORTALITY AND PATHOGENIC PROPERTIES
OF *Heterorhabditis bacteriophora* (POINAR 1976)
FROM NEMATOP BIOPREPARATION
AFTER CONTACT WITH AN INSECTICIDE**

**ŚMIERTELNOŚĆ I WŁAŚCIWOŚCI PATOGENNE
Heterorhabditis bacteriophora (POINAR 1976)
POCHODZĄCYCH Z BIOPREPARATU NEMATOP
PO KONTAKCIE Z INSEKTYCYDEM**

Abstract: The effect of Baycidal WP 25 insecticide on the mortality of *entomopathogenic nematodes* (EPN) *Heterorhabditis bacteriophora* from Nematop biopreparation was studied under laboratory conditions. The invasive larvae were kept in aquatic solutions of different concentrations of Baycidal WP 25. The concentration of 0.0013 g/cm³ of insecticide (recommended by the manufacturer) unfavourably affected the survival of IJs. Moreover, it was found that their mortality was the lowest in the highest concentrations of Baycidal WP 25, except for the control group. In this study the effect of different concentrations of Baycidal WP 25 on pathogenic properties of entomopathogenic nematodes was also analysed.

Keywords: entomopathogenic nematodes, EPN, *Heterorhabditis bacteriophora*, Nematop, insecticide, Baycidal WP 25, *Alphitobius diaperinus*

Entomopathogenic nematodes are used in the production of biological preparations for controlling various pests [1]. Nematode species show different tolerance to insecticides used in agriculture [2]. Studies confirmed the possibility of parallel application of biological and chemical means, which increases the mortality of pest populations. The effectiveness of integrated methods of pest control often exceeds the effectiveness of one of these methods used alone [3]. Application of biological insecticides based on nematodes together with chemical means may be used to control a beetle of the family Tenebrionidae – *Alphitobius diaperinus* (Panzer 1797). It is a dangerous pest and a vector of many diseases. It propagates with poultry fodder and

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the conditions in henhouses favour its development. The most threatened group of animals are bred birds which have contact with insects brought to farm buildings [4].

Material and methods

The effect of the Baycidal WP 25 insecticide on mortality and pathogenic properties of entomopathogenic nematodes *Heterorhabditis bacteriophora* was studied in experimental conditions. Baycidal WP 25 is an insecticide from the group of insects' growth and development regulators. It is produced by BAYER CropScience AG in Germany. The preparation is mainly used for the control of flies and the lesser mealworm in farm houses. Three concentrations of Baycidal WP 25 were used in experiments: the dose recommended by the producer (0.0013 g/cm^3), ten times lower (0.00013 g/cm^3) and ten times higher (0.013 g/cm^3) than the recommended. *H. bacteriophora* originated from the biopreparation Nematop made by the German firm E-nema.

The experiment was carried out during 7 consecutive days under laboratory conditions at $25 \text{ }^\circ\text{C}$. Larvae of the third invasive stadium (IJs) were placed in water solutions of the appropriate concentration of Baycidal WP 25. The control group consisted of the larvae kept in distilled water. Every day samples of the solution were taken and nematodes mortality was studied. Tests were made in 5 repetitions.

After 7 days the nematodes that survived the contact with the insecticide were separated by sedimentation. Live nematodes were used to infect various growth stages of *A. diaperinus* (four week larvae, pupae and adults). Experiments were performed in Petri dishes of the diameter of 9 cm lined with filter paper in which 10 insects from particular growth stages were placed. Each dish received 500 invasive larvae (IJs). Tests were made in 3 repetitions. Mortality was checked for 7 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 reason of their death. Experiment was carried out at $25 \text{ }^\circ\text{C}$ and 85–90 % relative moisture. The control consisted of insects in a respective growth stage infected with nematodes which did not contact Baycidal WP 25. The mortality, extensiveness and intensity of infection of insects by *H. bacteriophora* were analyzed.

The obtained results were statistically processed (ANOVA, chi square and Tukey test) with the SPSS 15 software. Statistical significance was tested at $p < 0.05$.

Results and discussion

Nematodes mortality in solutions of Baycidal WP 25 (0.00013 ; 0.0013 and 0.013 g/cm^3) was analysed every day during the 7 days of the experiment (Fig. 1). The highest concentration of 0.013 g/cm^3 caused the lowest (17 %) mortality in *H. bacteriophora*, the lowest (0.00013 g/cm^3) – 19 % mortality. A similar tendency for high heavy metal doses was observed for different groups of nematodes [5, 6]. It can be caused by excess of the threshold value of a toxic factor, in which defense mechanisms are activated. At the concentration recommended by the producer (0.0013 g/cm^3) nematodes mortality was the highest and amounted 28 %. Differences between

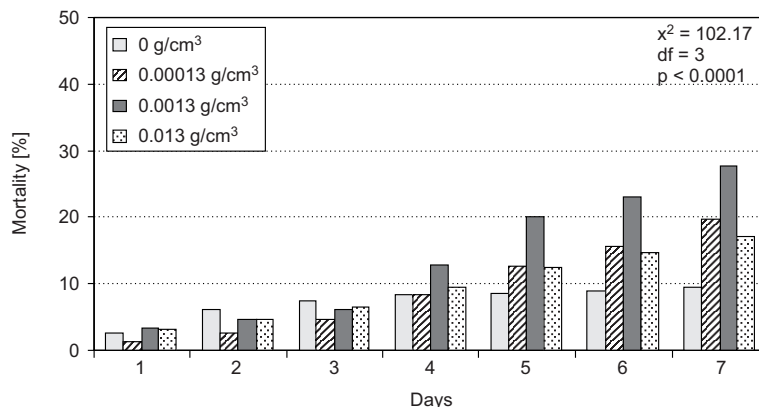


Fig. 1. The effect of various concentrations of Baycidal WP 25 on the mortality of *Heterorhabditis bacteriophora* larvae (test Chi² performed for the last day of experiment)

particular concentrations of Baycidal WP 25 measured on 7th day of the experiment were statistically significant in all cases.

The highest mortality and extensiveness of infection on the last day of the experiment were noted for the larvae of *A. diaperinus* (Fig. 2). A hundred per cent of dead insects were noted for nematodes exposed to 0.00013 g/cm³ solution of Baycidal WP 25, the extensiveness of infection was 93 % in that case. The lowest percentage of mortality and extensiveness (83 and 33 %, respectively) was found at the highest concentration of insecticide.

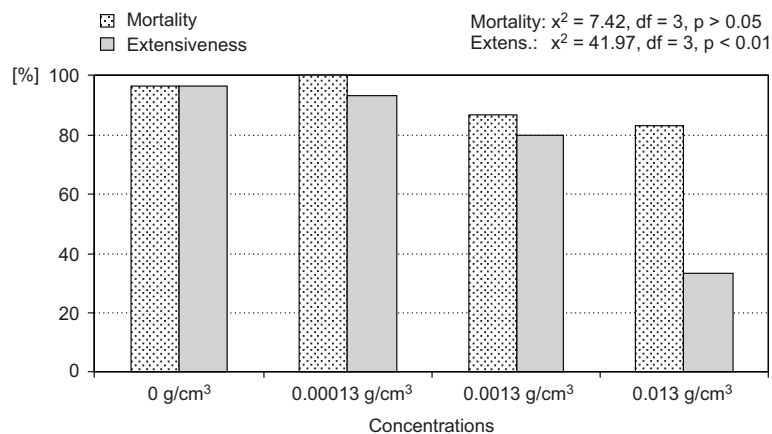


Fig. 2. The effect of Baycidal WP 25 on pathogenic properties of the nematode *Heterorhabditis bacteriophora* exposed for 7 days to solutions of various concentrations (the test of mortality percentage and extensiveness of infection of *Alphitobius diaperinus* larvae) (test Chi²)

High mortality (83 %) and extensiveness of infection (73 %) were noted in pupae after the contact of nematodes with the solution of a concentration of 0.0013 g/cm³

(Fig. 3). The lowest values (40 and 27 %, respectively) were observed in the control group. For the highest concentrations, mortality and extensiveness of infection were respectively 63 and 33 %, which could be caused by lowered ability of EPN to penetrate hosts' tissues (fe damaging cuticule).

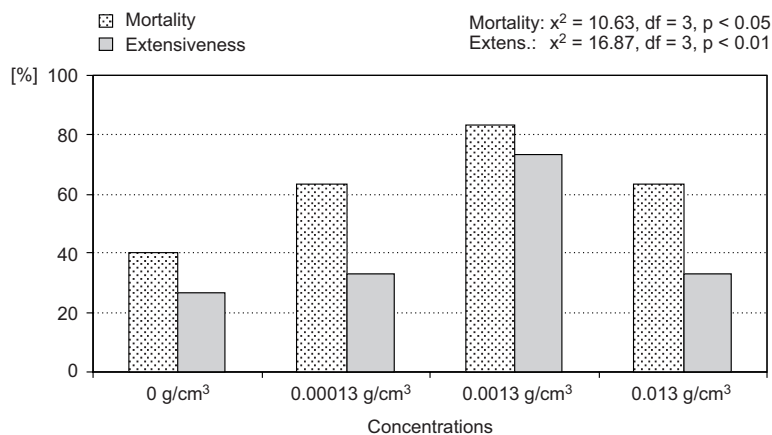


Fig. 3. The effect of Baycidal WP 25 on pathogenic properties of the nematode *Heterorhabditis bacteriophora* exposed for 7 days to solutions of various concentrations (the test of mortality percentage and extensiveness of infection of *Alphitobius diaperinus* pupae) (test Chi²)

The highest mortality of adult *A. diaperinus* (Fig. 4) was caused by nematodes exposed to concentrations of 0.013 and 0.0013 g/cm³ (47 and 43 %, respectively). The extensiveness of infection at the concentration recommended by the producer was similar to mortality (43 %). The lowest mortality and extensiveness of infection (both equal to 13 %) was noted in the control.

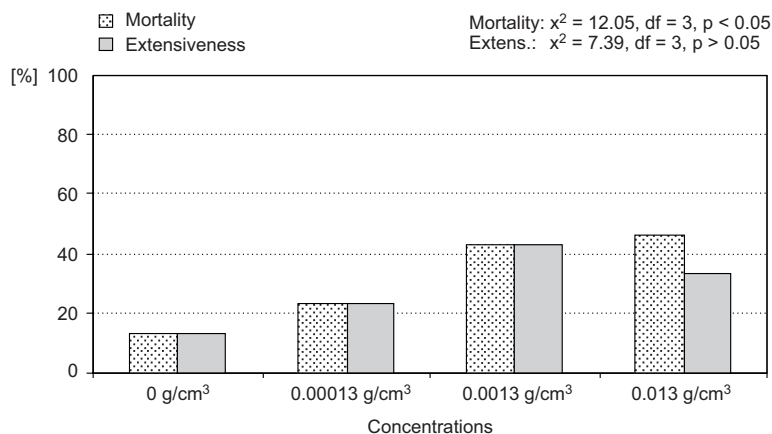


Fig. 4. The effect of Baycidal WP 25 on pathogenic properties of the nematode *Heterorhabditis bacteriophora* exposed for 7 days to solutions of various concentrations (the test of mortality percentage and extensiveness of infection of *Alphitobius diaperinus* imagines) (test Chi²)

The presence of nematodes found after dissection of an insect's body evidenced that bacteria were the cause of its death. Microorganisms are the food base for entomopathogenic nematodes. Due to the lack of bacteria they do not reproduce [7]. Usually mortality is higher than the extensiveness of infection. This is because nematodes sometimes release bacteria from the alimentary tract and do not reproduce further. Therefore, their presence could not be found during dissection.

Intensity of infection is the mean number of invasive larvae of the nematode that entered the insect and developed to the L4 form plus hermaphroditic individuals. The intensity of infection for insect larvae was 6.87 at a concentration of 0.0013 g/cm³ and 10.23 in the control (Table 1). Contribution of particular growth stages of nematodes to the population structure of the parasitic generation is shown in Table 2. Hermaphrodites dominated in the studied populations.

Table 1

The effect of Baycidal WP 25 on the intensity of infection of *Alphitobius diaperinus* by *Heterorhabditis bacteriophora* (Tukey test and ANOVA)

Insect developmental stage	Concentrations of Baycidal WP 25	Intensity of infection (Means)	ANOVA
Larvae	0 g/cm ³	10.23 B	F3.116 = 9.53, p < 0.01
	0.00013 g/cm ³	6.8 B	
	0.0013 g/cm ³	6.87 B	
	0.013 g/cm ³	0.97 A	
Pupae	0 g/cm ³	2.2 A	F3.116 = 3.69, p < 0.05
	0.00013 g/cm ³	4.87 AB	
	0.0013 g/cm ³	8.57 B	
	0.013 g/cm ³	1.87 A	
Adult insects	0 g/cm ³	0.97 A	F3.116 = 2.42, p > 0.05
	0.00013 g/cm ³	2.17 A	
	0.0013 g/cm ³	4.33 A	
	0.013 g/cm ³	1.7 A	

The highest intensity of infection (8.57) was noted for pupae (Table 1) at a concentration of 0.0013 g/cm³, the lowest (1.87) – for the concentration 10 times lower than the recommended one. Contribution of particular growth stages of nematodes to the population structure of the parasitic generation is presented in Table 2. Hermaphrodites were the main component of the studied populations except for L4 larvae which attained a slight majority (4.43) at a concentration of 0.0013 g/cm³.

The highest intensity of infection (4.33) in adult insects was noted at a concentration of 0.0013 g/cm³ and the lowest – in the control (0.97) (Table 1). Contribution of particular growth stages of nematodes to the population structure of the parasitic generation is shown in Table 2. L4 growth stage was the main component of the studied populations except for hermaphrodites in the control group.

Table 2

The effect of Baycidal WP 25 on the population structure of the parasitic generation of *Heterorhabditis bacteriophora* in *Alphitobius diaperinus*

Insect developmental stage	Concentrations of Baycidal WP 25	Population structure of the parasitic generation (Means)	
		Hermaphrodite	L4
Larvae	0 g/cm ³	10.23	0.00
	0.00013 g/cm ³	6.40	0.40
	0.0013 g/cm ³	5.80	1.07
	0.013 g/cm ³	0.23	0.73
Pupae	0 g/cm ³	2.17	0.03
	0.00013 g/cm ³	4.17	0.70
	0.0013 g/cm ³	4.13	4.43
	0.013 g/cm ³	1.10	0.77
Adult insects	0 g/cm ³	0.53	0.43
	0.00013 g/cm ³	0.63	1.53
	0.0013 g/cm ³	1.07	3.37
	0.013 g/cm ³	0.33	1.37

The number of nematodes in the insect's body is an evidence of its attractiveness as a food base and indicates nematodes' ability to infect the host. As an effect of the performed studies one may conclude that high mortality, extensiveness and intensity of infection of *A. diaperinus* larvae point to attractiveness of this growth stage for nematodes.

The study confirmed that it is possible to simultaneously apply biological and chemical control means which, when used at the recommended dose or ten times lower dose, increases the mortality of larvae, pupae and adult insects.

Conclusions

1. Mortality of the invasive larvae of *H. bacteriophora* exposed to Baycidal WP 25 depended on the concentration of solutions and on exposure time.
2. Mortality and extensiveness of infection by Baycidal WP 25 treated nematodes and control nematodes differed among various growth stages of the lesser mealworm. Larvae were most sensitive to nematodes.
3. Intensity of infection by nematodes was the highest in the larvae of the beetle.
4. Hermaphrodites dominated in the population structure of the parasitic generation in most larvae and pupae of *A. diaperinus* and L4 larvae dominated in adult insects.

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POCHODZĄCYCH Z BIOPREPARATU NEMATOP PO KONTAKCIE Z INSEKTYCYDEM

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Abstrakt: W warunkach laboratoryjnych badano wpływ insektycydu Baycidal WP 25 na śmiertelność nicieni entomopatogennych *Heterorhabditis bacteriophora* pochodzących z biopreparatu Nematop. Larwy inwazyjno-przetrwalnikowe (IJs) umieszczono w roztworach wodnych zawierających różne stężenia Baycidal WP 25. Dawka 0,0013 g/cm³ środka (dawka zalecana przez producenta) wpływała niekorzystnie na żywotność larw. Stwierdzono również, że śmiertelność ich jest najniższa, przy najwyższej ilości substancji owadobójczej rozpuszczonej w wodzie. Zbadano również wpływ różnych stężeń Baycidal WP 25 na patogenność nicieni.

Słowa kluczowe: nicienie entomopatogenne, *Heterorhabditis bacteriophora*, Nematop, insektycyd, Baycidal WP 25, *Alphitobius diaperinus*