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Efficacy of Entomopathogenic Nematodes Against *Arion distinctus* and *Deroceras reticulatum* in a Biological Plant Protection System

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Abstract

The current model of agricultural development, largely focused on the intensification of production, causes increased pressure on the natural environment and, at the same time, does not guarantee sufficient food supplies in the era of global demographic expansion. In light of current environmental changes and the escalating food shortage, the modern agricultural paradigm must strive to achieve a balance between productivity and the quality of agricultural products produced within an environmentally responsible production system. A promising and sustainable tool for future agriculture is a biorational model of agricultural production based, among other things, on the biological protection of agricultural products. The study aimed to assess the effectiveness of biological control agents containing entomopathogenic nematodes in controlling pests from the class Gastropoda. The tests showed that these preparations inhibited the feeding intensity of the analyzed pests. Among the insecticidal nematodes, the biological product containing *S. carpocapsae* at doses of 2000 and 4000 LJ/m^2 demonstrated the highest effectiveness (mass loss: *A. distinctus*: 0.61 g, 0.58 g; *D. reticulatum*: 0.60, 0.71 g). The research conducted indicates that preparations containing entomopathogenic nematodes have the potential to reduce damage caused by slugs in crops.

Keywords: biopesticides; parasitic nematodes; snail control; *Heterorhabditis bacteriophora*; *Steinernema carpocapsae*; *Steinernema feltiae; Phasmarhabditis californica*

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1. Introduction

The current world faces numerous, complex, and simultaneously increasing global threats and problems [1,2]. The destabilization of the food and nutrition system and the deepening climate crisis can be considered among the most important civilizational challenges of the contemporary century [1,3]. The current model of agricultural production is the foundation of the food system's efficiency and the cause of its instability. Modern agricultural management systems as market gaps are identified increasingly emphasize the search for effective and sustainable strategies to develop agricultural production [4–7]. Therefore, biological pest control has a growing significance in shaping contemporary agricultural systems [8–15]. Among low-risk biopesticides, preparations

containing parasitic nematodes play a particularly important role in the European market [16–19]. Their effectiveness has been confirmed primarily in controlling harmful entomofauna [19–23], but this is not the only threat to crops. Native and invasive snail species (Gastropoda: Pulmonata) are also a significant risk factor, especially slugs in the order Stylommatophora [24–26]. Due to their polyphagous nature and broad environmental tolerance, they cause losses in agroecosystems worldwide [27,28]. Slugs are most prevalent in winter wheat, winter corn, and soybeans, but may also infest other economically important plant species [25,27,29].

In accordance with the guidelines the European Union's shared agricultural policy, chemical control of Gastropoda was limited in 2025 to two active substances (metaldehyde and iron (III) phosphate) [30]. However, among nematode-based preparations for infecting and killing these pests, only one product is registered, commercialized under the brand name Nemaslug® [31,32]. This preparation is based on nematodes of the genus *Phasmarhabditis* that carry the bacterium *Moraxella osloens*is Bøvre and Henriksen, which is deadly to many snail species [32–35].

As pests become resistant to limited pest control agents (both chemical and biological), and due to the observed escalation in the scale of losses caused by snails, it is becoming necessary to search for effective tools for their control, especially in the field of low-risk plant protection products [28,36,37]. Therefore, this research assessed the feasibility of using preparations containing entomopathogenic nematodes, including *Steinernema feltiae* (Filipjev), *Steinernema carpocapsae* (Weiser), *Heterorhabditis bacteriophora* (Poinar), and *Phasmarhabditis californica* (Tandingan De Ley et al.) to control two snail species: *Arion distinctus* Mabille and *Deroceras reticulatum* O.F. Müller. The study also aimed to determine the optimal doses of the preparations and to assess whether the preparations control the studied snail species or merely limit their feeding. The research hypotheses presented above were verified by assessing:

- the feeding intensity of both snail species on butterhead lettuce (*Lactuca sativa* L. var.), measured as the average leaf mass loss per snail per day;
- snail survival throughout their feeding period;
- effectiveness of various doses of preparations (250, 500, 1000, 2000, 4000 LJ/m^2) containing nematodes.

2. Materials and Methods

2.1. Materials

The experiment used snails collected from experimental farms at the Agricultural Station in Bałcyny, in the vicinity of Ostróda, Poland (53°36′ N, 19°51′ E). Gastropoda faunal material was collected by hand in April 2024. Two species of adults were used in the study: *Arion distinctus* and *Deroceras reticulatum*. Taxonomic identification of the zoological material was performed using a diagnostic key based on morphological techniques [24]. The experiment was conducted in laboratory conditions using four preparations containing larvae of beneficial nematodes:

- Entonem (Koppert Polska Sp. z o.o., Dąbrówka, Poland), which contains the invasive nematode *Steinernema feltiae*. It is recommended for the control of Hemiptera, Diptera larvae, Coleoptera, Lepidoptera, and Thysanoptera pupae and larvae [38];
- Capsanem (Koppert Polska Sp. z o.o.), which contains insect-parasitic nematodes *Steinernema carpocapsae* to manage pests from Hemiptera, Lepidoptera juveniles, Coleoptera, Diptera, and Orthoptera [38];
- Larvanem (Koppert Polska Sp. z o.o.), which contains *Heterorhabditis bacteriophora* for the control of Coleoptera and Lepidoptera larvae [38];

- Nemaslug (BASF Agricultural Solutions), Warsaw, Poland, which is a product used for the biological control of small- and medium-sized snails that uses the parasitic nematode *Phasmarhabditis californica* [32].

Storage, suspension preparation, and application of the preparations containing the analyzed nematodes were carried out according to the manufacturers' recommendations [32,38].

2.2. Bioassays

To eliminate natural field infections, snails of both species were kept in an environmental chamber (SANYO MLR 351-H, Ōizumi-mach, Gunma, Japan) for 14 days prior to the experiment. During the pre-experimental period, the snails were fed moistened organic head lettuce leaves daily. All stages of the experiment were conducted under constant laboratory conditions at 18 °C, 70% relative humidity, and a 12-h light/12-h dark cycle in ventilated plastic containers (width 8 cm; height 3.3 cm; ventilation hole width 2.4 cm). Tests measuring the nematodes' effectiveness in controlling the tested pests were conducted in containers lined with filter paper soaked in nematode larvae diluted in 15 mL of distilled water. A simplified laboratory setup using filter paper was deliberately used to eliminate additional environmental factors (such as soil structure, microflora, or variable humidity) that could influence snail behavior and mask the direct effects of the tested preparations. Each tested preparation (Entonem, Capsanem, Larvanem, Nemaslug) was tested on both snail species at concentrations of 250, 500, 1000, 2000, and 4000 LJ/m^2 (2 snail species × 4 preparations × 5 concentrations). In the control, filter paper was soaked only with 15 mL of distilled water (control for *A. distinctus* and *D. reticulatum*). Each combination was provided with 5 adult snails and 20 g of fresh lettuce leaves. The food substrate was replaced daily with fresh, moistened substrate; the old substrate was removed from the culture containers and weighed using a WPS 220/C/2 laboratory scale (RADWAG, Radom, Poland). The experimental design included 5 replicates for each combination. Monitoring of feeding and mortality was conducted daily for 9 days. The duration and conditions of the experiment were designed, among other things, based on research by McDonnella et al. [39] and Hussein and Salem [40], as well as our own observations.

2.3. Statistical Analysis

The experimental results were subjected to multivariate analysis using the Statistica 13.1 and Canoco 4.51 software packages. The distributions of data on the mortality of *A. distinctus* and *D. reticulatum*, and on the loss of mean lettuce leaf mass (per individual) due to their feeding, were analyzed using the Shapiro–Wilk normality test. Based on the probability distribution function of the experimental data, their unimodal nature was demonstrated, and the distribution was then transformed to the natural logarithm of the variable shifted by a constant 1 (x + 1). One-way analysis of variance (ANOVA) was used to compare the significance of differences between the combinations used in the experiment. Homogeneous groups of mean snail development parameters obtained in the selected statistical model, separated by the Tukey HSD post hoc test, were marked with the same letters (a, b, c, …) for better readability. The relationship between variables related to snail development intensity and the dose levels of the preparations used was examined using the advanced RDA (Redundancy Analysis) technique [41]. Based on the analysis of Monte Carlo permutation tests conducted within the full model, the significance of the experimental variables, namely mortality of the studied snails and the loss of food substrate mass associated with their feeding, was also assessed. The RDA method used was selected based on the gradient range (SD) for each analysis. Additionally, a statistical analysis was performed to determine the linear correlation

(Pearson's *r*) between the doses of entomopathogenic nematodes used in the entomological experiment and the developmental parameters of the analyzed snails.

3. Results

The study assessed the effects of preparations containing entomopathogenic nematodes on the development and survival of the common and spotted slugs. The average weight loss of lettuce leaves per slug and the survival of slugs over a 9-day period were assessed.

3.1. Feeding Intensity and Survival of Arion Distinctus

Steinernema feltiae

In the case of combinations containing this nematode species, its presence and dose significantly affected the feeding intensity of *A. distinctus* (F = 3.59; $p = 0.00$) and its survival (F = 12.41; $p = 0.00$) (Table 1).

Table 1. Results of statistical analysis (ANOVA) of selected *Arion distinctus* development parameters.

	df *	ANOVA F Value	*p* **
	Steinernema feltiae		
Average weight loss of lettuce leaves/1 individual	5	3.59	0.00
Survival rate	5	12.41	0.00
	Steinernema carpocapsae		
Average weight loss of lettuce leaves/1 individual	5	10.52	0.00
Survival rate	5	12.88	0.00
	Heterorhabditis bacteriophora		
Average weight loss of lettuce leaves/1 individual	5	7.36	0.00
Survival rate	5	6.94	0.00
	Phasmarhabditis californica		
Average weight loss of lettuce leaves/1 individual	5	4.19	0.00
Survival rate	5	6.20	0.00

* Degrees of freedom. ** The value of the test probability *p*.

The highest average loss of lettuce leaves was observed in the R_2000 combination, averaging 0.94 g (Figure 1A). The snail fed the least on the combination with the highest applied nematode dose (R_4000), where the loss of lettuce leaves averaged 0.67 g. In the remaining combinations with lower nematode doses (R_250, R_500, and R_1000) and the control combination, the feeding intensity of *A. distinctus* did not differ statistically (Tukey's HSD test) and ranged from 0.78 to 0.86 g (Figure 1A).

The survival of *A. distinctus* in the conducted experiment was highest in the control combination (average 100.0%) and R_250 (average 99.6%) with the lowest nematode dose (Figure 1B). Significantly lower survival of these snails was recorded in combinations in which the nematode dose in the food increased (R_500, R_1000), and the highest survival was observed in R_4000 (93.8%, 96.4%, and 96.0%, respectively). The lowest survival of *A. distinctus* individuals was observed in the R_2000 combination (average 79.6%), on which the snail also fed most intensively (Figure 1B).

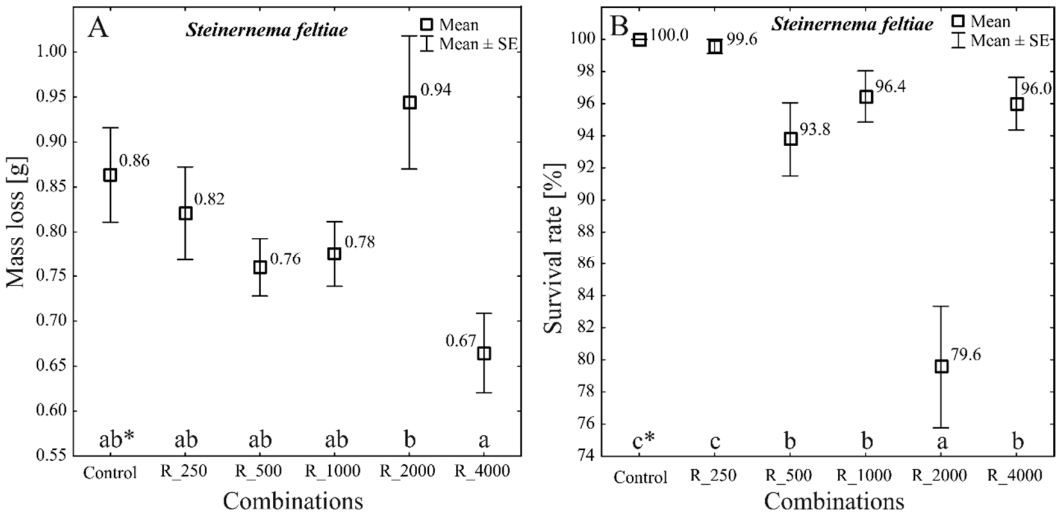

Figure 1. Average values of lettuce leaf loss per 1 individual of *A. distinctus* (**A**) and survival of this species (**B**) depending on different doses of the preparation containing the nematode *Steinernema feltiae*. * Groups of average parameters related to snail development that did not differ statistically were designated with the same letter index: a, b, c, … (Tukey's HSD test).

Steinernema carpocapsae

Analysis of the combinations containing the nematode *Steinernema carpocapsae* showed a significant effect of the tested nematodes on the feeding intensity of this species (F = 10.52; p = 0.00) and their survival (F = 12.88; p = 0.00) (Table 1).

The greatest loss in lettuce leaf weight (average 0.86 g) was observed in the control combination (Figure 2A). Increasing nematode concentrations resulted in a significant decrease in snail feeding intensity compared with the control, but differences between combinations with different nematode concentrations were not statistically significant and ranged from 0.66 g (R_250) to 0.56 g (R_1000).

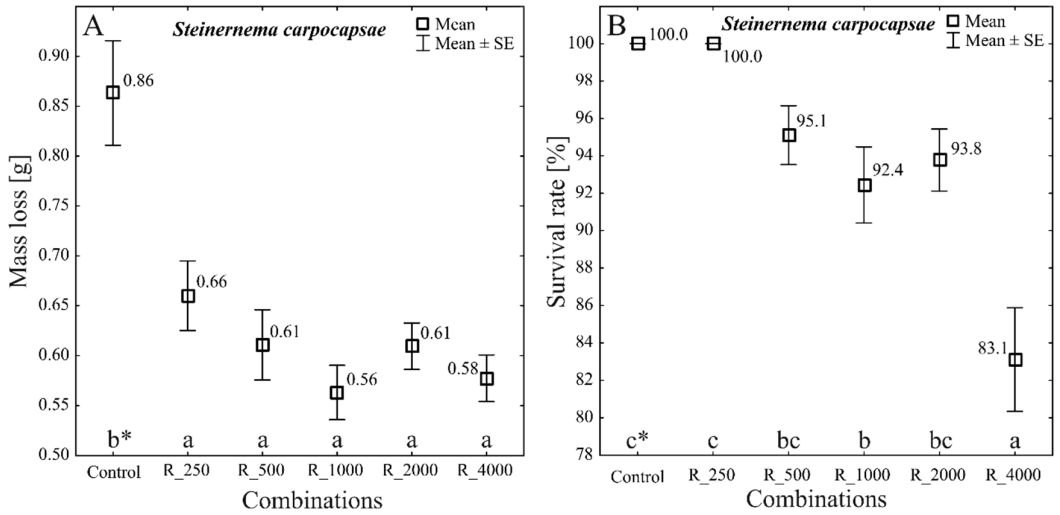

Figure 2. Average values of lettuce leaf loss per 1 individual of *A. distinctus* (**A**) and survival of this species (**B**) depending on different doses of the preparation containing the nematode *Steinernema carpocapsae*. * Groups of average parameters related to snail development that did not differ statistically were designated with the same letter index: a, b, c, … (Tukey's HSD test).

The highest (100%) survival of *A. distinctus* was observed in the control combination and the combination with the lowest nematode dose (R_250) (Figure 2B). A gradual

increase in the addition of nematodes across subsequent combinations (R_500, R_1000, and R_2000) resulted in a decrease in snail survival to 95.1%, 92.4%, and 93.8%, respectively. The lowest survival of *A. distinctus* was observed in combination with the highest nematode dose introduced into the food (R_4000) and amounted to 83.1% (Figure 2B).

Heterorhabditis bacteriophora

Another tested preparation containing *Heterorhabditis bacteriophora* nematodes also significantly (depending on the dose used) affected the feeding intensity of *A. distinctus* (F = 7.36; $p = 0.00$) and its survival (F = 6.94; $p = 0.00$) (Table 1).

Feeding of the tested snail species was most intense on the control treatment, averaging 0.86 g of lettuce leaves (Figure 3A).

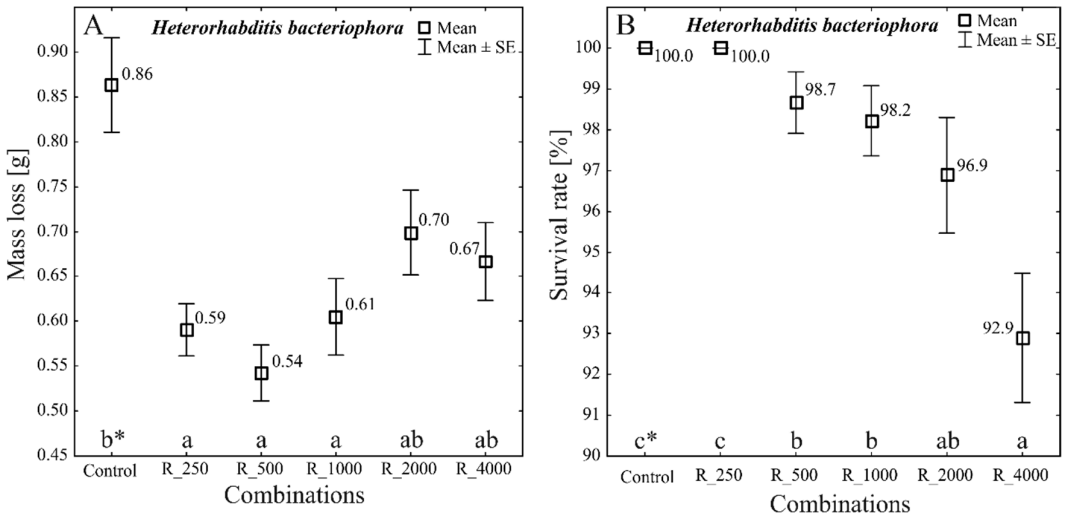

Figure 3. Average values of lettuce leaf loss per 1 individual of *A. distinctus* (**A**) and survival of this species (**B**) depending on different doses of the preparation containing the nematode *Heterorhabditis bacteriophora*. * Groups of average parameters related to snail development that did not differ statistically were designated with the same letter index: a, b, c, … (Tukey's HSD test).

The addition of nematodes resulted in a significant decrease in the weight of food consumed by *A. distinctus* compared to the control combination. The greatest decreases in feeding intensity were observed in combinations with low nematode doses (R_250, R_500, and R_1000), reaching 0.59 g, 0.54 g, and 0.61 g, respectively. However, a high nematode addition to lettuce leaves caused a slight increase in the weight of food eaten (R_2000 to 0.70 g and R_4000 to an average of 0.67 g) (Figure 3A).

However, analyzing the survival of *A. distinctus* across the tested combinations shows that the addition of the nematode *H. bacteriophora* at increasing doses linearly reduced *A. distinctus* survival (Figure 3B). Complete (100%) snail survival was observed in the control and R_500 combinations. Increasing the nematode dose (R_500, R_1000, and R_2000) decreased snail survival to 98.7%, 98.2%, and 96.9%, respectively. The lowest (92.9%) survival rate for *A. distinctus* feeding on lettuce leaves was observed in the combination with the highest addition of the tested nematode (R_4000).

Phasmarhabditis californica

The final formulation tested contained the nematode *P. californica*. The addition of this nematode at various doses, which *A. distinctus* fed on, also significantly affected the mean weight loss of lettuce leaves (F = 4.19; $p = 0.00$) and the survival of these snails (F = 6.20; $p = 0.00$) (Table 1). The highest mean weight loss of lettuce leaves was observed in the control treatment, where the snails consumed an average of 0.86 g of food (Figure 4A).

The addition of nematodes to the snails' food caused a decrease in their feeding intensity (Figure 4A). The greatest decrease was recorded in the combination with the lowest nematode dose (R_250)—an average of 0.58 g of lettuce leaves. In the remaining combinations (R_500, R_1000, R_2000, and R_4000), the decrease in *A. distinctus* feeding intensity was lower and amounted to 0.71 g, 0.73 g, 0.70 g, and 0.66 g, respectively.

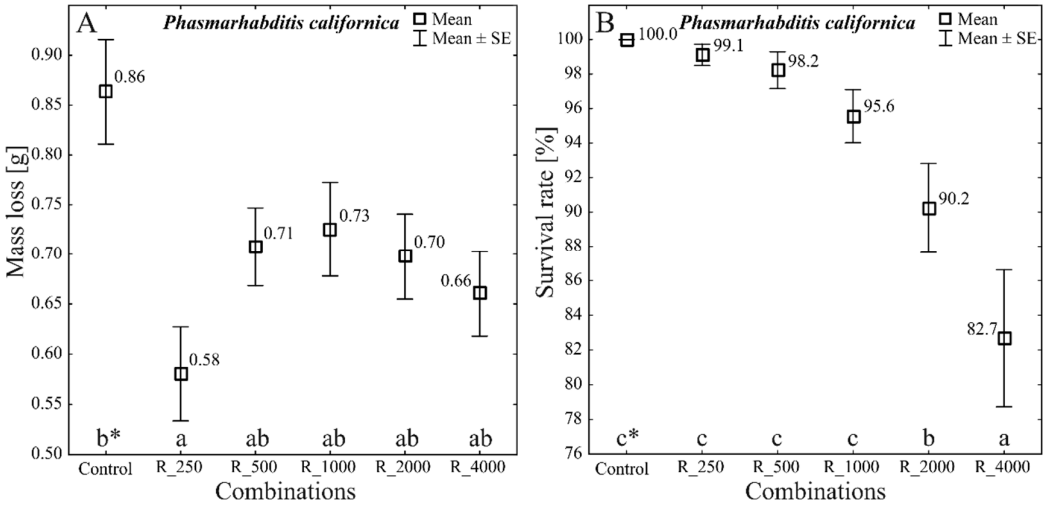

Figure 4. Average values of lettuce leaf loss per 1 individual of *A. distinctus* (**A**) and survival of this species (**B**) depending on different doses of the preparation containing the nematode *Phasmarhabditis californica*. * Groups of average parameters related to snail development that did not differ statistically were designated with the same letter index: a, b, c, … (Tukey's HSD test).

Analysis of the survival of the tested snail species on lettuce leaves with various additions of *P. californica* indicates an almost linear relationship between the nematode preparation dose and the decrease in snail survival (Figure 4B). The absence of nematodes (control) was the combination in which 100% of the snails survived. Lower concentrations of the added preparation (R_250, R_500, and R_1000) caused a similar decrease in the survival of *A. distinctus* (averages of 99.1%, 98.2%, and 95.6%, respectively). A significant decrease in feeding snail survival was observed in the combinations R_2000 (average 90.2%) and R_4000 (average 82.7%).

3.2. Feeding Intensity and Survival of Deroceras reticulatum

Steinernema feltiae

The addition of a preparation containing the nematode *S. feltiae* in various concentrations to the food on which *D. reticulatum* fed significantly influenced the feeding intensity of the snail (F = 2.79; $p = 0.02$) and its survival (F = 10.85; $p = 0.00$) (Table 2).

Table 2. Results of statistical analysis (ANOVA) of selected *Deroceras reticulatum* development parameters.

	df *	**ANOVA F Value**	p **
	Steinernema feltiae		
Average weight loss of lettuce leaves/1 individual	5	2.79	0.02
Survival rate	5	10.85	0.00
	Steinernema carpocapsae		
Average weight loss of lettuce leaves/1 individual	5	6.10	0.00
Survival rate	5	14.16	0.00

	Heterorhabditis bacteriophora		
Average weight loss of lettuce leaves/1 individual	5	3.54	0.00
Survival rate	5	15.31	0.00
	Phasmarhabditis californica		
Average weight loss of lettuce leaves/1 individual	5	4.18	0.00
Survival rate	5	8.36	0.00

* Degrees of freedom. ** The value of the test probability *p*.

The lowest mean weight loss of lettuce leaves was observed in the combination with the highest dose of the nematode preparation (R_4000)—0.67 g (Figure 5A). Significantly greater weight losses of lettuce leaves were observed in the combination R_500 (mean 0.82 g) and the control (mean 0.80 g). The survival of feeding snails was the highest (100%) in the control combination and in the combinations with the lowest nematode dose: R_250 and R_500 (Figure 5B). Increasing the dose (R_1000) decreased *D. reticulatum* survival to 94.7%. This trend was observed in subsequent combinations with increasing nematode dose (R_2000—87.1%; R_4000—90.7%).

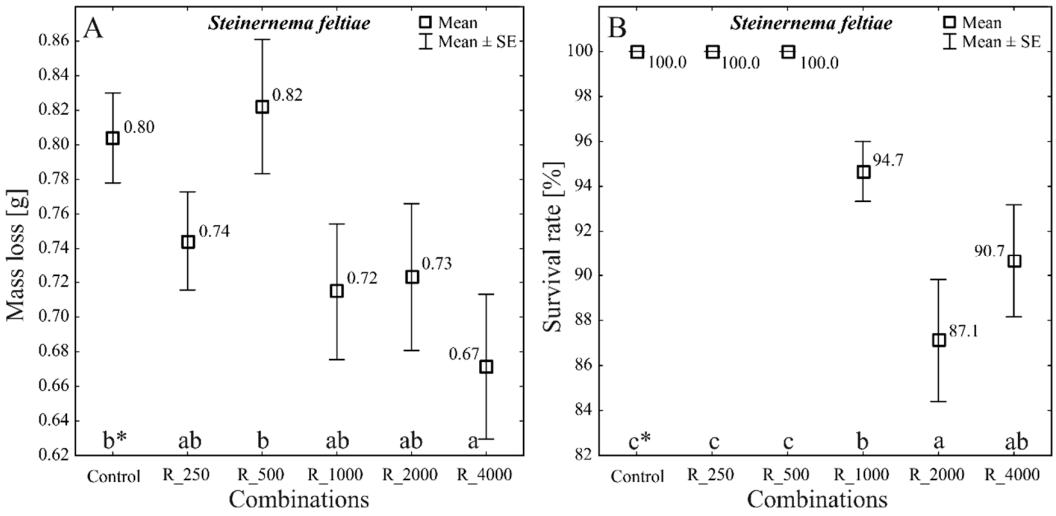

Figure 5. Average values of lettuce leaf loss per 1 individual of *D. reticulatum* (**A**) and survival of this species (**B**) depending on different doses of the preparation containing the nematode *Steinernema feltiae*. * Groups of average parameters related to snail development that did not differ statistically were designated with the same letter index: a, b, c, … (Tukey's HSD test).

Steinernema carpocapsae

Adding a preparation containing the nematode *S. carpocapsae* to lettuce leaves at various concentrations significantly affected the feeding intensity of *D. reticulatum* (F = 6.109; *p* = 0.00) and its survival (F = 14.16; *p* = 0.00) (Table 2).

The greatest reduction in the mass of food eaten by snails was observed in the combinations with the high doses of the nematode (R_2000, R_1000, and R_4000). The lettuce leaf mass losses in these combinations were 0.60 g, 0.70 g, and 0.71 g, respectively (Figure 6A). Snails fed significantly more intensively on the control combination (mean 0.80 g), R_250 (mean 0.84 g), and R_500 (mean 0.77 g).

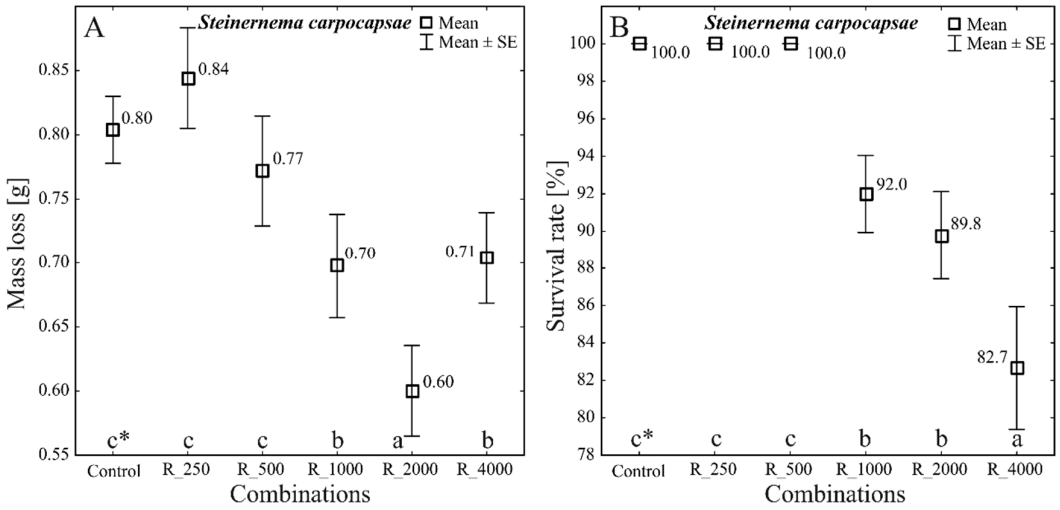

Figure 6. Average values of lettuce leaf loss per 1 individual of *D. reticulatum* (**A**) and survival of this species (**B**) depending on different doses of the preparation containing the nematode *Steinernema carpocapsae*. * Groups of average parameters related to snail development that did not differ statistically were designated with the same letter index: a, b, c, … (Tukey's HSD test).

D. reticulatum survival was correlated with increasing the dose of the preparation used. No snail death was observed in the control, R_250, and R_500 combinations. Survival decreased to 92.0% in the R_1000 combination, to 89.8% in the R_2000 combination, and to 82.7% in the R_4000 combination (Figure 6B).

Heterorhabditis bacteriophora

Another tested formulation, containing nematodes of the species *Heterorhabditis bacteriophora*, also significantly (depending on the dose used) affected the feeding intensity of *D. reticulatum* (F = 3.54; $p = 0.00$) and its survival (F = 15.31; $p = 0.00$) (Table 2).

For the tested formulation, a very strong inhibition of food intake in snails (mean 0.63 g) was observed with the highest dose, R_4000 (Figure 7A). In the remaining combinations, including the control, the average weight loss of lettuce leaves was significantly lower and similar (mean: 0.81 g to 0.73 g).

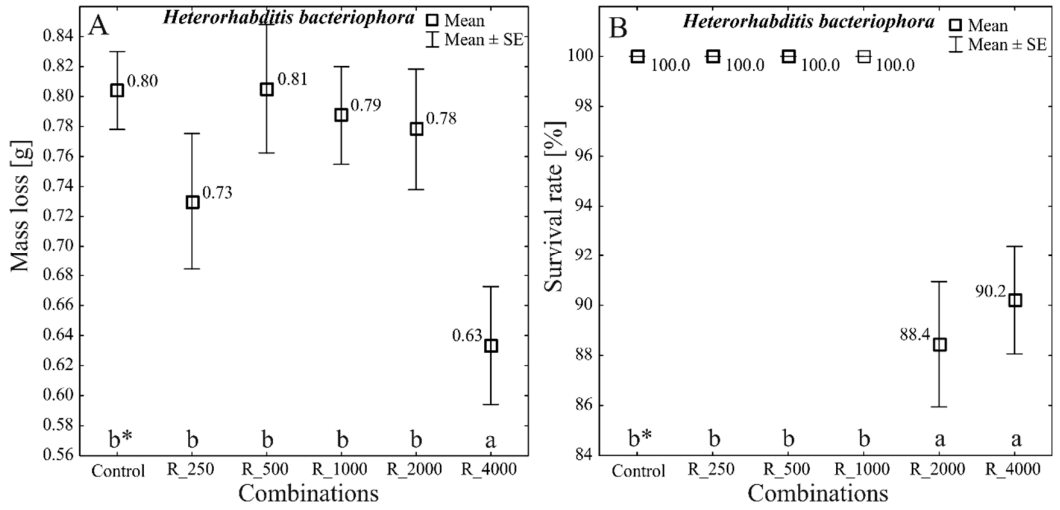

Figure 7. Average values of lettuce leaf loss per 1 individual of *D. reticulatum* (**A**) and survival of this species (**B**) depending on different doses of the preparation containing the nematode *Heterorhabditis bacteriophora*. * Groups of average parameters related to snail development that did not differ statistically were designated with the same letter index: a, b, … (Tukey's HSD test).

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In the control combination and with low doses of the preparation (R_250, R_500, and R_1000), the survival of *D. reticulatum* was 100% (Figure 7B). Dead snails were observed in the R_2000 and R_4000 combinations, with survival rates of 88.4% and 90.2%, respectively.

Phasmarhabditis californica

The fourth formulation tested contained the nematode *P. californica*. Adding this nematode at various doses to the diet fed on by *D. reticulatum* also significantly affected the mean weight loss of lettuce leaves ($F = 4.18$; $p = 0.00$) and the survival of these snails ($F = 8.36$; $p = 0.00$) (Table 2).

The snails fed most intensively on the control treatment (average 0.80 g) (Figure 8A). *A decrease* in *D. reticulatum* feeding intensity was observed in the nematode treatments compared to the control. The lowest mean weight loss of lettuce leaves was observed on the R_500 treatment, averaging 0.61 g (Figure 8A). In the remaining combinations, the average food weight loss was lower, ranging from 0.67 to 0.74 g.

In the control combination and at the lowest nematode dose (R_250), snail survival was 100% (Figure 8B). Increasing the nematode doses resulted in a linear decrease in *D. reticulatum* survival, from 93.7% in the R_500 combination to 76.0% in the R_4000 combination.

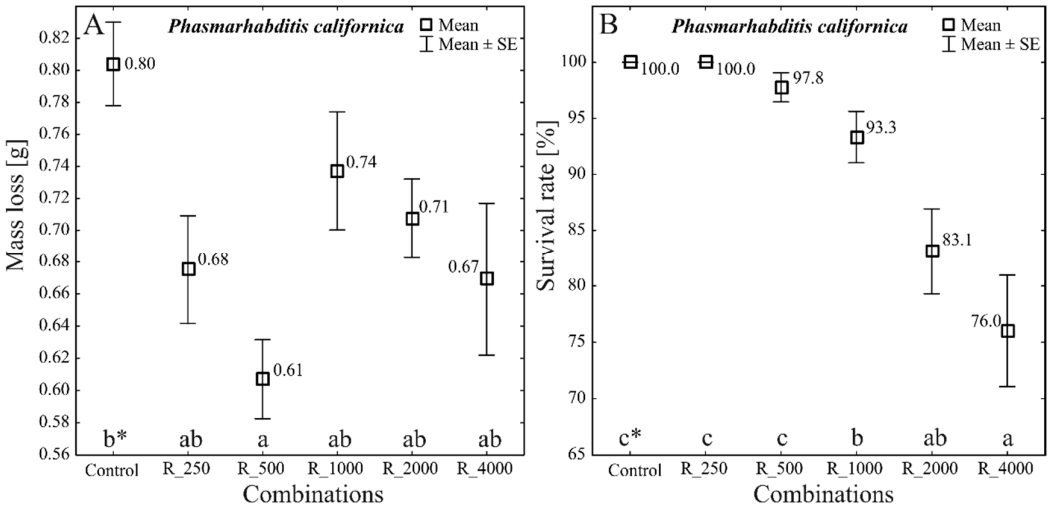

Figure 8. Average values of lettuce leaf loss per 1 individual of *D. reticulatum* (**A**) and survival of this species (**B**) depending on different doses of the preparation containing the nematode *Phasmarhabditis californica*. * Groups of average parameters related to snail development that did not differ statistically were designated with the same letter index: a, b, c, … (Tukey's HSD test).

3.3. The Relationship Between the Tested Parameters and the Development of Slugs

To determine the relationship between the dose of the preparations containing different nematode species and the average mass loss of lettuce leaves (understood as the intensity of snail feeding) and snail mortality, a correspondence analysis (RDA) was performed. The resulting ordination graphs present a graphical interpretation of these correlations. In the case of the preparation containing *S. feltiae*, increased mortality of *A. distinctus* was correlated with the use of nematodes at a dose of 2000 LJ/m^2 and increased snail feeding intensity (Figure 9A).

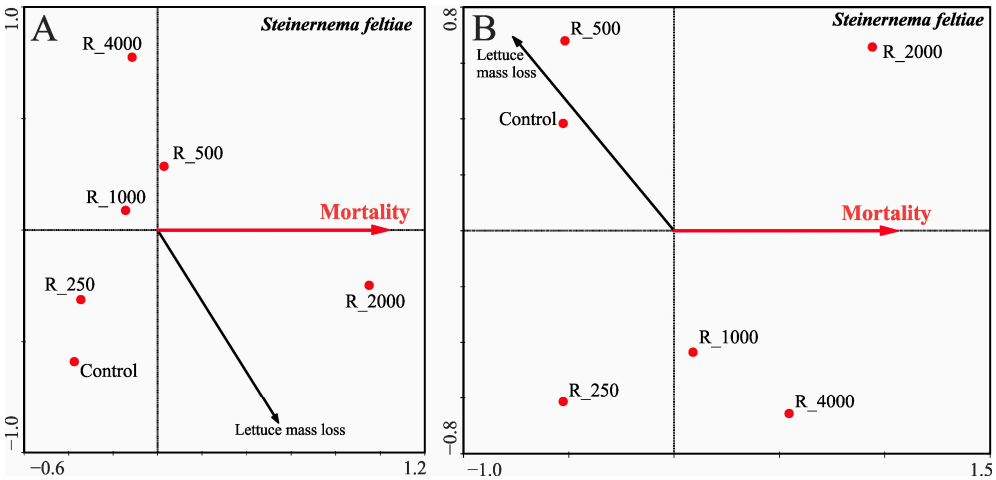

Figure 9. RDA diagram presenting the relationship between the tested parameters related to the development of *A. distinctus* (**A**) and *D. reticulatum* (**B**) treated with different doses of *S. feltiae*.

The second snail species, *D. reticulatum*, responded with an increase in mortality of individuals given high nematode doses (1000, 2000, and 4000 LJ/m^2), and a high feeding intensity was observed with the control combination and the combinations with low nematode doses (R_250, R_500) (Figure 9B). However, the observed trends were not statistically significant, as indicated by the calculated Pearson correlation coefficient (*r*) (Table 3).

Table 3. Pearson's linear correlation coefficient values (*r*) between the doses of tested nematodes and the development parameters of *A. distinctus* and *D. reticulatum*.

Development Parameter	Nematodes Doses			
	A. distinctus		***D. reticulatum***	
	r	***p* ***	***r***	***p***
	Steinernema feltiae			
Average weight loss of lettuce leaves/1 individual	−0.16	n. s.	−0.22	n. s.
Mortality	0.19	n. s.	0.23	n. s.
	Steinernema carpocapsae			
Average weight loss of lettuce leaves/1 individual	−0.29	n. s.	−0.24	n. s.
Mortality	0.57	0.05	−0.09	n. s.
	Heterorhabditis bacteriophora			
Average weight loss of lettuce leaves/1 individual	−0.02	n. s.	0.44	n. s.
Mortality	0.59	0.05	0.54	0.05
	Phasmarhabditis californica			
Average weight loss of lettuce leaves/1 individual	−0.11	n. s.	0.48	0.05
Mortality	0.49	0.05	0.51	0.05

* The value of the test probability *p*.

The second formulation tested contained the nematode *S. carpocapsae*. In the case of *A. distinctus*, the RDA diagram indicated a correlation between high doses of the nematode (1000, 2000, and 4000 LJ/m^2) and increasing mortality of this snail species and a decrease in its feeding intensity (Figure 10A). The Pearson correlation coefficient between dose of the formulation and mortality of *A. distinctus* was positive and statistically significant ($r = 0.57$; $p = 0.05$) (Table 3). Very similar relationships were also found for the second snail species tested (*D. reticulatum*), which was treated with the nematode *S. carpocapsae*. However, in this combination, the feeding intensity of this species was higher

in the low-nematode-dose treatments (Figure 10B). The described correlations were not confirmed in this case by the values of the calculated Pearson *r* coefficient (Table 3).

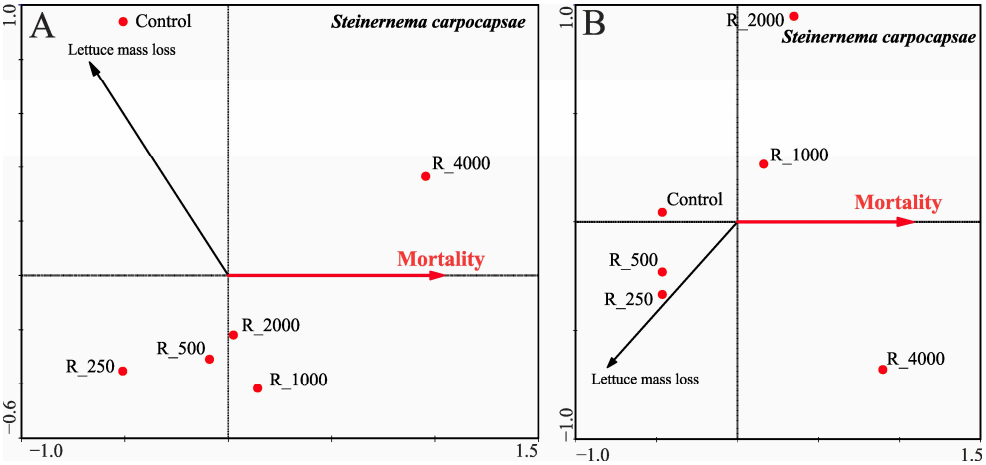

Figure 10. RDA diagram presenting the relationship between the tested parameters related to the development of *A. distinctus* (**A**) and *D. reticulatum* (**B**) in combinations treated with different doses of *S. carpocapsae*.

The next nematode species tested was *H. bacteriophora*. Ordination plots of RDA diagrams for both tested snail species showed that the vectors describing the mortality variable were correlated with high doses of the tested preparation (2000 and 4000 LJ/m^2) (Figure 11A,B). This is confirmed by the calculated value of the Pearson coefficient (*A. distinctus*: $r = 0.59$, $p = 0.05$; *D. reticulatum*: $r = 0.54$, $p = 0.05$) (Table 3).

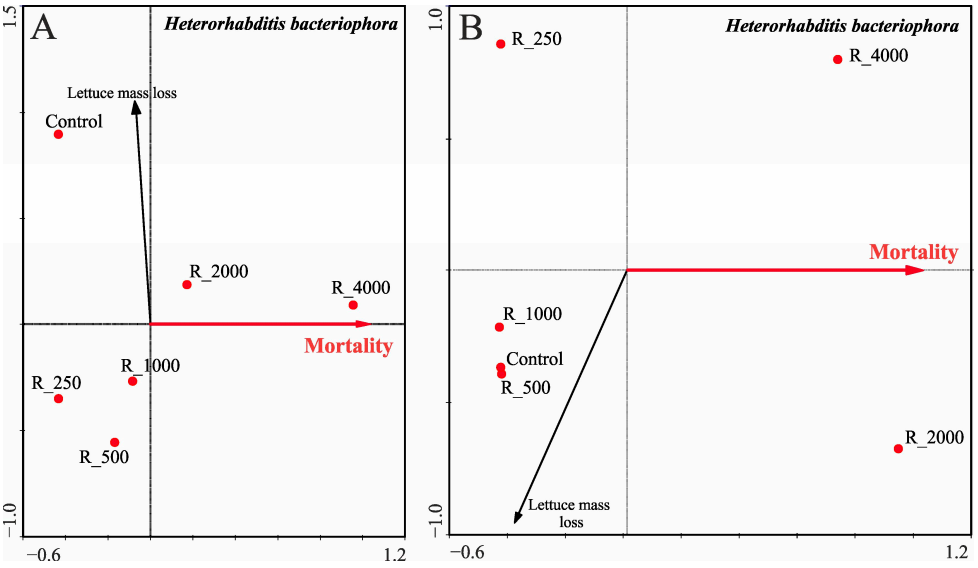

Figure 11. RDA diagram presenting the relationship between the tested parameters related to the development of *A. distinctus* (**A**) and *D. reticulatum* (**B**) in combinations treated with different doses of *H. bacteriophora*.

However, no significant correlations were found between the nematode doses used and the feeding intensity of the studied snail species (Table 3).

The final formulation tested contained the nematode *P. californica*. Analysis of the RDA diagram indicated a high correlation between the highest nematode doses (2000 and

4000 LJ/m²) and the vector describing the increased mortality of both snail species (Figure 12A,B).

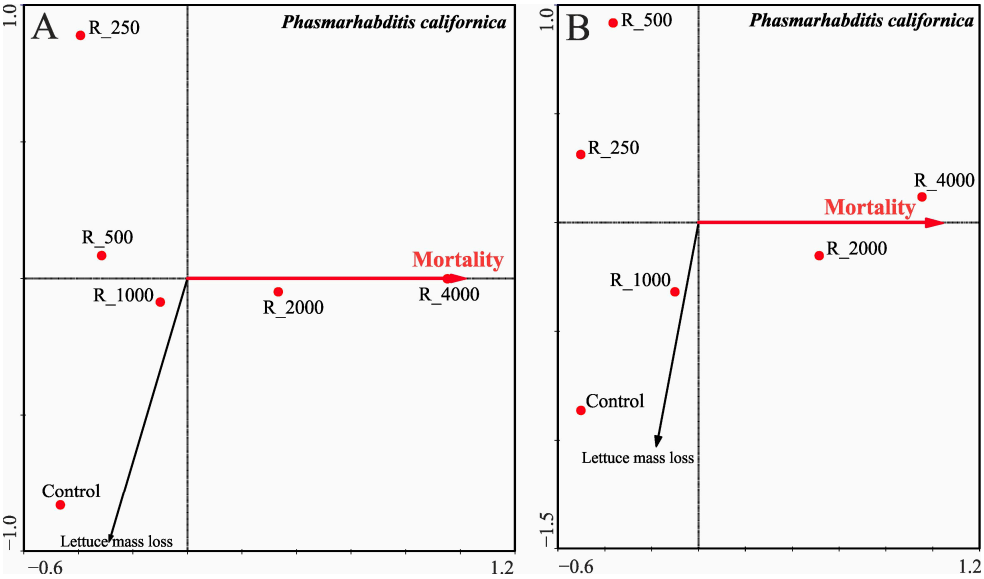

Figure 12. RDA diagram presenting the relationship between the tested parameters related to the development of *A. distinctus* (**A**) and *D. reticulatum* (**B**) in combinations treated with different doses of *P.californica*.

Adding the preparation to the food and increasing the dose had different effects on the studied snail species. In the case of *A. distinctus*, increasing the dose decreased snail feeding intensity (without statistical significance), whereas in the case of *D. reticulatum*, a significant correlation (r = 0.51, $p = 0.05$) was observed (Table 3).

4. Discussion

Entomopathogenic nematodes adapted to infect invertebrates from the class Insecta can also parasitize snails. This was confirmed in studies using nematodes from the families Heterorhabditidae and Steinernematidae to control *Theba pisana* (Müller) [42] and the observed reduction in damage caused by *Parmacella ibera* using the nematodes *S. carpocapsae*, *H. bacteriophora*, and *S. feltiae* [21]. Our study showed that the pathogenicity of the tested entomopathogenic nematodes can also affect the vital parameters of the snails tested (Table 1).

The success of applying using roundworm-based preparations depends on many factors, the most important of which is the selection of the correct nematode species for the type of pest being controlled [22]. Statistical analysis showed that, among the entomopathogenic preparations used, the lowest average survival of the tested snails, and therefore the highest potential for their control, was achieved using a biopesticide containing nematodes of the *S. carpocapsae* species (*A. distinctus*, *D. reticulatum*—93% survival) (Figures 1–4). Compared to the registered molluscicide Nemaslug, the preparation containing these nematodes demonstrated higher effectiveness in controlling snails of the *A. distinctus* species (Capsanem 93%, Nemaslug 95% survival), and slightly lower effectiveness in controlling *D. reticulatum* (Capsanem: 93% survival, Nemaslug: 91% survival) (Figures 2, 4, 6 and 8).

The effectiveness of plant protection, whether chemical or biological, is largely dependent on the dose of the preparation used [43,44]. Observations confirmed the effect of the tested biopesticides' concentrations on the vital parameters of both snail species

used in the experiment (Figures 1–8). The lowest average survival was observed with entomopathogenic nematodes *S. feltiae* (79.6%) at a dose of 2000 LJ/m^2 (Figure 1B). *Steinernema* nematodes, which serve as vectors for *Xenorhabdus* and *Photorhabdus* bacteria, form a highly effective symbiosis when used in crop protection [45]. Studies by El-Ashry and El-Aal [46] demonstrated complete mortality of *D. reticulatum* and *Deroceras laeve* Müller after 14 days of exposure to *S. carpocapsae* at a dose of 2000 LJ/m^2. A lethal effect of this nematode was also demonstrated on populations of *P. ibera* [21] and *Monacha cantiana* [47]. El-Mahdi and Eid [42] demonstrated no mortality of *Theba pisana* Müller when only using low doses of a preparation containing nematodes from the families Heterorhabditidae and Steinernematidae.

The use of entomopathogenic nematodes, similarly to the biomolluscicide Nemaslug, did not achieve 100% lethal effects in the pest population but significantly reduced feeding intensity (Figures 1–8). According to BASF SE guidelines, the application mixture containing the marketed nematode *P. californica* is lethal to small- and medium-sized individuals of many snail species, while significantly reducing the feeding of large snails [32], as was observed in our experiment (Figures 4 and 8). The lowest survival rate of the second snail species, *D. reticulatum*, was recorded after the application of Capsanem at a dose of 4000 LJ/m^2 (82.7%) (Figure 6B). Canonical redundancy analysis confirmed a correlation between the mortality of these snails and higher doses of nematological biopreparations (Figures 9–12). Furthermore, the lowest pest survival rates observed following treatment with insect-parasitic nematodes were similar to those obtained with the registered biomolluscicide Nemaslug at the maximum dose of 4000 LJ/m^2 (*A. distinctus*: 82.7%; *D. reticulatum*: 76.0%) (Figures 4 and 8).

The conducted research has shown that preparations containing entomopathogenic nematodes have the potential to reduce the feeding intensity not only of harmful insects but also of snails belonging to the species *A. distinctus* and *D. reticulatum*. Expanding the scope of application of the analyzed preparations to new pest groups could be an important element of sustainable agricultural practices, which are particularly sought after on organic farms and in home gardens. Despite the promising potential of EPN in biological plant protection against snails, existing limitations must also be considered. The experiment conducted in the laboratory did not fully reflect the conditions prevailing in real agricultural systems (including temperature, humidity, host density, interactions with other organisms, and EPN mobility and survival in soil). Therefore, further experiments are necessary to assess their effectiveness in field conditions.

5. Conclusions

1. The feeding intensity and mortality of the studied snail species were significantly dependent on the type of preparation containing entomopathogenic nematodes used and the dose used.
2. In *A. distinctus*, all tested preparations significantly reduced feeding intensity and increased mortality compared with the control group, with the greatest effect observed with preparations containing *Steinernema carpocapsae*.
3. *Deroceras reticulatum* showed a similar response to the preparations used as *A. distinctus*, with the highest mortality and lowest feeding intensity recorded in combinations with preparations containing *S. carpocapsae*, particularly at the highest doses.
4. All analyzed preparations containing entomopathogenic nematodes significantly reduced the feeding intensity of both snail species. However, even the highest doses did not lead to complete elimination of individuals, and the maximum reduction in survival was 76%.

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5. The obtained results indicate that preparations containing entomopathogenic nematodes of the species *Steinernema carpocapsae*, used in doses of 2000 and 4000 LJ/m^2, can be considered as an effective method for limiting the feeding intensity and increasing the mortality of *A. distinctus* and *D. reticulatum*.

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