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INFLUENCE OF LOW DOSES OF IONIZING RADIATION ON YOUNG AND TWO WEEK OLD INVASIVE LARVAE OF ENTOMOPATHOGENIC NEMATODES (*Heterorhabditidae*, *Steinernematidae*)

WPŁYW NISKICH DAWEK PROMIENIOWANIA JONIZUJĄCEGO NA MŁODE ORAZ DWUTYGODNIOWE LARWY NICIENI ENTOMOPATOGENNYCH (*Heterorhabditidae*, *Steinernematidae*)

Abstract: The performed experiments were aimed at checking the effect of ionising radiation as an abiotic factor on the bionomics of young and two week old larvae of *Steinernema feltiae* and *Heterorhabditis megidis*. Features associated with nematode pathogenicity (intensity of infection) and morphometry (length and width of individuals of the giant generation) were analysed. Three doses of ionising radiation (0.1, 0.05, and 0.01 kGy) were applied. The obtained results indicate the modifying effect of ionising radiation on each of the studied features of entomopathogenic nematodes.

Keywords: ionizing radiation, entomopathogenic nematodes, *Steinernema feltiae*

Together with the advancement of ecological farming and nature protection, plant protection chemicals are being withdrawn from use. Subsidies for farmers and producers associated with ecological farming have opened a new market for biological plant protection measures and consequently for mass production of biopreparations. Rapid growth of the economic importance of entomopathogenic nematodes for agricultural practice creates a constant demand for new strains. These strains should have high potential for reproduction on artificial substrata, resistance to storage and transport and high efficiency of pest insect control competitive with that of chemical

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plant protection measures. Biological methods of plant protection were known already in 1900 but their wide application started not earlier than 30 years ago. In orchards, fields, vineyards and greenhouses it is recommended to restrict the use of plant protection chemicals due to environmental protection and for economic reasons [1]. Unfortunately, under specific field conditions the commercial biopreparations are not efficient.

One of the possible solutions is an attempt of genetic improvement through artificial selection, strain hybridisation and genetic engineering. One of abiotic factors affecting nematodes is ionising radiation which interferes in cell processes and leads to biological and chemical changes.

The invasive stage, due to its unique characteristic, plays an exceptional role in the growth cycle of entomopathogenic nematodes and in survival and expansion of their populations. These features are also the basis of the commercial value of nematodes. Even small morphologic modifications may have a significant impact on the activity of invasive larvae of entomopathogenic nematodes.

The aim of the study was to check whether low doses of ionising radiation produce different effects in young and in two weeks old larvae of entomopathogenic nematodes and if the changes in nematode features could be been used in commercial production of biopreparations.

Material and methods

Invasive larvae of *Steinernema feltiae* from Olsztyn and of *Heterorhabditis megidis* in two age groups were used in experiments. The first group consisted of young (2–3 days old) invasive larvae devoid of cuticulum [2]. After irradiation the larvae were left for at least two weeks until they gained infective properties [2]. The second group was composed of invasive larvae at least two weeks old but not older than two months. The larvae had double cuticulum [2].

The Cobalt bomb (RChM-g-20) was the source of radiation used in the experiment. The bomb emits gamma radiation from cobalt isotope ^{60}Co. Radiation doses of 0.1, 0.05 and 0.01 kGy were applied to all nematode species and strains. Selection of these radiation doses was preceded by preliminary studies on nematode reproduction abilities as the main criterion.

The experimental plan was based on using irradiation in the following set-up:

1. Non-irradiated insect + irradiated nematode.

Irradiated nematodes were used to infect the host insect *G. mellonella* (90 caterpillars – 30 in each of the three measurement series). Infected insects were dissected to estimate the invasiveness and to measure hermaphroditic individuals (*Heterorhabditidae*) or giant females (*Steinernematidae*).

2. Non-irradiated insect + non-irradiated nematode (control).

The obtained results were statistically processed with the SPSS v. 12.0 software. Statistical significance of differences was tested with non-parametric ANOVA.

Results and discussion

The intensity of infection is an important parasitic parameter which determines nematode invasiveness towards insects. It was observed during dissection that all caterpillars infected by irradiated nematodes contained giant individuals of *Steinernematidae* or hermaphroditic individuals of *Heterorhabditidae*. It means that gamma irradiation did not hamper the development of invasive larvae. Within-strain comparison between the young and two weeks old invasive larvae showed that two doses of 0.1 and 0.01 kGy applied to *S. feltiae* Olsztyn affected the intensity of infection. The differences were statistically significant (Table 1).

Table 1

Comparison of the mean intensity of infection of *G. mellonella* by *S. feltiae* Olsztyn larvae

Dose	*S. feltiae* Olsztyn two-weeks old L_3	*S. feltiae* Olsztyn young L_3
0.1 kGy	12.4 a	27.3 b
0.05 kGy	18.5 a	15.6 a
0.01 kGy	16.6 a	24.6 b
Control	18.0 a	18.0 a

Different letters denote significant differences at $p \leq 0.05$.

In *H. megidis* only the dose of 0.01 kGy resulted in highly significant difference. Two week old larvae irradiated with the lowest dose showed high intensity of infection but in young nematode larvae this effect was not found (Table 2).

Table 2

Comparison of the mean intensity of infection of *G. mellonella* by *H. megidis* larvae

Dose	*H. megidis* two weeks old L_3	*H. megidis* young L_3
0.1 kGy	8.6 a	9.7 a
0.05 kGy	7.2 a	5.8 a
0.01 kGy	17.3 a	5.3 b
Control	6.4 a	6.4 a

Different letters denote significant differences at $p \leq 0.05$.

The performed measurements of hermaphroditic individuals of *H. megidis* revealed that irradiation of young nematode larvae generated adults of smaller body sizes. The effect was visible at the doses of 0.01 and 0.05 kGy but at the dose of 0.1 kGy the calculated parameters did not differ statistically from the control (Table 3).

Table 3

Comparison of the body size of giant females of *S. feltiae* Olsztyn

Dose	Size [mm]	*S. feltiae* Olsztyn two weeks old L_3	*S. feltiae* Olsztyn young L_3
0.1 kGy	length	10.35 a	11.42 a
	width	0.56 a	0.65 b
0.05 kGy	length	10.61 a	10.25 a
	width	0.52 a	0.60 b
0.01 kGy	length	12.33 a	13.06 a
	width	0.50 a	0.64 b
Control	length	11.64 a	11.64 a
	width	0.51 a	0.51 a

Different letters denote significant differences at $p \leq 0.05$.

In *S. feltiae* Olsztyn the irradiation of young invasive larvae resulted in obtaining adults of body size different from those obtained by the irradiation of two weeks old L_3 larvae. The differences manifested themselves in body widths of the analysed individuals of *S. feltiae* Olsztyn; their body length remained the same (Table 4).

Table 4

Comparison of the body size of hermaphroditic individuals of *H. megidis*

Dose	Size [mm]	*H. megidis* two weeks old L_3	*H. megidis* young L_3
0.1 kGy	length	7.63 a	7.07 a
	width	0.54 a	0.51 a
0.05 kGy	length	8.28 a	7.31 b
	width	0.63 a	0.49 b
0.01 kGy	length	10.35 a	8.27 b
	width	0.69 a	0.53 b
Control	length	11.60 a	11.60 a
	width	0.76 a	0.76 a

Different letters denote significant differences at $p \leq 0.05$.

Conclusions

1. Ionising radiation is the factor affecting the bionomics of entomopathogenic nematodes.
2. The intensity of infection increased when young larvae of *S. feltiae* were irradiated. This effect was not observed in *H. megidis*.
3. Irradiation of young larvae of *S. feltiae* and *H. megidis* increased body sizes of giant nematodes as compared with the irradiation of two week old larvae.

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Abstrakt: Przeprowadzone doświadczenie miało na celu sprawdzenie wpływu promieniowania jonizującego, jako czynnika abiotycznego, na bionomię młodych oraz dwutygodniowych larw inwazyjno-przetrwalnikowych *Steinernema feltiae* oraz *Heterorhabditis megidis*. Badano cechy związane z patogenicznością nicieni (intensywność zarażenia) oraz morfometrią (długość oraz szerokość osobników pokolenia olbrzymiego). Zastosowano trzy dawki promieniowania jonizujacego: 0,1; 0,05; 0,01 kGy.

Otrzymane wyniki wskazują na modyfikujący charakter promieniowania jonizującego u każdej z badanych cech nicieni entomopatogenicznych.

Słowa kluczowe: nicienie entomopatogeniczne, *Steinernema feltiae*, promieniowanie jonizujące