



# Sensitivity of *Pieris brassicae*, *P. napi* and *P. rapae* (Lepidoptera: Pieridae) larvae to native strains of *Steinernema feltiae* (Filipjev, 1934)

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**Abstract** The study was aimed at determining the sensitivity of three species of *Pieris* sp. to native strains of *Steinernema feltiae*. Studied strains were highly pathogenic to all *Pieris* species (the extensity varied from 80 to 100%).

**Keywords** Native strains · *Steinernema feltiae* · *Pieris* · Biological control

## Introduction

Larvae of butterflies of the genus *Pieris* (Lepidoptera: Pieridae) may substantially decrease yields and market value of plants of the family Brassicaceae, in particular of various varieties of *Brassica oleracea* such as: cabbage (*B. oleracea* var. *capitata*), common turnip (*Brassica napus* var. *napobrassica*), cauliflower (*B. oleracea* var. *botrytis*), rape (*B. napus* ssp. *oleifera*) and horse radish (*Armoracia rusticana*). As other pests of the cabbage family, they are commonly controlled with chemical insecticides (Kochman and Węgorzek 1997).

In Poland there are four species of the genus *Pieris*: the cabbage butterfly (*P. brassicae*), small white butterfly (*P. rapae*), green-veined white butterfly (*P. napi*) and typically mountain dark-veined white butterfly (*P. bryoniae*) (Warecki 2010). Economic losses are mainly caused by larvae of the II generation of *P. brassicae* and *P. rapae*. Gregarious feeding of the caterpillars of the cabbage

butterfly leads to leaves skeletonizing while single caterpillars of the small white butterfly gnaw holes and enter the loose heads of cabbage, and cauliflower and flower buds of broccoli (Boczek and Lewandowski 2016; Kochman and Węgorzek 1997). Moreover, caterpillars of both species leave many feces on plants (Jõgar et al. 2008).

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* are obligatory and lethal insect parasites associated, in at least one of their growth stages, with soil (Adams et al. 2006). Commercial preparations containing EPNs of three species: *Steinernema feltiae*, *S. carpocapsae*, and *Heterorhabditis bacteriophora* are now commonly used in the practice of plant pests control. These species are mainly used to control pests living in soil, which is associated with biology of these nematodes. Studies performed by Arthurs et al. (2004) indicate that EPNs may be equally effective in controlling ground pests. They demonstrated the importance of selection of appropriate species and even strains since each of them has different optimum temperature for activity and respond differently to habitat humidity.

Laboratory studies provide information necessary to estimate the specificity of species/strains of EPNs to a given crop pest (Tóth 2006). The study was undertaken to estimate the effectiveness of two native strains of EPNs *S. feltiae* (ZAG 15 and K 11) in controlling larvae of three species of *Pieris*. The sensitivity of *Pieris* sp. larvae to infection by these strains was assessed based on extensity and intensity of infection.

## Materials and methods

Fertilized females of *P. rapae*, *P. napi* and *P. brassicae* butterflies were caught in summer 2013. Butterflies were caught on the experimental vegetable plots of the Warsaw

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University of Life Sciences located in the southern part of Warsaw (N52°9.0306' E21°3.0246'). Butterflies were placed in plastic containers till the time of egg laying. Hatched larvae were fed with leaves of cabbage (*Brassica oleracea* var. *capitata*). Larvae of the III stage were used in the study.

Two native strains of *S. feltiae* (Filipjev, 1934) isolated from soil samples collected in autumn 2011 were used in experiments. The strain of *S. feltiae* Zag15 was isolated from the meadows in the valley of Zwolenka river flowing through the Kozienska Forest in Central Poland (N51°23'10.4820" E21°33'15.5412") and the strain of *S. feltiae* K11 from wheat crop growing in the vicinity of Katowice in Southern Poland (N50°20'5.5968" E19°2'14.0388"). EPNs were isolated with the trap method using live bait (larvae of *Galeria mellonella* L.) (Bedding and Akhurst 1975). Nematodes were identified to species based on morphometric criteria (Adams and Nguyen 2002) and genetic methods (Tumialis et al. 2016). *Pieris* larvae together with a piece of cabbage leaf were placed onto Petri dishes lined with filter paper, 5 larvae per dish. Nematodes were applied on filter paper at a dose of 50 IJs/*Pieris* larva. Then, Petri dishes were placed in the incubation chamber at a temperature of 20 °C. Each of the strains was used to infect 30 larvae of every butterfly species (in two repetitions). The caterpillars of tested species (30 individuals for each species) were used as a control variant. As similarly as in variants with nematodes, control insects were placed together with a piece of cabbage leaf onto Petri dishes lined with filter paper moistened with distilled water, five larvae per dish.

Forty eight hours since infection dead larvae were sectioned and the following parameters were determined with the use of binocular (magnification  $\times 3$ –5):

- extensity of infection (percent of infected larvae in analyzed sample),
- intensity of infection (mean number of nematodes per larva).

Obtained results were statistically processed with STATISTICA 10.0 software. Chi-square test was used in the analysis of extensity. Nonparametric Kruskal–Wallis test was used to analyze the intensity of infection.

## Results

There was no mortality of *Pieris* larvae in the control variant. The results showed high extensity ( $\geq 80\%$ ) of infection regardless of the insect species and *S. feltiae* strain (Table 1). Significant differences were found between the extensity of infection of particular insect species. In the case of strain ZAG 15, the extensity of

infection of *P. brassicae* caterpillars statistically differed from the lower extensity observed in other insect species. In the groups of insect infected with strain K 11, the nematodes infected nearly 90% of *P. napi* caterpillars, which was statistically different from the extensity observed in other insect species.

The numbers of nematodes found in one caterpillar ranged from 2 to 132 individuals, on average  $34.1 \pm 25.9$  IJs. The comparison of mean numbers of IJs between nematode strains indicated the highest intensity of infection in caterpillars treated with K 11 strain (average  $42.6 \pm 25.0$  IJs) compared to the results obtained with ZAG 15 (average  $25.3 \pm 23.0$  IJs).

Analysis of the intensity of infection in relation to the host species showed significant differences (Kruskal–Wallis test:  $H = 50.1598$ ;  $p = 0.0001$ ) between the highest number of nematodes in *P. brassicae* caterpillars (average  $46.6 \pm 12.0$  IJs), followed by those in *P. rapae* (average  $37.1 \pm 28.2$  IJs) and by the lowest number in *P. napi* caterpillars (average  $18.7 \pm 15.4$  IJs). In the group of caterpillars treated with ZAG 15, the intensity of infection significantly differed between insect species ( $H = 43.0403$ ;  $p = 0.0001$ ) (Table 2). The use of K 11 strain resulted in a similar intensity of infection of *P. rapae* and *P. brassicae* caterpillars, and significantly lower intensity of infection of *P. napi* caterpillars ( $H = 15.6022$ ;  $p = 0.0004$ ) (Table 2).

## Discussion

Laboratory tests are aimed at indicating appropriate doses of nematodes, temperature and such strains that would cause the highest mortality in pests. In presented laboratory studies, it was shown that two strains of *S. feltiae*, ZAG 15 and K 11 were characterized by high pathogenicity to all three species of butterflies. High extensity of infection ( $\geq 89.7\%$ ) of three *Pieris* species was demonstrated for two studied strains. The extensity of infection of *P. rapae* with

**Table 1** Extensity of infection (%) of *Pieris* spp. with two strains of *S. feltiae*

Strain	Insect	Extensity*	$\chi^2$	$p^{**}$
ZAG 15	<i>P. rapae</i>	80.0 <sup>a</sup>	7.6829	0.0215
	<i>P. napi</i>	93.3 <sup>a</sup>		
	<i>P. brassicae</i>	100 <sup>b</sup>		
K 11	<i>P. rapae</i>	100 <sup>a</sup>	7.1546	0.028
	<i>P. napi</i>	89.7 <sup>b</sup>		
	<i>P. brassicae</i>	100 <sup>a</sup>		

\* Different letters show significant differences at  $p \leq 0.05$

\*\* Significant differences at  $p \leq 0.05$

**Table 2** Intensity of infection (mean numbers of IJs/caterpillar) of *Pieris* spp. with two strains of *S. feltiae*

Strain	Insect species	Intensity*	Median	<i>p</i> **
ZAG 15	<i>P. rapae</i>	31.1 ± 27.1 <sup>a</sup>	17.5	0.0109
	<i>P. napi</i>	7.1 ± 4.7 <sup>b</sup>	6.5	0.0008
	<i>P. brassicae</i>	41.8 ± 12.0 <sup>c</sup>	42.0	0.0001
K 11	<i>P. rapae</i>	45.2 ± 23.1 <sup>a</sup>	45.0	0.0423
	<i>P. napi</i>	32.7 ± 32.0 <sup>b</sup>	20.0	0.0215
	<i>P. brassicae</i>	52.3 ± 9.4 <sup>a</sup>	53.5	0.4232

\* Different letters show significant differences at  $p \leq 0.05$

\*\* Significant differences at  $p \leq 0.05$

strain ZAG 15 was comparable to that obtained by Bélair et al. (2003) who used two strains of *S. feltiae* at 20 °C and obtained insect mortality of 57.9 and 70.8% at a dose of 100 IJs. Similar mortality was noted by Wu and Chow (1989). Mortality of *P. rapae* larvae infected by *S. feltiae* varied between 75 and 97.5% after 3 days of exposition. Bélair et al. (2003) demonstrated also a significant relationship between the mortality of *P. rapae* and the time of their exposition to IJs. After 6-h-long exposition to *S. feltiae* they obtained 50% mortality while after 12 h the mortality among studied larvae was 78%.

Much smaller mortality after 48 h of exposition at the same dose (50 IJs) was found by Ramliana and Yadav (2009). Two species of EPNs: *S. thermophilidium* and *S. glaseri* (both not present in Poland) caused 45 and 35% mortality, respectively, in *P. brassicae* larvae. Not before 72 h the authors obtained results comparable to those presented here (80% for *S. glaseri* and 85% for *S. thermophilidium*). After 120 h cited authors found 100% mortality. Similarly, Sandner and Pezowicz (1983) found 100% mortality 96 h since infection of *P. brassicae* larvae by *S. carpocapsae*.

In studies presented here, larval extensity was determined after 48 h. Obtained results of 100% mortality of *P. rapae* and *P. brassicae* with the use of strain K 11 and 93.3% mortality of *P. napi* and 100% of *P. brassicae* with the use of strain ZAG 15 evidence their greater effectiveness compared with other strains of *S. feltiae* and of other EPNs species noted in the literature.

Performed studies on the intensity of infection showed statistically significant differences ( $p < 0.05$ ) between analyzed *Pieris* species. Tests showed that *P. brassicae* was most intensively infected by both strains of nematodes. High intensity of infection indicates high reproductive potential of nematodes in studied host species. Mahar et al. (2005) observed the number of IJs larvae obtained from *P. brassicae* larvae infected by various species of EPNs. The highest number of IJs was found in larvae infected by *S.*

*carpocapsae* and *S. feltiae*, which seems to show their greatest affinity to host. Less IJs larvae were noted after infection by *H. indica* and *H. bacteriophora*.

Bélair et al. (2003) proved in their studies that temperature might exert significant effect on the mortality of *P. rapae* larvae. At a temperature of 25 °C the mortality of *P. rapae* larvae after infection with *S. feltiae* strain UK and *S. riobrave* (95.8 and 89.2%, respectively) was much higher than that after infection with *S. carpocapsae* (65.8%). At temperatures of 20 °C and 15 °C the mortality after infection with *S. feltiae* was 70.8 and 19.2%, respectively.

Gupta et al. (2009) assessed in the field the effectiveness of preparations containing EPNs against *P. brassicae*. They used *S. carpocapsae* (strain PDBC), *S. carpocapsae* (strain JMU) and *H. indica*. The highest mortality was noted after application of *S. carpocapsae* (JMU) at a dose of 2 billion IJs/ha. After one day since application the mortality was 7.5% and increased to 41.8% 13 days later. Similar trend of increasing mortality was observed with other strains/species of EPNs.

High pathogenicity of native strains observed in our study permits us to assume their equally high effectiveness in field studies. Such studies on selected strains will be performed to establish practical guidelines of using EPNs to control *Pieris* species.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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