Article

Biological Control Potential of Native Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) against *Mamestra brassicae* L. (Lepidoptera: Noctuidae)

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Abstract: The largest group of cabbage plant pests are the species in the owlet moth family (Lepidoptera: Noctuidae), the most dangerous species of which is the cabbage moth (*Mamestra brassicae* L.). In cases of heavy infestation by this insect, the surface of plants may be reduced to 30%, with a main yield loss of 10–15%. The aim of the present study was to assess the susceptibility of *M. brassicae* larvae to nine native nematode isolates of the species *Steinernema feltiae* (Filipjev) and *Heterorhabditis megidis* Poinar, Jackson and Klein under laboratory conditions. The most pathogenic strains were *S. feltiae* K11, *S. feltiae* K13, *S. feltiae* ZAG11, and *S. feltiae* ZWO21, which resulted in 100% mortality at a temperature of 22 °C and a dosage of 100 infective juveniles (IJs)/larva. The least effective was *H. megidis* Wispowo, which did not exceed 35% mortality under any experimental condition. For most strains, there were significant differences ($p \leq 0.05$) in the mortality for dosages between 25 IJs and 50 IJs, and between 25 IJs and 100 IJs, at a temperature of 22 °C. Statistical analysis of the effect of temperature on mortality showed that only strain *H. megidis* Wispowo exhibited significant differences ($p \leq 0.05$) when applied at dosages of 50 IJs and 100 IJs.

Keywords: bioassay; biological control; *Heterorhabditis megidis*; *Mamestra brassicae*; *Steinernema feltiae*

1. Introduction

Plants of the mustard family (Brassicaceae) are predated on by many insect species, the largest group of which is the owlet moth (Noctuidae) family. One of the most dangerous species among this family is the cabbage moth *Mamestra brassicae* L. (Lepidoptera: Noctuidae), which can inflict major economic losses. *Mamestra brassicae* larvae are polyphagous, and apart from feeding on plants in the mustard family (cabbage, broccoli, cauliflower, Brussels sprout etc.), they also consume more than 70 plant species from 22 families (e.g., lettuce, beets, onion, potatoes, peas, tomatoes, and chrysanthemum) [1]. The first generation of *M. brassicae* appears in May and June. At this time, females lay eggs on the undersides of leaves, and the resulting larvae feed starting from mid-July. They initially damage external leaves by gnawing holes between veins, and later gnaw into the heads of cabbage, cauliflower, and broccoli. The larvae pass through six stages of development over the course of four to six weeks [2]. The second generation of *M. brassicae* is then observed in July and August. Their larvae feed from September to October [3].

On average, pests damaged 3% of the cabbage crops in Poland in 2017 and 4.5% in 2016, with the long-term (1993–2017) mean plant losses amounted to 7.2% [2]. In cases of heavy infestation,

the surface area of plants may be reduced to 30%, while the main yield losses may reach 10–15%. The economic threshold for *M. brassicae* infestation on cabbage is four to five larvae per 50 plants. Although environmentally friendly pest control options are available, such as the biological insecticide Dipel WG (with *Bacillus thuringiensis* var. Kurstaki (54%) as the active substance) that targets early larval stages, pests are largely controlled using chemical treatments [4]. However, as the latter insecticides pose a risk to the environment and human health, there is an urgent need to develop alternative strategies for controlling owl moths. One emerging option utilizes entomopathogenic nematodes (EPNs) [5–8]. EPNs are soil-inhabiting parasites that belong to the phylum Nematoda and the families Steinernematidae and Heterorhabditidae, and have been proven to be very effective in controlling soil and above-ground insect populations [9–13]. EPNs are mutually associated with bacteria of the family Enterobacteriaceae; while EPNs of the family Steinernematidae usually carry bacteria of the genus *Xenorhabdus*, EPNs of the family Heterorhabditidae typically carry bacteria of genus *Photorhabdus* [14]. The third juvenile stage of EPNs is referred to as the “infective juvenile” (IJ). Once IJs of either genera have infected an insect host, they release their bacterial symbionts into the host’s body and develop into fourth-stage juveniles and adults. The insect hosts die primarily because of septicaemia [15]. When their food source is depleted, the next generation of IJs exits the host insect cadaver, moves into the surrounding environment, and searches for a new host.

In this study, we examined eight strains (K10, K11, K13, ZAG11, ZAG15, ZWO4, ZWO21, and ZWO23) of *Steinernema feltiae* (Filipjev) and one strain (Wipsowo) of *Heterorhabditis megidis* Poinar, Jackson and Klein for their lethal activity against the larvae of *M. brassicae*. We then determined how the infectivity of these strains was impacted by temperature and concentration.

2. Materials and Methods

2.1. Test Insects

Larvae of *M. brassicae* in growth stages from L₄ to L₅ were used in laboratory tests. Observations of larval growth were carried out in a phytotron cabin with artificial light with a photoperiod L:D = 16:8, temperature of about 22 °C ± 2 °C, and relative air humidity 65 ± 5% [3,16,17]. A light trap was used to catch adult individuals from June to the end of August. The trap was systematically checked every 2–3 days.

Moths in pairs were released into glass isolators (600 cm³) placed in the phytotron and covered with bolting cloth, to prevent the moths from escaping. The bottoms of the isolators were covered with discs of filter paper. The isolators were equipped with vials filled with moth feed and washed clumps of grass (perennial ryegrass—*Lolium perenne* L.) or inflorescences (common chickweed—*Stellaria media* L.) with intact root systems as a substratum for egg laying. The moth feed consisted of a 10% honey solution with drops of fruit essence. The food was replaced every second day. Eggs were checked for every day, and plant regions with eggs were removed with scissors, transferred to separate plastic containers lined with damp discs of filter paper, and then covered with cabbage or lettuce leaves. The larval development was observed daily at the same time each day. Larvae from hatching (stage L₁) to stage L₃ were kept in plastic boxes (160 × 120 mm) with ventilated caps, each containing 10 larvae. To provide appropriate sanitary and hygienic conditions, the filter paper was replaced every day. At the beginning of stage L₄, the containers were half-filled with wet sand. Fresh food was provided to the larvae every day.

2.2. Nematodes

Nine strains of EPNs, isolated in 2010 and 2011, were used for experiments. These strains included eight strains of *S. feltiae* (K10, K11, K13, ZAG11, ZAG15, ZWO4, ZWO21, and ZWO23) (Table 1), collected from soils in southern and central Poland, and one strain of *H. megidis* (Wipsowo), from northern Poland [18]. EPNs were maintained on the larvae of *Galleria mellonella* L. (Lepidoptera:

Pyralidae) in the laboratory, following the technique from Kaya and Stock [19]. The IJs were collected using White traps [20] and kept in water at 4 °C for later use.

Table 1. Isolates used in experiments.

Species	Isolate	Sampling Site	Geographic Coordinates
<i>Steinernema feltiae</i>	K10	fallow lands near Katowice	N 50°10'27.5" E 18°56'51.3"
<i>S. feltiae</i>	K11	wheat crop near Katowice	N 50°20'5.5968" E 19°2'14.0388"
<i>S. feltiae</i>	K13	field (<i>Miscanthus giganteus</i> crop) (Silesia Region)	N 50°15'58.68" E 19°5'52.08"
<i>S. feltiae</i>	ZAG11	deciduous forest, the Zagożdżonka River valley (Kozienicka Forest)	N 51°30'19.3" E 21°29'13.9"
<i>S. feltiae</i>	ZAG15	meadow, the Zagożdżonka River valley (Kozienicka Forest)	N 51°23'10.4820" E 21°33'15.5412"
<i>S. feltiae</i>	ZWO21	meadow, the Zwolenka River valley (Kozienicka Forest)	N 51°23'21.7" E 21°33'38.9"
<i>S. feltiae</i>	ZWO23	meadow, the Zwolenka River valley (Kozienicka Forest)	N 51°23'11.2" E 21°33'10.1"
<i>S. feltiae</i>	ZWO4	meadow, the Zwolenka River valley (Kozienicka Forest)	N 51°23'10.5" E 21°33'33.7"
<i>Heterorhabditis megidis</i>	Wipsowo	wheat field (Pojezierze Olsztyńskie)	N 53°54'32.0" E 20°47'54.4"

2.3. Bioassay

The nematode strains were tested at two temperatures (17 °C and 22 °C) and three dosages (25 IJs, 50 IJs, 100 IJs per larva).

Mamestra brassicae larvae, together with a piece of cabbage leaf, were placed on wet filter paper in a Petri dishes (Ø 9cm). Thirty-five larvae (with 5 individuals per dish) were used for each experimental condition. Nematodes were applied to the dishes at dosages of 25 IJs, 50 IJs, or 100 IJs per larva, and the dishes were placed in Sanyo incubation chambers at temperatures of 17 °C and 22 °C, in the dark. Each EPN strain was used to infect 210 insects: 35 larvae × 3 dosages × 2 temperatures.

The number of IJs was determined by counting the number of IJs in five droplets (5 µL) for a previously prepared nematode suspension in water. The selected concentrations of nematode suspension were obtained by diluting the suspension with tap water or concentrating the suspension through centrifugation [21].

Control larvae, together with a piece of cabbage leaf, were placed onto Petri dishes lined with filter paper moistened with distilled water. Five larvae were used for each dish, with 7 dishes 35 insects for each temperature, giving 35 insects per temperature and a total of 70 individuals.

The mortality rate (percent of parasitized larvae in each analyzed sample) was determined 72 h after infection by dissecting the dead larvae under an Olympus microscope at a 3–5× magnification.

2.4. Statistical Analyses

The data were statistically processed with the use of Statistica (version 10; StatSoft, Kraków, Poland). A Pearson chi-square (χ^2) test was used to determine the relationship between two variables i.e., between temperature and the mortality induced by particular nematode isolates in two categories (17 °C and 22 °C, versus mortality expressed as “yes” or “no”). A chi-square goodness of fit test was used to determine the effect of the initial IJ dosage (25 IJs, 50 IJs, or 100 IJs) on the mortality.

3. Results and Discussion

Mamestra brassicae larvae in the control condition exhibited no mortality during the course of the experiment. In contrast, seventy-two hours after application, EPN strains *S. feltiae* K11, *S. feltiae* K13, *S. feltiae* ZAG11, and *S. feltiae* ZWO21, resulted in 100% larval mortality at 22 °C and a dosage of 100 IJs. These strains also resulted in high mortalities of between 88.6% and 100% at the lower dosage of 50 IJs. Equally high mortalities of 94.3%, 94.3%, 97.1%, and 97.1% were observed when the samples were incubated at 17 °C at a dosage of 100 IJs for strains *S. feltiae* K11, *S. feltiae* ZAG11, *S. feltiae* K13, and *S. feltiae* ZWO21, respectively. The same strains also produced high mortalities of 85.7% to 91.4% at a dosage of 50 IJs.

Only two strains, *S. feltiae* K13 and *S. feltiae* ZWO21, exhibited a mortality that exceeded 50% at 22 °C at the lowest dosage of 25 IJs. For the remaining strains, at either 17 °C or 22 °C, the mortality varied between 0–48.6%. The least effective strains were *H. megidis* Wispowo, whose mortality did not exceed 35% in any of the experimental conditions, and *S. feltiae* ZWO4, which did not exceed 52% mortality (Table 2).

Table 2. The effect of entomopathogenic nematode (EPN) dosages applied at two temperatures on the mortality of the cabbage moth parasitism (%).

Isolate	Temperature and Dosages of EPNs					
	17 °C			22 °C		
	25 IJs	50 IJs	100 IJs	25 IJs	50 IJs	100 IJs
K10	40	82.9	88.6	48.6	88.6	91.4
K11	45.7	91.4	94.3	48.6	94.3	100
K13	54.3	91.4	97.1	57.1	100	100
ZWO 4	11.4	31.4	51.4	20	42.9	48.6
ZWO 21	48.6	91.4	97.1	54.3	97.1	100
ZWO 23	20	45.7	85.7	22.9	37.1	80
ZAG 11	48.6	85.7	94.3	40	88.6	100
ZAG15	31.4	57.1	71.4	37.1	51.4	65.7
Wipsowo	0	5.7	8.6	2.9	31.4	34.3

IJs: Infective juveniles.

Statistical analysis of the effect of temperature on mortality showed that only *H. megidis* Wipsowo, applied at dosages of 50 and 100 IJs, was statistically significant ($p \leq 0.05$) (Table 3). The effect of nematode dosage on mortality was significantly ($p \leq 0.05$) different for dosages of 25 IJs versus 50 IJs, and 25 IJs versus 100 IJs, at 22 °C for most of the strains. At 17 °C, significant differences were found in the mortality between dosages of 25 IJs and 100 IJs (for all studied strains), and between 25 IJs and 50 IJs (only for *S. feltiae* K10, *S. feltiae* K11, and *S. feltiae* ZWO21). Except for one strain, *S. feltiae* ZWO23, no significant differences in the mortality were noted between dosages of 50 and 100 IJs (Table 4).

Table 3. The effect of temperature on the mortality of the cabbage moth parasitism (%) by EPNs applied at three dosages.

Isolate	Temperature and Dosages of EPNs											
	25 IJs				50 IJs				100 IJs			
	17 °C	22 °C	χ^2	p^*	17 °C	22 °C	χ^2	p^*	17 °C	22 °C	χ^2	p^*
K10	40	48.6	0.52	0.4704	82.9	88.6	0.47	0.4945	88.6	91.4	0.16	0.6903
K11	45.7	48.6	0.06	0.8108	91.4	94.3	0.22	0.6426	94.3	100	2.06	0.1513
K13	54.3	57.1	0.16	0.6903	91.4	100	3.13	0.0767	97.1	100	1.01	0.3138
ZWO 4	11.4	20	0.97	0.3245	31.4	42.9	0.98	0.3224	51.4	48.6	0.06	0.8111
ZWO 21	48.6	54.3	0.23	0.6324	91.4	97.1	1.06	0.3031	97.1	100	1.01	0.3138
ZWO 23	20	22.9	0.08	0.7708	45.7	37.1	0.53	0.4667	85.7	80	0.40	0.5259
ZAG 11	48.6	40	0.52	0.4704	85.7	88.6	0.13	0.7210	94.3	100	2.06	0.1513
ZAG15	31.4	37.1	0.78	0.3758	57.1	51.4	2.32	0.1277	71.4	65.7	0.27	0.6066
Wipsowo	0	2.9	1.01	0.3138	5.7	31.4	7.65	0.0057	8.6	34.3	6.87	0.0088

* Statistical differences at $p \leq 0.05$. χ^2 : chi-square**Table 4.** The effect of EPN dosages applied at two temperatures on the mortality of the cabbage moth parasitism (%).

Isolate	17 °C						22 °C					
	25 × 50 IJs		50 × 100 IJs		25 × 100 IJs		25 × 50 IJs		50 × 100 IJs		25 × 100 IJs	
	χ^2	p^*	χ^2	p^*	χ^2	p^*	χ^2	p^*	χ^2	p^*	χ^2	p^*
K10	5.233	0.022	0.067	0.796	6.422	0.011	4.083	0.043	0.016	0.900	4.592	0.032
K11	5.333	0.021	0.015	0.901	5.898	0.015	5.120	0.024	0.059	0.808	6.231	0.013
K13	3.314	0.069	0.061	0.806	4.245	0.039	4.091	0.043	0.000	1.000	4.091	0.043
ZWO 4	3.267	0.071	1.690	0.194	8.909	0.003	2.909	0.088	0.125	0.724	4.167	0.041
ZWO 21	4.592	0.032	0.061	0.806	5.667	0.017	4.245	0.039	0.014	0.904	4.741	0.029
ZWO 23	3.522	0.061	4.261	0.039	14.297	<0.001	1.190	0.275	5.488	0.019	11.111	0.001
ZAG 11	3.596	0.058	0.143	0.705	5.120	0.024	6.422	0.011	0.242	0.622	9.000	0.003
ZAG15	2.613	0.106	0.556	0.456	5.444	0.020	0.806	0.369	0.610	0.435	2.778	0.096
Wipsowo	-	-	0.200	0.655	-	-	8.333	0.004	0.043	0.835	9.308	0.002

* Statistical differences at $p \leq 0.05$.

Many studies have sought to optimize the application of EPNs for use as a potential insecticide. These studies have focused on selecting the appropriate initial dosage of nematodes, the temperature, and most importantly, the strain that induces the highest mortality in the target insect. Indeed, our previous studies showed that the susceptibility of different insects to native isolates of EPNs is highly dependent on the type of isolate used [22,23].

In this study on the susceptibility of cabbage moth larvae to EPNs, strains *S. feltiae* K11, *S. feltiae* K13, *S. feltiae* ZAG11, and *S. feltiae* ZWO21 were found to be the most pathogenic. Similarly, in our earlier studies on the susceptibility of *Agrotis exclamationis* L. (Lepidoptera: Noctuidae) larvae to EPNs, the most pathogenic nematode strains were *S. feltiae* K13 and *S. feltiae* ZWO21. Isolate *S. feltiae* ZAG11 was more efficient in controlling the larvae of *M. brassicae*, with a mortality following infection of 85.7–100%, than the larvae of *A. exclamationis*, with a mortality of 81% [22]. In a previous study, *S. feltiae* K11 was highly effective (89.7–100% mortality) against *Pieris* spp. (Lepidoptera: Pieridae) larvae [23]. Strains *S. feltiae* K11 and *S. feltiae* K13 were highly pathogenic (96.7–100%) to the larvae of *Dendrolimus pini* L. (Lepidoptera: Lasiocampidae) [24]. Of note, the pathogenic properties of *S. feltiae* ZWO4 differ depending on the infected host insect species. For *M. brassicae*, this strain did not induce greater than 52% mortality, and was the weakest among the strains tested in this study. In contrast, this strain induced 100% mortality in *D. pini* larvae [24]. A remarkable discrepancy in mortality was also demonstrated for *S. feltiae* ZAG15, which caused 100% mortality in the case of *Pieris* spp. [23], but less

than 72% mortality when used against cabbage moth larvae in this study. Our studies clearly indicate that, while some strains are always effective, the pathogenicity of others is more dependent on the host.

Although *M. brassicae* is one of the most harmful pests for field crops, there have been no studies to date examining the potential use of native strains of EPNs, or of commercial preparations based on EPNs, to control this insect. As there is scarce data on the susceptibility of larvae of the genus *Mamestra* to EPNs, our results were also referred to those pertaining to other foliage feeders.

The aboveground applications of EPNs may be limited due to the harmful effects of ultraviolet radiation or desiccation on the nematodes [12]. However, optimized EPN formulations that protect against harmful environmental conditions may improve the efficacy of aboveground applications [25]. For example, the addition of anti-desiccants or other adjuvants has been reported to provide improved aboveground control of various foliar pests, including the diamond back moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) [26,27], the sweet potato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) [28], *Spodoptera littoralis* (Boisduval), and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) [29].

For isolates of *S. feltiae*, temperature did not significantly affect the mortality of the cabbage moth; in contrast, there was a temperature effect noted for *H. megidis* Wipsowo. This may be due to the fact that *S. feltiae* is adapted to cooler climates but also present in warmer areas [30]. Nevertheless, some studies [11,31,32] found that *S. feltiae* isolates exhibited the strongest effect against the cereal leaf beetle *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae) and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) at temperatures between 20 and 25 °C. Similarly, Bélair et al. [33] showed that temperature might significantly affect the susceptibility of *Pieris rapae* L. larvae. Indeed, *P. rapae* showed a markedly higher mortality rate at 25 °C after infection with *S. feltiae* UK (95.8%) or *Steinernema riobrave* Cabanillas, Poinar and Raulston (89.2%), than after infection with *Steinernema carpocapsae* Weiser (65.8%). In contrast, the mortality rates after infection with *S. feltiae* were 70.8% at 20 °C and only 19.2% at 15 °C. The efficiency of nematode species of the genus *Heterorhabditis* exhibit greater variability in response to temperature, initial IJ dosage, and host species [34].

We observed no differences in mortality between dosages of 50 IJs and 100 IJs, while marked differences were found between dosages 25 IJs and 50 IJs. Laznik [11] showed that higher nematode dosages caused higher mortality, but Steinernematidae suggested that satisfactory mortality rates were achieved even at lower dosages, which is important from an economics point of view. This conclusion was confirmed in another study [31], which did not reveal the influence of dosage on the efficacy of EPNs against the Colorado potato beetle. A similar trend was described by Andaló et al. [35], who found greater differences in the mortality between dosages of 100 IJs and 250 IJs than between 250 IJs and 500 IJs when studying the susceptibility of armyworm *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) larvae to various EPN isolates. Furthermore, as shown by Lewis et al. [36], an excessively high density of EPNs in the host's body can increase competition, resulting in the first IJs that penetrate the host sending chemical cues to discourage further IJ invasion.

Our study indicated markedly higher mortality rates induced by isolates of *Steinernema* than by those of *Heterorhabditis*. Similarly, Gulcu et al. [37] in their study of the susceptibility of *Spodoptera ciliium* Guenée (Lepidoptera: Noctuidae) to native EPN isolates, showed higher pathogenicity of *Steinernema* (77%) than of *Heterorhabditis* (29%) isolates. On the other hand, Salvadori et al. [38] found that the mortality of *S. frugiperda*, caused by four strains of Steinernematidae, ranged from 28 to 70%, while the respective efficacy of four strains of Heterorhabditidae varied between 55 and 77%. Yuksel et al. [39], in their study on the susceptibility of *Peridroma saucia* (Hubner) (Lepidoptera: Noctuidae) larvae to four native isolates of *Heterorhabditis* and *Steinernema*, found that the former were more effective in controlling the pest. The higher efficacy of *Heterorhabditis* noted by the authors cited above may be explained by the higher temperature of the experimental conditions (25–30 °C), which is favorable for the development of *Heterorhabditis*. Gözel and Güneş [40] confirmed this temperature effect and showed that the mortality of corn stalk borer *Sesamia cretica* Led. (Lepidoptera: Noctuidae) larvae treated with *H. bacteriophora* Poinar at 15 °C was 14%, but reached 94% at 30 °C. No differences

were noted in the mortality of *S. frugiperda* larvae treated with either *Heterorhabditis* or *Steinernema*, although differences were observed between isolates within the same nematode species.

Current experiments were carried out at lower temperatures (17 °C and 22 °C), because most measures to control larvae of the genus *Mamestra* in Poland should be performed in spring and autumn. For biological pest control, it is important to select the most appropriate species or specific EPN isolate, and therefore the parasitic properties of these organisms must be first examined under laboratory conditions. These studies provide valuable information with on which species/strains of EPNs are most effective against a given insect, as the selection of the most pathogenic strains will translate into the successful biological control of harmful crop pests. This study sets the basis for future studies using EPNs for cabbage moth control.

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