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# The influence of temperature on the biological activity of selected nematode species (Steinernematidae and Heterorhabditidae) under the conditions of their coexistence

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**Abstract:** The presented study is concerned with infectivity of select entomopathogenic nematode species under different temperature conditions (15, 20 and 25 °C) in the presence of competing species. Two species of nematodes of the genus *Steinernema* (*S. kraussei*, *S. feltiae*) and two of the genus *Heterorhabditis* (*H. bacteriophora* and *H. megidis*) were included in the analysis. Different experimental variants were adopted in which the selected two entomopathogenic nematode species were mixed between *Steinernema* and *Heterorhabditis*. The study showed that *Heterorhabditis* and *Steinernema* cannot coexist together in a single host and one genus will always prevail. *H. megidis*, under co-occurrence with *S. feltiae* separately infected *Galleria mellonella* larvae most commonly at 15 °C, while *H. bacteriophora* at 20 °C. The study showed that the main determinant of nematode activity towards the host is not temperature, but the presence of co-existing nematode species. The results of the experiment encourage further research to determine the effects of a variety of concurrent biotic and abiotic factors on entomopathogenic nematodes and their biological activity.

**Keywords:** entomopathogenic nematodes; coexistence of different species; infective abilities; interactions

Entomopathogenic nematodes (EPNs) from the Steinernematidae and Heterorhabditidae families are widely distributed in soils in the world (Lewis & Clarke 2012; Bhat et al. 2020). All their developmental stages occur inside the host's body, and only invasive larvae of the third/infective stage juveniles (IJs) can survive in the soil outside of the host organism (Shapiro-Ilan et al. 2017). These larvae (IJs) are particularly well adapted to the changing conditions of their external environment. Both the exceptional adaptations of nematodes from the Steinernematidae and Heterorhabditidae families – such as high mobility, high infectivity to a wide range of host species, propensity to mass reproduction – and, above all, their harmlessness to the environment, led to the wide recognition of the Steinernematidae and Heterorhabditidae in the protection against a large group of plant pests (Lacey & Georgis 2012; Istkhar et al. 2019). The presence and activity of nematodes in the environment may be subject to limitation by many biotic and abiotic factors (Shapiro-Ilan et al. 2012a; Campos-Herrera et al. 2014). Temperature is among the most important abiotic factors determining the mobility of nematodes, their further

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development, and reproductive ability (Lacey et al. 2006). Among the biotic factors, a principal factor affecting the EPN species is competition for host (Stuart et al. 2015). In the natural environment, entomopathogenic nematodes encounter many biotic and abiotic factors that affect them simultaneously. However, laboratory conditions allow creating conditions for a one-factor effect on a living organism. While there are many studies on competition between EPN species, none have addressed the determination of the effect of temperature on the activity of different EPN species under competitive conditions. Management entomopathogenic nematodes of local insect populations, including many plant pests, is one of the important links in the trophic chain, essential for a functional ecosystem (Lewis et al. 2015). Therefore, the aim of the research was to determine the possibility of effective use of various species of entomopathogenic nematodes in the practice of plant protection against pests, considering their competition for food and various temperature conditions. The global increase in temperature and the drought observed in many parts of the world have a significant impact on crop quality and yield (Zhao et al. 2017). At the same time, they can adversely affect the effectiveness of biological methods used to protect crops from pests. Therefore, the search for solutions to increase the effectiveness of biopreparations in plant protection, including those containing entomopathogenic nematodes, seems justified and necessary.

## MATERIAL AND METHODS

**Multiplication and maintenance of the entomopathogenic nematodes.** The nematodes in this study were represented by four entomopathogenic nematode species from biopreparations produced by Biobest Group NV, Belgium – *Steinernema feltiae* Filipjev, 1934 (Steinernema-System), *S. kraussei* Steiner, 1923 (Kraussei-System) and *Heterorhabditis bacteriophora* Poinar, 1976 (B-Green) and by Becker Underwood Ltd, UK – *H. megidis* Poinar, Jackson & Klein, 1987 (Nemasys). The biopreparations used for the experiment are recommended for pest control in various crop types (Lacey & Georgis 2012; Koppenhöfer et al. 2020).

The nematodes used in the experiment were propagated on the larvae of the fifth-stage wax moth (*Galleria mellonella* L.). The *G. mellonella* larvae were reared on artificial media in transparent plastic jars at 28 ± 2 °C in the laboratory (Kassab & Entsar 2016). They are the most common insect hosts used *in vivo* for the mass rearing of nematodes (Woodring & Kaya 1988). Insect larvae of. *G. mellonella* were placed in a 9-cm-diameter Petri dish lined with a moistened filter paper and exposed to about 100 IJs of EPN at 25 °C. After two days, infected insect cadavers were transferred to the white traps, which consisted of a dish covered with a filter paper on which the cadavers rest surrounded by water (Shapiro-Ilan et al. 2012b). After 7–10 days, all number of IJs was collected. Multiplied third-stage infective juveniles (IJs) of EPN were stored at 7 °C (*Steinernema* spp.) and at 10 °C (*Heterorhabditis* spp.) which were used in the experiment one week after harvesting (Kaya & Stock 1997). Before using, the viability of the infective juveniles of EPNs was assessed under binoculars.

**Experimental protocol.** Under laboratory conditions, a biological activity of EPNs was tested in host insects (larvae of greater waxmoth *Galleria mellonella*) in three variants of coexistence (Table 1). The variants have been chosen based on coexistence observed in the natural environment, in various types of agro- and biocenoses (Karbowska-Dzięgielewska 2013). For each variant the biological activity was analyzed separately under different temperatures: 15, 20 and 25 °C in the frame time of five days. Analysed parameters were: the extensity of infection – number of the *Galleria* cadavers with nematodes discovered after dissection (Table 2), the host mortality – the number of dead larvae of *G. mellonella* after contact with each variant of EPNs and was assessed after each 24 h of observations (Table 3), and the intensity of infection – number of nematodes found that have entered the host and developed into females and males in the case of Steinernematidae or hermaphroditic individuals in the case of Heterorhabditidae

Table 1. Variants with tested species of coexisting entomopathogenic nematodes (including abbreviations used in test results)

| Variant experiment | Species 1 | Species 2 |
|---|---|---|
| I | *S. feltiae* | *H. megidis* |
| II | *S. feltiae* | *H. bacteriophora* |
| III | *S. kraussei* | *H. megidis* |

*H. – Heterorhabditis*; *S. – Steinernema*

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Table 2. Number of host insects (larvae of *Galleria mellonella*) infected by various species of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae)

| Variant | Temperature (°C) | Total number of dead larvae | *P*-value | Number of the *Galleria* cadaver infected by: | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | species 1 | species 2 | both species | no species | *P*-value |
| *S. feltiae*[1] + *H. megidis*[2] | 15 | 26 | 0.018 | 15 | 11 | 0 | 4 | 0.044 |
| | 20 | 26 | | 26 | 0 | 0 | 4 | |
| | 25 | 22 | | 20 | 2 | 0 | 8 | |
| *S. feltiae*[1] + *H. bacteriophora*[2] | 15 | 27 | 0.017 | 27 | 0 | 0 | 3 | 0.233 |
| | 20 | 28 | | 22 | 6 | 0 | 2 | |
| | 25 | 26 | | 23 | 3 | 0 | 4 | |
| *S. kraussei*[1] + *H. megidis*[2] | 15 | 27 | 0.034 | 20 | 7 | 0 | 3 | 0.319 |
| | 20 | 23 | | 23 | 0 | 0 | 7 | |
| | 25 | 26 | | 25 | 1 | 0 | 4 | |

*H.* – *Heterorhabditis*; *S.* – *Steinernema*

[1]Species 1; [2]species 2

(Table 4). Infection of *Galleria* larvae by nematodes was assessed when the first adult generation of EPNs could be obtained. It means for steinernematids and for heterorhabditis nematodes the degree of infection was assessed on the third and fourth day of the experiment, respectively. Dissections of dead larvae to assess the degree of infection were done in Ringer's solution to avoid excessive osmotic stress to the adult stages of nematodes. Firstly, all specimens of the first-stage generation adults isolated from *G. mellonella* cadavers were examined live or heat-killed in 60 °C Ringer's solution (Kaya & Stock 1997). Nematodes were fixed in triethanolamine formalin and processed to anhydrous glycerine for mounting (Seinhorst 1959). Specimens were mounted on glass slides supported with glass rods to avoid their flattening. Observations were made from live and mounted specimens using an Olympus BX60 microscope equipped with differential interference contrast optics (Olympus, Japan). Selection of morphometric characters was done according to Hominick et al. (1997) and Nguyen (2007). Further the isolated nematodes were counted to detailed evaluate degree of infection. Following the biological activity of each EPNs in selected variants was compared to the control groups where larvae of *G. mellonella* were exposed to single species of EPN*s* in the same temperature conditions.

The *in vivo* culture method for both steinernematid and heterorhabditis nematodes was used (Woodring & Kaya 1988). In the experimental and control conditions ten *G. mellonella* larvae (each with a mass of approximately 180 mg), were exposed in a Petri dish (100 × 15 mm) lined with two moistened filter papers. In the experimental arm, 1 000 infective juveniles (IJs) of EPNs were used (500 IJs of a *Steinernema* species and 500 IJs of a *Heterorhabditis* species). This means that there were 50 IJs of nematodes per host from each species used in the experimental variant. Similarly, in the control groups each *Galleria* larva was exposed to 50 IJs of the specified single species of EPNs. The whole experiment was triplicated and repeated in time. It means that each variant of coexistence in the experimental arm or a single species of EPNs in control arm was tested in three dishes with ten *G. mellonella* larvae at the same time. The following experiment was repeated twice, each time with fresh nematodes larvae (IJs) and 10 larvae of *G. mellonella* per a Petri dish. Finally, we have done three independent tests.

**Statistical analysis.** A chi-squared test was used to assess the disproportion in the number of dead *G. mellonella* individuals after contact with each variant of EPNs under different temperature conditions. The Kruskal-Wallis test was performed to determine the number of host insects infected by various species of EPS in each tested variant. A *t*-test for independent samples was used to determine the differences between the control and experimental groups in the numbers of adult individuals of EPS in dead *G. mellonella* larvae. Testing for normality of distribution was performed with a Shapiro-Wilk test. The Levene's test was used to check for homogeneity of variance. In case

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Table 3. Differences between experimental and control groups in the number of death *Galleria mellonella* larvae within the timeframe of the observation

| Time (h) | 15 °C | | | | | 20 °C | | | | | 25 °C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sf + Hm | Sf | *P*-value | Hm | *P*-value | Sf + Hm | Sf | *P*-value | Hm | *P*-value | Sf + Hm | Sf | *P*-value | Hm | *P*-value |
| 24 | 0 | 0 | 1.000 | 0 | 1.000 | 0 | 0 | 1.000 | 0 | 1.000 | 0 | 0 | 1.000 | 0 | 1.000 |
| 48 | 0 | 26 | < 0.001 | 0 | 1.000 | 0 | 30 | < 0.001 | 0 | 1.000 | 0 | 30 | < 0.001 | 30 | < 0.001 |
| 72 | 6 | 30 | < 0.001 | 16 | 0.004 | 30 | 30 | 1.000 | 30 | 1.000 | 30 | 30 | 1.000 | 30 | 1.000 |
| 96 | 30 | 30 | 1.000 | 28 | 0.070 | 30 | 30 | 1.000 | 30 | 1.000 | 30 | 30 | 1.000 | 30 | 1.000 |
| 120 | 30 | 30 | 1.000 | 30 | 1.000 | 30 | 30 | 1.000 | 30 | 1.000 | 30 | 30 | 1.000 | 30 | 1.000 |
| | Sf + Hb | Sf | | Hb | | Sf + Hb | Sf | | Hb | | Sf + Hb | Sf | | Hb | |
| 24 | 0 | 0 | 1.000 | 0 | 1.000 | 0 | 0 | 1.000 | 0 | 1.000 | 0 | 0 | 1.000 | 0 | 1.000 |
| 48 | 0 | 26 | 0.021 | 0 | 1.000 | 30 | 30 | < 0.001 | 14 | < 0.001 | 30 | 30 | 1.000 | 30 | 1.000 |
| 72 | 30 | 30 | 1.000 | 9 | < 0.001 | 30 | 30 | 1.000 | 30 | 1.000 | 30 | 30 | 1.000 | 30 | 1.000 |
| 96 | 30 | 30 | 1.000 | 25 | 0.009 | 30 | 30 | 1.000 | 30 | 1.000 | 30 | 30 | 1.000 | 30 | 1.000 |
| 120 | 30 | 30 | 1.000 | 30 | 1.000 | 30 | 30 | 1.000 | 30 | 1.000 | 30 | 30 | 1.000 | 30 | 1.000 |
| | Skr + Hm | Skr | | Hm | | Skr + Hm | Skr | | Hm | | Skr + Hm | Skr | | Hm | |
| 24 | 0 | 0 | 1.000 | 0 | 1.000 | 0 | 0 | 1.000 | 0 | 1.000 | 0 | 0 | 1.000 | 0 | 1.000 |
| 48 | 9 | 26 | 0.283 | 0 | 0.006 | 15 | 30 | < 0.001 | 0 | < 0.001 | 0 | 30 | < 0.001 | 30 | < 0.001 |
| 72 | 21 | 30 | 0.006 | 16 | 0.088 | 30 | 30 | 1.000 | 30 | 1.000 | 30 | 30 | 1.000 | 30 | 1.000 |
| 96 | 30 | 30 | 1.000 | 28 | 0.070 | 30 | 30 | 1.000 | 30 | 1.000 | 30 | 30 | 1.000 | 30 | 1.000 |
| 120 | 30 | 30 | 1.000 | 30 | 1.000 | 30 | 30 | 1.000 | 30 | 1.000 | 30 | 30 | 1.000 | 30 | 1.000 |

Hb – *Heterorhabditis bacteriophora*; Hm – *H. megidis*; Sf – *Steinernema feltiae*; Skr – *S. kraussei*

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Table 4. Sex differences in nematodes under the investigated temperature conditions and different experimental variants

| Variant | Species | 15 °C | | | | | 20 °C | | | | | 25 °C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | experiment | | control | | *P*-value | experiment | | control | | *P*-value | experiment | | control | | *P*-value |
| | | mean | SD | mean | SD | | mean | SD | mean | SD | | mean | SD | mean | SD | |
| I | ***S. feltiae*** | | | | | | | | | | | | | | | |
| | all | 53.067 | 37.471 | 85.964 | 22.876 | 0.006 | 76.692 | 34.559 | 82.233 | 14.692 | 0.453 | 74.850 | 25.789 | 55.500 | 12.434 | 0.004 |
| | female | 35.733 | 24.915 | 57.607 | 15.543 | 0.001 | 49.885 | 23.483 | 53.933 | 10.062 | 0.420 | 50.200 | 16.994 | 35.667 | 8.770 | 0.002 |
| | male | 17.333 | 12.888 | 28.357 | 8.753 | 0.001 | 26.808 | 28.300 | 28.300 | 7.905 | 0.632 | 24.650 | 9.051 | 19.833 | 5.325 | 0.041 |
| | ***H. megidis*** | | | | | | | | | | | | | | | |
| | hermaphrodites | 58.182 | 12.311 | 46.840 | 11.459 | 0.011 | 0.000 | 0.000 | 47.667 | 12.721 | NA | 35.000 | 2.828 | 38.769 | 17.180 | 0.763 |
| II | ***S. feltiae*** | | | | | | | | | | | | | | | |
| | all | 50.889 | 23.707 | 85.964 | 22.876 | < 0.001 | 75.773 | 20.398 | 82.233 | 14.692 | 0.190 | 69.087 | 17.830 | 55.500 | 12.434 | 0.002 |
| | female | 35.444 | 16.388 | 57.607 | 15.543 | < 0.001 | 48.000 | 12.083 | 53.933 | 10.062 | 0.059 | 47.174 | 11.699 | 35.667 | 8.770 | < 0.001 |
| | male | 15.444 | 7.678 | 28.357 | 8.753 | < 0.001 | 27.773 | 82.233 | 28.300 | 7.905 | 0.823 | 21.913 | 6.273 | 19.833 | 5.325 | 0.198 |
| | ***H. bacteriophora*** | | | | | | | | | | | | | | | |
| | hermaphrodites | 0.000 | 0.000 | 36.815 | 10.902 | NA | 32.000 | 11.696 | 41.867 | 7.811 | 0.014 | 20.000 | 5.568 | 34.185 | 7.011 | 0.002 |
| III | ***S. kraussei*** | | | | | | | | | | | | | | | |
| | all | 57.250 | 14.664 | 76.750 | 18.548 | < 0.001 | 71.652 | 16.140 | 69.321 | 13.551 | 0.578 | 59.440 | 19.210 | 56.107 | 10.493 | 0.446 |
| | female | 42.050 | 10.560 | 51.607 | 11.464 | 0.005 | 47.913 | 10.518 | 49.036 | 10.251 | 0.702 | 38.800 | 12.234 | 38.643 | 7.329 | 0.956 |
| | male | 15.200 | 4.618 | 25.143 | 7.934 | < 0.001 | 23.739 | 6.129 | 20.286 | 4.569 | 0.025 | 20.640 | 7.216 | 17.464 | 3.554 | 0.079 |
| | ***H. megidis*** | | | | | | | | | | | | | | | |
| | hermaphrodites | 55.857 | 10.684 | 46.840 | 11.459 | 0.072 | 0.000 | 0.000 | 47.667 | 12.721 | NA | 42.000 | 0.000 | 38.769 | 17.180 | 0.855 |

*H.* – *Heterorhabditis*; *S.* – *Steinernema*; SD – standard deviation; NA – not applicable

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of failure to meet the assumptions of the *t*-test, the Box-Cox transformation was used. Additionally, differences between experimental and control groups in the number of death *G. mellonella* larvae on each day of observation was compered using a chi-squared test. All statistical tests were two-sided, and *P*-values < 0.05 were considered statistically significant. Tests were performed with Statistica software (version 13.5).

## RESULTS

On the basis of the conducted observations, it was found that all tested nematode species (*S. feltiae, S. kraussei, H. megidis and H. bacteriophora*) were the fastest to infect *G. mellonella* larvae under control conditions, without competition at 25 °C and after 48 h from the beginning of the experiment (Table 3). However, in conditions of coexistence of two nematodes species at 25 °C, II (*S. feltiae* + *H. megidis*) and V (*S. kraussei* + *H. megidis*) of the experiment, 100% mortality of *G. mellonella* was observed a day later than under the control conditions, that is 72 h after the nematodes had contact with the host. On the other hand, with the coexistence of *S. feltiae* with *H. bacteriophora* (the variant III) at 25 °C the infection rate of the insects tested was similar to that of the control group. At 15 °C, the total infestation of insects caused by EPNs was extended in time, depending on the species of nematode and on the experiment variant. The 100% mortality rate of *G. mellonella* larvae was observed at least 72 h after beginning of the experiment, i.e. for *S. fetiae*, and even after five days for *H. megidis* and *H. bacteriophora* (Table 3). On the other hand, at 15°C *Heterorhabditis* spp. infected insects faster in conditions of coexistence with other EPNs species. At 20 °C, under control conditions, *S. feltiae* and *S. kraussei* infected all *G. mellonella* larvae the fastest, after only two days. *Heterorhabditis* species took longer to infect all tested insects, usually three days (Table 3). In all variants of the experiment, *Steinernema* spp. were never found in insect cadavers simultaneously with *Heterorhabditis* spp., regardless of the temperature (Tables 1 and 2). The study shows that *H. megidis* under conditions of co-occurrence with *Steinernema* spp. (The variants: *S. feltiae* + *H. megidis* and *S. kraussei* + *H. megidis*) were more likely to infect *G. mellonella* larvae alone and at 15 °C than at higher temperatures (Table 2). In contrast, *H. bacteriophora* in variant with *S. feltiae* infected larvae only at higher temperatures i.e. 20 °C and 25 °C, but never at 15 °C. However, the mean of infected insects by *H. bacteriophora* was always lower than that of *S. feltiae* (Table 2).

The mean number of females and male's nematode species from *Steinernema* in control conditions at different temperatures was approximate: twice as many females as males were found in the cadaver (Table 4). At 20 °C and 25 °C the mean numbers of *S. kraussei* females and males, both in the control conditions and in the presence of a competitor *H. megidis* (the variant V of experiment) were comparable, while males were always less than females (Table 4). Under conditions of co-occurrence of *H. megidis* with *S. feltiae* (variant II of the experiment) or *S. kraussei*, at 20 °C, *H. megidis* did not infect any of the tested insects under conditions of co-occurrence with *S. feltiae* (Table 4). Similarly, *H. bacteriophora* did not infect any *Galleria* larvae except at 15 °C in the co-occurrence variant with *S. feltiae*. In the variants of co-occurrence of *S. feltiae* with species of the family Heterorhabditidae, the mean number of male and female *S. feltiae* found in the host was lower compared to the control at 15 and 20 °C but significantly higher at 25 °C (Table 4).

## DISCUSSION

The biological activity of EPN depends on many abiotic and biotic factors. One of the abiotic factors is temperature, which may affect the activity of entomopathogenic nematodes, constituting a barrier to their effective use in the protection of plants against pests (Tarasco et al. 2015). Conducting the experiment on a substrate such as filter paper allowed us to minimize abiotic factors that could affect the results obtained. The optimal development of most species of entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae is observed at 20–25 °C (Saunders & Webster 1999). The conducted experiment demonstrated that both the temperature and the presence or absence of competing species influenced the tested nematodes and their activity toward the host. The closely related *S. feltiae* and *S. kraussei* in control conditions (without a competitor) showed a very high percentage of effectiveness

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in attacking the host (90–100%), in all temperature variants. However, in the presence of a competitor, the result was not obvious. The conducted studies show that at higher temperatures (20, 25 °C) under coexistence conditions, *Steinernema* spp. showed greater efficiency in colonization of the host compared to *Heterorhabditis* spp.

It is generally assumed that Steinernematidae nematodes retain their infectivity over a wide temperature range, from 2 to 30 °C (Kaya 1990; Mráček et al. 1998; Hazir et al. 2001). However, the range of temperature tolerance depends on the nematode species and its geographic location (Hazir et al. 2001; Tarasco et al. 2015; El-Khoury et al. 2018). Mráček et al. (1998) observed that isolates of the same species of nematodes from geographically distant locations differ in their infectious abilities and their reaction to the same hosts. On the other hand, it was noticed that at lower temperatures, below 15 °C, the infectious abilities of nematodes weaken and their reproduction is inhibited (Saunders & Webster 1999; Radová & Trnková 2010). Low temperature also limits the infectivity of thermophilic species, which include nematodes from the Heterorhabditidae family (Brown & Gaugler 1997). However, some species from this family, such as *H. bacteriophora* and *H. megidis*, occurring naturally in soils in the temperate climate zone, are able to live in lower temperatures and successfully compete for host with other species of entomopathogenic nematodes, as was established in our research.

In the case of coexistence of nematodes, which is a phenomenon relatively often observed in the environment (Mráček et al. 2005), an important factor influencing the effectiveness of EPNs infectivity is their feeding strategy (Campbell & Gaugler 1997; Půža & Mráček 2010). Competition is related to the domination of one species over another. Nematodes will have varying reproductive success depending on a combination of co-infective species of one host, relative inoculum size, and other environmental factors (Bashey et al. 2012; O'Callaghan et al. 2014). Moreover, recent studies show that entomopathogenic nematodes can change foraging strategies depending on environmental conditions and the availability of potential hosts (Bal et al. 2014). Increased nematode activity in the soil can also be stimulated by various chemical stimuli and temperature (Lewis et al. 2006; Dillman et al. 2012). As a consequence, the biological activity of nematodes and their effectiveness in insect infestation reflects the sum of the influence of various biotic and abiotic factors present in the environment (Lortkipanidze et al. 2016). It also depends on the adaptability of the nematodes themselves to different habitat conditions. Laboratory assays conducted for the purposes of the presented study show that the temperature can significantly affect the activity of nematodes, especially when they coexist with competing species. Although it was already observed in the literature that the feeding strategies of *S. feltiae*, *H. megidis* or *H. bacteriophora* are similar and are based on active penetration of the environment in search of a host (Půža & Mráček 2010), the conducted research has shown that in all temperature variants (15, 20 and 25 °C) *S. feltiae* infected *Galleria* larvae more effectively than *H. megidis or H. bacteriophora.* On the other hand, *S. kraussei* in the presence of *H. megidis* was more effective in infecting of the host than heterorhabditid nematodes, regardless of temperature conditions. The sex ratio of the *Steinernema* spp. in the host's body remained unvaried in all temperature variants and it was female-biased. It is noted that inside the host's cadaver, depending on the nematode species tested and the variant of the experiment, males were usually markedly less-represented. O'Callaghan et al. (2014) presented an interesting relationship in which the males can compete directly for resources, both for food (host) and females, by injuring or eliminating also males of their own species. Many authors suggest that in the initial stage of infection, the ratio of females to males is unbalanced in favour of females (Campos-Herrera et al. 2006; Alsaiyah et al. 2009; Campos-Herrera & Gutierrez 2014). Alsaiyah et al. (2009) emphasize that, under competitive conditions, the sex ratio of "pioneer" nematodes that are first to enter a host's body may favour females. Their research shows that males of the genus *Steinernema* show a greater tendency to disperse in the environment to forage for a new host, accepting the risk of finding and attacking the host. However, as emphasized by Alsaiyah et al. (2009), this trend is not consistent for all nematode species. For example, in *S. feltiae*, females may be the first to invade the host, initially dominating the host's body. Similar observations were obtained in our research, where females were always dominant both in control conditions and in variants of the coexistence of *S. feltiae* with other competing species. Some authors suggest that differences in sex ratio may be due to the difference in time of assay since the host was colonized by successive

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nematode invasive larvae and also as a result of the adopted feeding strategy (Lewis & Gaugler 1994; Alsaiyah et al. 2009). In addition, it is emphasized that differences in the behaviour and body size of the EPNs infective juveniles can be affected by their success in locating and penetrating the host (Lewis & Gaugler 1994; Campos-Herrera & Gutierrez 2014). Perhaps the greater proportion of females observed in the first generation of nematodes developing inside the host's body is intended to ensure the reproductive success of the species and is an evolutionary response to inbreeding, as suggested by Alsaiyah et al. (2009). In nature, populations of nematodes of the same species from varying habitats may show different adaptability to specific environmental conditions (Campos-Herrera & Gutierrez 2014). The present research suggests that different temperature conditions (abiotic factor) combined with the presence of a competitor (biotic factor) may stimulate or limit nematode activity. The simultaneous influence of several factors on an organism may cause unexpected reactions both within a single individual and within the entire population.

## CONCLUSION

The present research suggests that different temperature conditions (abiotic factor) combined with the presence of a competitor (biotic factor) may stimulate or limit nematode activity. The simultaneous influence of several factors on an organism may cause unexpected reactions both within a single individual and within the entire population. Understanding the intra- and extra-population mechanisms responsible for the biological activity of insecticidal nematodes and influencing their infectious capacity, survival and reproduction are keys to the further effective use of these beneficial organisms in the protection of plants against pests and should be continued.

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