

Effect of Temperature on the Virulence of Entomopathogenic Nematodes

Manana Lortkipanidze¹, Kazim Hwseynov², Mzia Kokhia¹, Oleg Gorgadze¹, Madona Kuchava¹

1. Department of entomopathogens, Ilia State University, Institute of Zoology, 3/5 Kakuca Cholokashvili Ave, Tbilisi 0162, Georgia.

2. Scientific-Research Institute of Plant Protection and Technical Cultures, 91 St. Aziz Aliyev, Ganja, AZ2002, Azerbaijan.

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Abstract: Nowadays, the use of entomopathogenic nematodes as a biological control agent is a key component in IPM system. Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* (Nematoda: Rhabditida) are extraordinarily lethal to many important insect pests, yet are safe for plants and animals. They are the only insect-parasitic nematodes possessing an optimal balance of biological control attributes.

The effect of temperature on the virulence of three species of entomopathogenic nematodes, *Steinernema thesami*, *Heterorhabditis bacteriophora* and *Steinernema feltiae* was investigated. Last instar of *Tenebrio molitor* larvae were chosen for experiment. In the laboratory, all three nematode species successfully reproduced inside *T. molitor* larvae. *H. bacteriophora* produced the highest number of infective juveniles per larva at 30°C than *S. feltiae* and *S. thesami*. *S. feltiae* caused the highest mortality of larvae at 20°C, whereas *S. thesami* infected *T. molitor* larvae at the widest temperature range and killed insects between 8-35°C. Based on the present study, we indicate that entomopathogenic nematodes have well-defined thermal breadths for their development and reproduction.

Key words: Entomopathogenic nematodes, *Heterorhabditis*, *Steinernema*, *Tenebrio molitor*, biocontrol.

1. Introduction

Entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) are soil-inhabiting insect parasites that possess potential as biological control agents (Gaugler and Kaya, 1990; Kaya and Gaugler, 1993). These nematodes have a symbiotic association with bacteria of the genus *Xenorhabdus* (Akhurst and Boemare, 1990). The bacteria convert the insects into a suitable environment for development and reproduction of the nematodes' parasitic stages (Poinar, 1990). The only function of the infective juveniles is to locate and parasitize new host (Grewal et al., 1994). The aim of this work was determined thermal factor for infection,

Corresponding author: Manana Lortkipanidze, Department of entomopathogens, Ilia State University, Institute of Zoology, 3/5 Kakuca Cholokashvili Ave, Tbilisi 0162, Georgia.

establishment and reproductions of three species of entomopathogenic nematode: *Steinernema thesami*, *Heterorhabditis bacteriophora* and *Steinernema feltiae*) in laboratory conduction. The species *Neaplectana* (= *Steinernema*) *thesami* was isolated in Mtskheta-Mtianeti Region of Georgia, from infected pupa of a winter moth, *Operophtera brumata* Linnaeus, 1758 (Geometridae: Lepidoptera). The search of endemic and new species of entomopathogenic nematodes is very important for revealing their genetic diversity and optimization because the Caucasus regions have a rich diversity of biologic species which are well adapted to the local climatic conditions and insect organisms.

The isolate of *S. thesami* was maintained in the laboratory of entomopathogenic nematodes of the Institute of Zoology of Ilia State University, Tbilisi, Georgia (Gorgadze et al., 2016).

2. Methodology

Nematodes were reared at 25°C in last instar larvae of the bread beetle *Tenebrio molitor*, according to procedures described by (Woodring and Kaya 1998). The infective juveniles (IJs) that emerged from cadavers were recovered using modified White traps (White, 1927), and stored at 7°C for 7-14 days before use (Kaya & Stock, 1997).

Infectivity of nematodes to last instar *T. molitor* at 8-35°C was tested in a sand –based assay (Grewal et al., 1993a).

Fifty infective juveniles of a nematode species in 200 µl of distilled water were inoculated into a 10 cm diameter Petri dish containing 3g dry sand.

All dishes were incubated at respective temperatures for 2 hours for acclimatization of nematodes prior to the introduction of last instar *T. molitor*. The dishes were wrapped with parafilm to reduce desiccation (Grewal 1994).

The mortality of insects was assessed after 3, 5 and 7 days and the presence of nematodes inside the insects served as the indicator of nematode infection. Dead larvae were transferred to White traps for the recovery of a new generation of IJs and incubated at 25°C until the emergence of a new generation of IJs.

The emerging IJs were harvested and counted after 11 to 15 days. Total number of IJs produced per host insect was then determined.

All treatments were replicated four times, included untreated control dishes, which received only distilled water. Mortality percentage was recorded and corrected with Abbott formula (Abbott, 1925). The penetration assay calculated the percentage of IJs entering the larvae. The assays of time of exposure and exposure to different temperature were organized on a completely randomized design (CRD). One-way ANOVA was used to compare the mortality of last instar of *Tenebrio molitor*. Means were compared at the P= 0.05 level, and

Tukey's test was used to separate means.

Experiments were carried out in the laboratory conditions at a temperature of 22°C and 80% RH.

3. Results

S. thesami and *H. bacteriophora* infected *T. molitor* larvae at the wide temperature range between 8-35°C and 8-32°C, both nematode species infected and killed host insects between 10-33°C and 10-30°C, whereas *S. feltiae* infected host at the narrow temperature between 10-25°C, compare with *S. thesami* and *H. bacteriophora* and caused the highest mortality to larvae at 20°C. There was no significant difference between *S. thesami* and *H. bacteriophora* species, significantly was observed between *S. thesami* and *S. feltiae* P <0.05 (Figure 1).

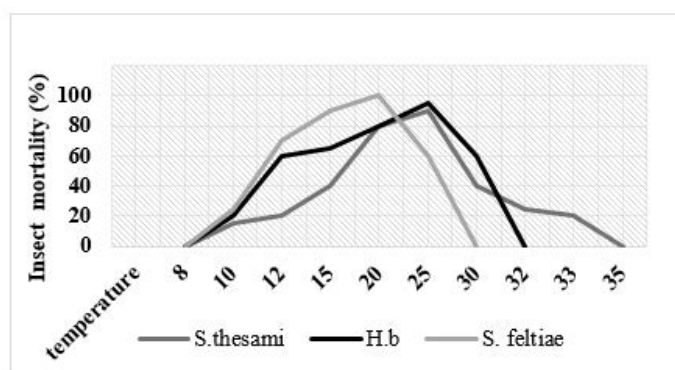


Figure. 1. Mean (\pm SE) percent mortality of *Tenebrio molitor* larvae by entomopathogenic nematodes at different temperature.

Temperature ranges for establishment of *S. thesami* and *H. bacteriophora* in insects were between 12-30°C and 15-33°C, however *S. feltiae* between 12- 30 °C. No significant difference was found between *S. thesami* and *S. feltiae* species, difference was observed between *H. bacteriophora* and *S. feltiae* P 0.05 (Figure 2).

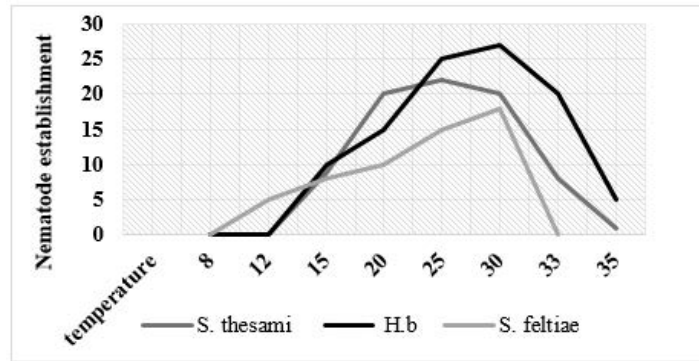


Figure 2. Mean (\pm SE) number of infective juveniles established per *Tenebrio molitor* larvae at different temperature.

After 5 days' exposure, each larva was transferred to a separate White trap containing filter paper with distilled water and the total number of emerging IJs, were counted every two days until there was no further recovery. *H. bacteriophora* produced the highest number of infective juveniles – 200, 000 per cadaver at 30°C compared with *S. thesami* and *S. feltiae*. The number of infective juveniles produced by *S. thesami* was 90.000, whereas for *S. feltiae* – 85.000 per insects (Figure 3).

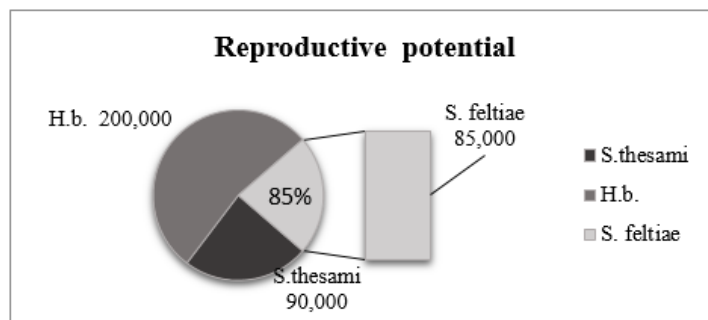
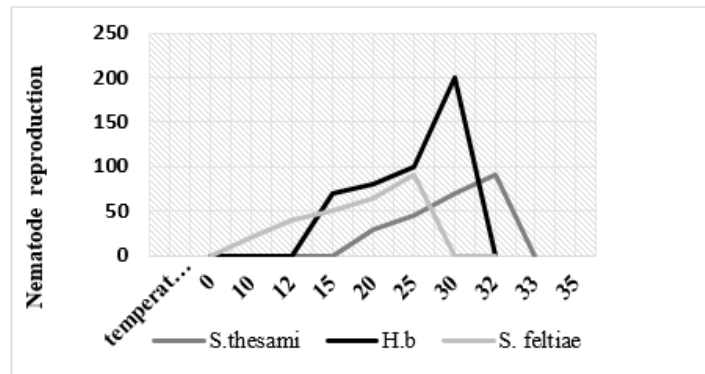


Figure 3. Mean (\pm SE) number of infective juveniles produced per *T. molitor* larvae

4. Conclusion

Biological control is a method of controlling insect pests. It relies on predation, parasitism, herbivory, or other natural mechanisms, but typically also involves an active human management role. It can be an important component of integrated pest management (IPM) programs.

The aim of biocontrol is use of living organisms - entomopathogens (entomopathogenic nematodes, entomopathogenic fungi, bacteria, virus etc.), to prevent or reduce damage caused by insect pests (Selçuk Hazir et.al 2003).

Temperature influences the nematodes' survival, infection, and reproduction, is one of the most important factors limiting the practical uses of the nematodes as biocontrol agents (Jagdale and Gordon 1998a). It has been established that the nematodes are able to adapt physiologically to environmental temperatures.

The results of the presented study show that thermal niche breadth for infection consisted of temperature range over which nematodes caused insect mortality. Establishment thermal niche was defined as the temperature range over which the nematodes developed to adults following infection.

Reproduction thermal niche breadth consisted of temperature range over which nematodes penetrated, established and produced next generation infective juveniles (Grewal 1994).

Thermal effect for infection differed among nematode species. Both species *S. thesami* and *H. bacteriophora* were more adapted to warm temperature reproduction whereas *S. feltiae* to cooler temperatures. In the laboratory, all three nematode species successfully reproduced inside *T. molitor* larvae, *H. bacteriophora* produced the highest number of infective juveniles per larva at 30°C, followed *S. thesami* and *S. feltiae*. *S. feltiae* caused the highest mortality of larvae at 20°C, whereas *S. thesami* infected *T. molitor* larvae at the widest temperature range and killed insects between 8-35°C.

All nematodes were significantly different from each other in effectiveness against last instar of *T. molitor* larvae. Temperature is the most influential environmental factor, which has great biological significance. Mortality of larvae and production of IJs in *T. molitor* increased with increasing exposure time and temperature in both experiment. In future research, for field tests will be used the most suitable nematode species for biological control of different pest insects.

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