EFFECT OF TEMPERATURE ON THE VIRULENCE OF ENTOMOPATHOGENIC NEMATODES

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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 speciesof entomopathogenic nematodes, *Steinernema thesami*, *Heterorhabditis bacteriophora* and *Steinernema feltiae* was investigated. Last instar of *Tenebrio molitor* Linnaeus, 1758 larvae were choosing 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 10–33°C. Based on the present study, we indicate that entomopathogenic nematodes have well-defined thermal breadths for their development and reproduction.

Keywords: entomopathogenic nematodes, *Heterorhabditis*, *Steinernema*, *Tenebrio molitor*, biocontrol.

INTRODUCTION

Entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) are soil-inhabiting insect parasites that possess potential as biological control agents (Gaugler & Kaya, 1990; Kaya & Gaugler, 1993). These nematodes have a symbiotic association with bacteria of the genus *Xenorhabdus* (Akhurst & 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). Variation among species of entomoparhogenic nematodes for temperature tolerance has been reported (Grewal *et al.*, 1993). The aim of this work was determined thermal factor for infection and reproductions of three species of entomopathogenic nematode: *Steinernema thesami*, *Heterorhabditis bacteriophora* and *Steinernema feltiae* (Gorgadze *et al.*, 2016) in laboratory conduction. The species *Neoaplectana*

(= *Steinernema*) *thesami* was isolated in Mtskheta-Mtianeti Region of Georgia, from infected pupa of a winter moth, *Operophtera brumata* Linnaeus, 1758 (Lepidoptera: Geometridae). The isolate of *S. thesami* was maintained in the laboratory of enthomopathogenic nematodes of the Institute of Zoology of Ilia State University, Tbilisi, Georgia.

MATERIAL AND METHODS

Nematodes were reared at 25°C in last instar larvae of the wax moth, *Galleria mellonella,* according to procedures described by (Woodring & 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 of *Tenebrio molitor* at 8–35°C was tested in a sand – based assay (Grewal *et al*., 1994). Fifty infective juveniles of a nematode species in 200 μl of distilled water were inoculated into a 5 cm diameter Petri dish containing 3 g dry sand. One last instar larva of *T. molitor* was placed on dish. The dishes were wrapped with parafilm to reduce desiccation. Insect mortality was recorded during 20 days. Dead larvae were removed from sand, washed in distilled water, dissected and the number of nematodes established recorded. Three Petri dishes were prepared for each species and for each temperature.

Nematode reproductive potential was evaluated in *T. molitor* larvae. Five last instars larvae were exposed to 500 IJs of each species on a filter paper in 10 cm diameter Petri dish. Dead larvae were transferred to White traps for the recovery of a new generation of IJs. After the start of emergence (from 5 to 14 days), IJs were collected and counted. Total number of IJs produced per host was then determined.

Both treatment was replicated four times, included untreated control dishes, which received only distilled water. Mortality percentage was recorded and corrected with Abbott formula (Abbott, 1925).

RESULTS AND DISCUSSION

Thermal effect for infection and reproduction differed among nematode species.

Infection. *S. thesami* infected *T. molitor* larvae at the widest temperature range and killed insects at 10–33°C, *H. bacteriophora* infected host at between 10–32°C. The two species of nematodes *S. thesami* and *H. bacteriophora* wide killed insects between 20–32°C and 10–30°C, whereas *S. feltiae* infected host at the narrow temperature range (10–25°C) and caused the highest mortality to larvae at 20°C (Fig. 1).

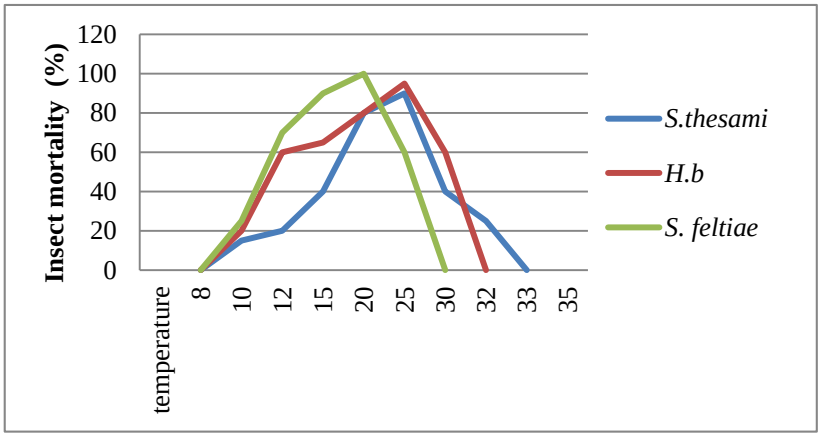

Fig. 1. Mortality of *Tenebrio molitor* larvae by entomopathogenic nematodes at different temperature.

Reproduction. Thermal breadth for reproduction was for *S. thesami* (20–32°C), for *H. bacteriophora* from 15–30°C, whereas *S. feltiae* reproduced at cooler temperatures (12–25°C) (Fig. 2).

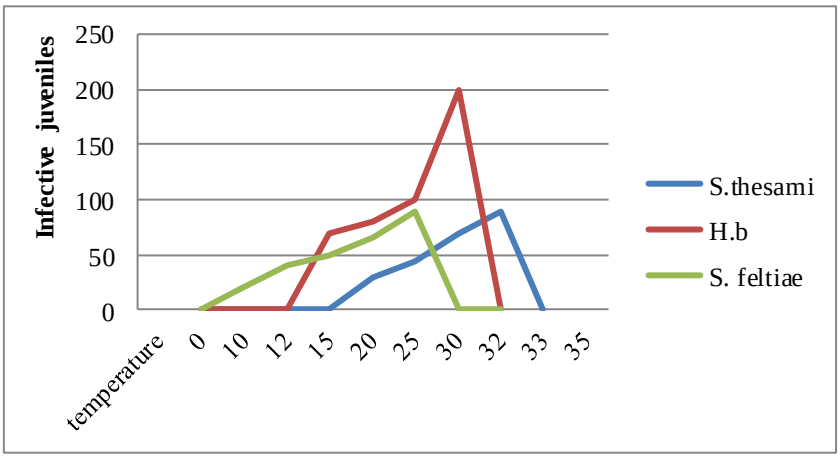

Fig. 2. Number of infective juveniles produced per *Tenebrio molitor* larvae at different temperature.

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 (Fig. 3).

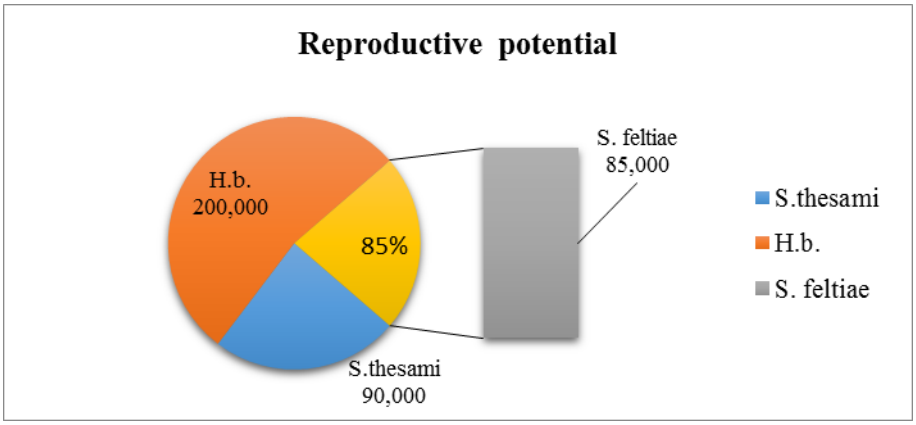

Fig. 3. Reproductive potential of entomopathogenic nematode species.

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 25°C, whereas *S. thesami* infected *T. molitor* larvae at the widest temperature range and killed insects between 12–32°C. *S. feltiae* was the only species that killed the larvae at 10°C, *S. thesami* and *H. bacteriophora* were effective at 15–30°C. The temperature significantly affected the host searching ability of all tested species.

CONCLUSIONS

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, followed *S. thesami* and *S. feltiae*. *H. bacteriophora* treatment generally caused the highest mortality levels to *T. molitor* at high temperature (30°C), whereas *S. feltiae* caused the highest mortality to *T. molitor* larvae at low temperature (25°C).

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 & Gordon, 1998). It has been established that the nematodes are able to adapt physiologically to environmental temperatures. In future research, for field tests will be used the most suitable nematode species for biological control of different pest insects.

Recommendations. All nematodes were significantly different from each other in effectiveness against last instars 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.

As an environmental factor, temperature is variable both in space and time (Prosser, 1973). Temperature influences nematode mobility, reproduction and development (Mason & Hominik, 1995).

Farmer through the activities should be acquire knowledge and choose the time and appropriate conditions for the application of entomopathogens in the field, i.e. season of the year, a-biotic factors such as temperature, humidity of soils etc. and biotic factors including living organisms. Environmental conditions – cool weather, dry conditions, UV radiation during application, climatic variation, pests, disease and price risks as well as natural disasters such as droughts and floods, free information about the weather and temperature.

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