

Assessment of Pathogenicity and Infectivity of *Heterorhabditis Bacteriophora* Against *Galleria Mellonella*

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Abstract: Entomopathogenic nematodes (EPNs) belonging to the families *Steinernematidae* and *Heterorhabditidae* are widely recognized as effective biological control agents against insect pests of agricultural importance. In the present study, entomopathogenic nematodes were isolated from soil samples collected in the Vere Valley, Tbilisi, Georgia, and their pathogenicity and infectivity were evaluated under laboratory conditions using *Galleria mellonella* larvae as a model host. Isolation was conducted using an insect baiting technique, followed by nematode recovery through a modified White trap method. Larvae were exposed to a standardized dose of eight infective juveniles per individual. Successful infection by *Heterorhabditis* spp. was confirmed by the appearance of characteristic brick-red coloration in larval cadavers, absence of putrefactive odor, and preservation of cadaver integrity. The results demonstrated high infectivity and rapid host mortality within 48–72 hours. These findings support the potential application of entomopathogenic nematodes as environmentally friendly alternatives to chemical pesticides and highlight their relevance in integrated pest management programs.

Keywords: Entomopathogenic Nematodes; *Heterorhabditis Bacteriophora*; *Galleria Mellonella*; Pathogenicity; Biological Control.

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I. INTRODUCTION

The intensive use of chemical insecticides has resulted in significant environmental concerns, including contamination of ecosystems and adverse effects on non-target organisms. Consequently, the development of sustainable and environmentally safe pest control strategies has become increasingly important. Among biological control agents, entomopathogenic nematodes (EPNs) have gained considerable attention due to their effectiveness, safety, and compatibility with integrated pest management (IPM) systems. Entomopathogenic nematodes belonging to the genera *Heterorhabditis*, *Steinernema*, and *Neosteinernema* are capable of infecting a wide range of insect hosts. The genera *Heterorhabditis* and *Steinernema* are the most extensively studied and commercially utilized because of their high virulence and broad host range. These nematodes maintain an obligate symbiotic relationship with insect-pathogenic bacteria, namely *Photorhabdus* spp. in *Heterorhabditis* and *Xenorhabdus* spp. in *Steinernema*. After penetrating the insect host, the nematodes release their symbiotic bacteria into the hemocoel, leading to septicemia and host mortality within two to three days. Entomopathogenic nematodes can be applied under field

conditions using conventional spraying equipment and are compatible with various chemical pesticides, making them suitable for integration into existing pest management programs. Although their effectiveness against insect pests from several taxonomic orders has been well documented, their practical application remains limited in many regions. Therefore, the present study aimed to isolate native entomopathogenic nematodes and assess their pathogenicity and infectivity using *Galleria mellonella* larvae as a model organism.

II. MATERIALS AND METHODS

➤ Isolation of Entomopathogenic Nematodes from Soil Samples

Soil samples were collected from different locations within the Vere Valley, Tbilisi, Georgia, at a depth of 15–20 cm. The samples were sieved to remove stones and organic debris and transferred into 200 mL plastic containers. Six late-instar larvae of *Galleria mellonella* were introduced into each container as bait. Containers were covered with perforated lids to ensure adequate aeration and incubated at room temperature. Larval mortality was monitored daily. Dead larvae were collected after three days of incubation. The

presence of brick-red coloration in larval cadavers was used as a preliminary indicator of infection by *Heterorhabditis* spp.

containers and stored at 8–12 °C. Nematode viability and activity were monitored periodically.

➤ *Recovery of Nematodes Using a Modified White Trap Method*

Entomopathogenic nematodes were recovered from infected larvae using a modified White trap technique following Kaya and Stock (1997). Individual infected larvae were placed on filter paper positioned in a small Petri dish within a larger Petri dish containing sterile distilled water. After seven days of incubation at room temperature, emerging infective juveniles were collected from the surrounding water. The nematodes were transferred to sterile

III. RESULTS

➤ *Recovery of Entomopathogenic Nematodes*

Infective juveniles were successfully recovered from soil samples using the insect baiting technique. Larval mortality occurred within approximately three days after exposure. Infected cadavers exhibited the absence of putrefactive odor and maintained structural integrity. A distinct brick-red coloration of the larvae confirmed infection by *Heterorhabditis* spp. (Figure 1).



Fig 1 Visual Diagram of the Isolation Process of Entomopathogenic Nematodes from Soil.

➤ *Bioassay of Nematode Infectivity*

Bioassays revealed a high level of infectivity of the isolated entomopathogenic nematodes against *Galleria mellonella* larvae. Mortality was observed within 48–72

hours post-inoculation. Infected larvae exhibited progressive color changes to brick red, indicating successful colonization by *Heterorhabditis* spp. nematodes (Figure 2).

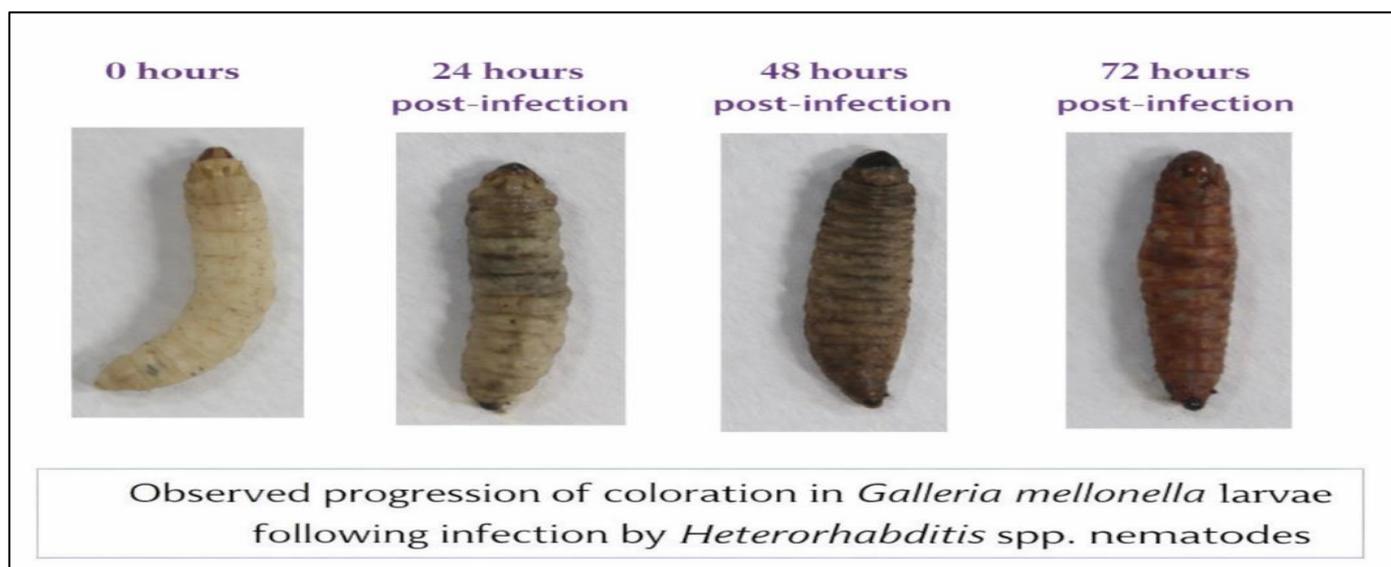


Fig 2 Color Transformation of *Galleria Mellonella* Larvae After Exposure to *Heterorhabditis* Spp.

IV. DISCUSSION

The findings of this study confirm the high pathogenicity and infectivity of *Heterorhabditis* nematodes against *Galleria mellonella* larvae. Entomopathogenic nematodes are recognized as effective biological control agents that are environmentally safe and compatible with IPM strategies. Their infective juvenile stage actively migrates through soil in search of suitable hosts, resulting in rapid insect mortality. Environmental factors such as temperature, soil moisture, and host availability may influence nematode performance; therefore, the use of locally adapted isolates can enhance their effectiveness under field conditions. Isolation using insect baiting techniques remains one of the most reliable methods for detecting entomopathogenic nematodes in soil. The present study highlights the potential of native *Heterorhabditis* isolates for sustainable pest management applications.

V. CONCLUSION

The present study demonstrated a high level of pathogenicity and infectivity of the isolated *Heterorhabditis bacteriophora* strain against the model insect *Galleria mellonella*. These results indicate the potential of this nematode for application as a biological control agent. Further studies will focus on evaluating its effectiveness against economically important agricultural insect pests under field conditions.

REFERENCES

- [1]. D. Pimentel, "Amounts of pesticides reaching target pests: Environmental impacts and ethics," *Journal of Agricultural and Environmental Ethics*, vol. 8, no. 1, pp. 17–29, 1995.
- [2]. G. O. Poinar Jr., "Description and biology of a new insect parasitic rhabditoid, *Heterorhabditis bacteriophora* n. gen., n. sp. (Rhabditida: Heterorhabditidae n. fam.)," *Nematologica*, vol. 21, pp. 463–470, 1976.
- [3]. L. Travassos, "Sobre o gênero *Oxystomatium*," *Boletim Biológico*, São Paulo, vol. 5, pp. 20–21, 1927.
- [4]. K. B. Nguyen and G. C. Smart Jr., "*Neosteinerinema longicurvicauda* n. gen., n. sp. (Rhabditida: Steinernematidae), a parasite of the termite *Reticulitermes flavipes* (Koller)," *Journal of Nematology*, vol. 26, no. 2, pp. 162–174, 1994.
- [5]. A. Burnell and S. P. Stock, "*Heterorhabditis*, *Steinernema* and their bacterial symbionts: Lethal pathogens of insects," *Nematology*, vol. 2, no. 1, pp. 31–42, 2000.
- [6]. N. Boemare, C. Laumond, and H. Mauleon, "The entomopathogenic nematode–bacterium complex: Biology, life cycle and vertebrate safety," *Biocontrol Science and Technology*, vol. 6, no. 3, pp. 333–346, 1996.
- [7]. L. Rovesti and K. V. Deseö, "Compatibility of chemical pesticides with the entomopathogenic nematodes *Steinernema carpocapsae* Weiser and *S.*

- feltiae* Filipjev (Nematoda: Steinernematidae)," *Nematologica*, vol. 36, no. 2, pp. 237–245, 1990.
- [8]. R. U. Ehlers, "Mass production of entomopathogenic nematodes for plant protection," *Applied Microbiology and Biotechnology*, vol. 56, no. 5–6, pp. 623–633, 2001.
- [9]. R. Gaugler, "Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes," *Agriculture, Ecosystems & Environment*, vol. 24, no. 1–3, pp. 351–360, 1988.
- [10]. D. Segal and I. Glazer, "Genetic approaches for enhancing beneficial traits in entomopathogenic nematodes," *Nematological Research (Japanese Journal of Nematology)*, vol. 28, pp. 61–67, 1998.
- [11]. R. Gaugler, *Entomopathogenic Nematodes in Biological Control*. Boca Raton, FL, USA: CRC Press, 2018.
- [12]. H. K. Kaya and S. P. Stock, "Techniques in insect nematology," in *Manual of Techniques in Insect Pathology*, L. A. Lacey, Ed. San Diego, CA, USA: Academic Press, 1997, pp. 281–324.
- [13]. R. J. Stuart, M. E. Barbercheck, and P. S. Grewal, "Entomopathogenic nematodes in the soil environment: Distributions, interactions and the influence of biotic and abiotic factors," in *Nematode Pathogenesis of Insects and Other Pests*. Cham, Switzerland: Springer, 2015, pp. 97–137.