INTERNATIONAL CONFERENCE ON GLOBAL RESEARCH, INNOVATION AND EMERGING TRENDS

PUBLISHED DATE: - 03-09-2026

PAGES: - 67-69

CONFERENCE ARTICLE

Biological Activity Of Steinernema And Heterorhabditis Species Isolated From Soil Using The Baermann Funnel Against The Diamondback Moth

Karimova Rikhsiniso Miratkhamovna
Doctoral student, Research Institute of Plant Quarantine and Protection, Uzbekistan

Abstract: The article presents laboratory assessment of the biological activity of local entomopathogenic nematode isolates, Steinernema carpocapsae, S. feltiae and Heterorhabditis bacteriophora, extracted from cabbage field soils by the Baermann funnel technique, against larvae of the diamondback moth (Plutella xylostella L.). Entomopathogenic nematodes were detected in 14.2% of 120 soil samples. At doses of 25–200 IJs/larva, mortality after 72 h ranged from 31.7% to 96.7%. The highest virulence was recorded for S. carpocapsae isolate UZ-S1 (LC50 = 28.4 IJs/larva).

Keywords: Entomopathogenic nematodes, Steinernema, Heterorhabditis, Baermann funnel, Plutella xylostella, biological control, infective juvenile, LC50.

Introduction

The diamondback moth (Plutella xylostella L., Lepidoptera: Plutellidae) is one of the most widespread and economically most damaging pests of cruciferous crops worldwide. The damage caused by this species and the cost of its control are estimated at about USD 4–5 billion per year [1]. The pest's short development cycle, multiple generations per year and high adaptability have led to the development of resistance to almost all major classes of insecticides [2; 3]. This makes the development of environmentally safe alternative methods an urgent task.

Among such alternatives, entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae occupy a special place. Their infective juveniles (IJs) enter the insect body through natural openings, release symbiotic bacteria (Xenorhabdus and Photorhabdus) into the haemolymph and kill the host by septicaemia within 24–72 hours [4]. The broad host range of EPNs, their safety for warm-blooded animals and plants, and the ease of their mass production have made them a promising component of integrated pest management systems [5; 6].

At the same time, the efficacy of imported commercial strains often declines under local climatic and soil conditions, since the adaptation of nematodes to temperature and moisture is strain-specific [7]. Isolates obtained from local populations, in contrast, are better adapted to regional conditions [8]. High efficacy of EPNs against the diamondback moth has previously been demonstrated even with foliar application [9].

The aim of the study was to obtain local isolates of the genera Steinernema and Heterorhabditis from the soil of cabbage agrocenoses using the Baermann funnel technique and to evaluate their biological activity against diamondback moth larvae.

Materials and methods

Soil samples were collected by the envelope method at a depth of 0–15 cm from cabbage fields in Urtachirchik and Yukorichirchik districts of Tashkent region during the 2024–2025 growing seasons (120 samples in total, 500 g each).

Nematodes were extracted using the Baermann funnel technique [10]. A rubber tube with a clamp was attached to the stem of a glass funnel 10 cm in diameter; 50 g of soil wrapped in two layers of filter paper was placed on a nylon mesh inside the funnel, and water was added up to the level of the sample. After 48 h at 22–25 °C, 10 ml of suspension was drawn off from the lower end of the tube and examined under a stereomicroscope. The extracted IJs were propagated in Galleria mellonella L. larvae and collected using White traps [11]. Species were identified on the basis of morphometric characters and by comparing ITS rDNA sequences with GenBank data.

To assess biological activity, third- to fourth-instar larvae of P. xylostella reared on cabbage leaves in the laboratory were used. Filter paper and a cabbage leaf disc (Ø 1.5 cm) were placed in each well of 24-well plates, and doses of 25, 50, 100 and 200 IJs/larva were applied in 100 µl of water; the control received distilled water. Each treatment was tested in three replicates (20 larvae per replicate) at 25 ± 1 °C and 75–80% relative humidity. Larval mortality was recorded after 24, 48 and 72 h and corrected using Abbott's formula [12]. LC50 values were estimated by probit analysis [13], and differences between treatments were assessed by one-way analysis of variance and Tukey's test ($p < 0.05$) in R 4.3.

Results

Entomopathogenic nematodes were detected in 17 of the 120 soil samples analysed (14.2%): 11 of them belonged to the genus Steinernema and 6 to the genus Heterorhabditis. Three active isolates were selected for further tests: S. carpocapsae UZ-S1, S. feltiae UZ-S3 and H. bacteriophora UZ-H2.

In all isolates, larval mortality increased consistently with increasing dose (Table 1). Natural mortality in the control did not exceed 5.0% at 72 h.

Table 1. Effect of local EPN isolates at different doses on the mortality of diamondback moth larvae (72 h, Abbott-corrected, %)

Isolate	25 IJ	50 IJ	100 IJ	200 IJ	LC50 (95% CI)
S. carpocapsae UZ-S1	46.7 ± 3.3a	68.3 ± 4.4a	86.7 ± 3.3a	96.7 ± 1.7a	28.4 (21.6–35.9)
S. feltiae UZ-S3	38.3 ± 4.4ab	58.3 ± 3.3ab	76.7 ± 4.4ab	90.0 ± 2.9ab	39.7 (31.2–49.5)
H. bacteriophora UZ-H2	31.7 ± 3.3b	51.7 ± 4.4b	71.7 ± 3.3b	85.0 ± 2.9b	48.6 (38.9–60.3)

Note: values within a column followed by different letters differ significantly (Tukey, $p < 0.05$); LC50 is expressed in IJs/larva; CI – confidence interval.

S. carpocapsae UZ-S1 showed the lowest LC50 value and caused almost complete (96.7%) mortality at 200 IJs/larva. The LC50 of H. bacteriophora UZ-H2 was 1.7 times higher. Analysis of mortality dynamics (Figure 1) showed that the isolates also differed in speed of action: at 100 IJs/larva, S. carpocapsae killed 38.3% of the larvae within the first 24 h, whereas for H. bacteriophora this figure was 16.7%. By 72 h the difference had narrowed but remained significant.

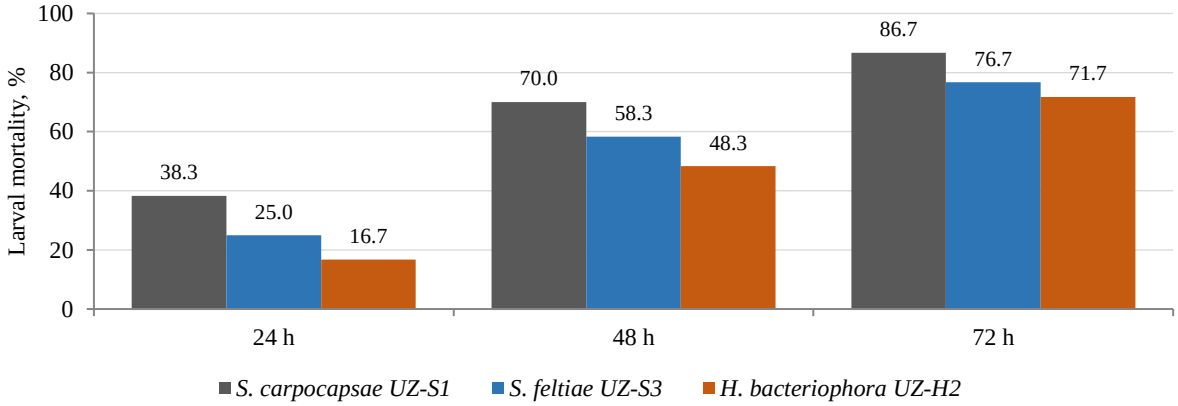

Figure 1. Time course of diamondback moth larval mortality at 100 IJs/larva, %

Discussion

The results are consistent with literature data on the high efficacy of S. carpocapsae against pests actively moving on leaf and soil surfaces [4; 9]. The "ambusher" foraging strategy of this species, i.e. its ability to wait on the substrate surface and attach to a passing mobile host, gives it an advantage against diamondback moth larvae feeding on leaves. H. bacteriophora, in contrast, is a "cruiser" species adapted mainly to less mobile insects in the soil [5]; this explains its relatively slower action on leaves. The intermediate performance of S. feltiae may be related to its adaptation to lower temperatures, since the bioassay was conducted at 25 °C [7].

The frequency of EPN occurrence in the samples (14.2%) falls within the range of 2–45% reported in studies conducted in temperate and subtropical regions [8]. Although the Baermann funnel technique is inexpensive and simple, it extracts only mobile IJs; therefore, combining it with the G. mellonella trap method increases the probability of detection [10; 11]. Since EPNs are sensitive to ultraviolet radiation and desiccation in the field, application in the evening with moisture-retaining adjuvants is recommended [6; 9].

Conclusion

Entomopathogenic nematodes of the genera Steinernema and Heterorhabditis are widespread in the soil of cabbage agrocenoses, and the Baermann funnel technique is an effective and convenient means of isolating them. All local isolates showed high biological activity against diamondback moth larvae; in terms of virulence they ranked as follows: S. carpocapsae UZ-S1 > S. feltiae UZ-S3 > H. bacteriophora UZ-H2. The S. carpocapsae UZ-S1 isolate is promising for developing a biological preparation against the diamondback moth; its field testing and the development of mass-production technology are the main directions of further research.

References

1. Zalucki M.P., Shabbir A., Silva R. et al. Estimating the economic cost of one of the world's major insect pests, Plutella xylostella (Lepidoptera: Plutellidae): just how long is a piece of string? // Journal of Economic Entomology. – 2012. – Vol. 105, No. 4. – P. 1115–1129.
2. Talekar N.S., Shelton A.M. Biology, ecology, and management of the diamondback moth // Annual Review of Entomology. – 1993. – Vol. 38. – P. 275–301.
3. Furlong M.J., Wright D.J., Dosdall L.M. Diamondback moth ecology and management: problems, progress, and prospects // Annual Review of Entomology. – 2013. – Vol. 58. – P. 517–541.
4. Kaya H.K., Gaugler R. Entomopathogenic nematodes // Annual Review of Entomology. – 1993. – Vol. 38. – P. 181–206.
5. Lacey L.A., Georgis R. Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production // Journal of Nematology. – 2012. – Vol. 44, No. 2. – P. 218–225.
6. Shapiro-Ilan D.I., Hazir S., Glazer I. Basic and applied research: entomopathogenic nematodes // Microbial Control of Insect and Mite Pests / ed. L.A. Lacey. – London: Academic Press, 2017. – P. 91–105.
7. Grewal P.S., Selvan S., Gaugler R. Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment, and reproduction // Journal of Thermal Biology. – 1994. – Vol. 19, No. 4. – P. 245–253.
8. Hominick W.M. Biogeography // Entomopathogenic Nematology / ed. R. Gaugler. – Wallingford: CABI Publishing, 2002. – P. 115–143.
9. Schroer S., Ehlers R.-U. Foliar application of the entomopathogenic nematode Steinernema carpocapsae for biological control of diamondback moth larvae (Plutella xylostella) // Biological Control. – 2005. – Vol. 33. – P. 81–86.
10. Baermann G. Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben // Geneeskundig Tijdschrift voor Nederlandsch-Indië. – 1917. – Vol. 57. – P. 131–137.
11. Kaya H.K., Stock S.P. Techniques in insect nematology //

Manual of Techniques in Insect Pathology / ed. L.A. Lacey. – San Diego: Academic Press, 1997. – P. 281–324.

12. Abbott W.S. A method of computing the effectiveness of an insecticide // Journal of Economic Entomology. – 1925. – Vol. 18. – P. 265–267.

13. Finney D.J. Probit Analysis. 3rd ed. – Cambridge: Cambridge University Press, 1971. – 333 p.