ANADOLU UNIVERSITY
FACULTY OF PHARMACY

European Journal of Life Sciences 2025; 4(2): 55-63

Research Article

Characterization of native entomopathogenic nematode isolates from Bilecik, Türkiye: Dispersal, heat tolerance and virulence

Aydan Terzioğlu[1], Rabia Nur Aksu[1], Tufan Can Ulu✉[1], Filiz Randa Zelyüt[1]

[1]Department of Plant Protection, Faculty of Agriculture and Natural Sciences, Bilecik Şeyh Edebali University, Bilecik, Türkiye

✉ Tufan Can Ulu
tufan.ulu@bilecik.edu.tr

https://doi.org/10.55971/EJLS.1696030

Received: 09.05.2025
Accepted: 19.06.2025
Available online: 19.07.2025

ABSTRACT

This study aimed to evaluate the biological and ecological characteristics of entomopathogenic nematode isolates collected from soil samples in Bilecik, Türkiye, to assess their potential for use in biological pest control. The isolates were tested for their dispersal ability in olfactometers, pathogenicity against greater wax moth larvae, and tolerance to elevated temperatures. A commercial strain and a hybrid strain were included as references for comparison. The experiments revealed significant variability among the isolates. One local isolate exhibited strong dispersal capacity and heat tolerance, outperforming the reference strains in some parameters. Conversely, certain isolates showed lower levels of virulence, indicating limited pest control potential. Lethal dose assays demonstrated that pathogenicity varied substantially, with some local isolates matching or exceeding the performance of commercial strains under laboratory conditions. Mortality assessments across increasing temperature levels revealed that sensitivity to heat stress was not uniform and differed considerably among isolates. The findings confirm that biological performance is highly isolate-dependent and influenced by multiple interacting traits. While some local isolates showed promise in one or more evaluated traits, no single isolate combined superior performance in all parameters. Therefore, the results provide a valuable foundation for further characterization studies involving additional traits such as reproductive capacity, storage potential, and field efficacy. Overall, this study emphasizes the importance of locally adapted isolates in developing effective and environmentally sustainable pest management strategies. Selecting and improving such isolates may contribute to the broader adoption of biological control practices and reduce dependency on chemical pesticides..

Keywords: Biological control, *Heterorhabditis*, pest, *Steinernema*

1. INTRODUCTION

In modern agriculture, the overuse of synthetic pesticides has raised serious concerns regarding environmental safety, human health, and pest resistance. As a result, interest in alternative, eco-friendly strategies has grown. Among these, entomopathogenic nematodes (EPNs) have gained attention as effective biological control agents. They offer several advantages, including environmental compatibility, safety for non-target organisms, and reduced risk of resistance development in insect pests [1, 2]. Entomopathogenic nematodes are soil-dwelling microscopic roundworms. Due to their biology, they require an insect host to complete their development, and in the process, they kill the host [3]. This characteristic gives them considerable potential as agents for biological control of insect

pests. Today, the most commonly used EPN species in biological control belong to Steinernematidae and Heterorhabditidae families [4]. EPNs enter their hosts through natural body openings or the intersegmental membrane, reach the hemocoel, and release their symbiotic bacteria into the host's hemolymph, ultimately causing the host's death [5]. The nematodes then reproduce inside the cadaver and return to the soil as infective juveniles (IJs) to seek new hosts [5]. EPNs are effective against hundreds of insect species, particularly soil-dwelling pests from various insect orders [6]. Their ability to actively seek out hosts, survive for extended periods in the soil, and be mass-produced in liquid culture contributes to their high biological efficacy [7,8]. However, high production costs and shorter shelf life compared to chemical pesticides limit their commercial competitiveness [9].

Despite their potential, EPNs often show inconsistent performance under field conditions, which limits their practical use in pest control programs [10]. Various biological and environmental factors, such as temperature, soil moisture, UV exposure, and host availability, can significantly influence their effectiveness. In addition, challenges like the need for skilled application techniques and high production costs further reduce their adoption. To overcome these limitations, efforts have been made to improve mass production methods, application technologies, and formulation stability [11-16]. Another important strategy is the identification and use of locally adapted or stress-tolerant isolates to enhance field performance and reliability [17-21]. These approaches continue to guide experimental efforts aimed at improving the practical use of EPNs under variable environmental conditions.

Numerous studies have focused on isolating native EPN strains and evaluating their biological and ecological characteristics to determine their potential for commercialization. Researchers have characterized local EPN populations by examining key factors such as tolerance to high temperatures, infectivity, reproductive capacity, and adaptability to different environments [22-24]. The goal of these studies is to identify superior strains that can enhance the effectiveness and reliability of EPN-based biological control strategies. Selecting and developing high-performing isolates is considered a crucial step toward expanding the practical use of EPNs in pest management, especially in diverse environmental conditions [25]. Such efforts contribute to advancing sustainable agriculture by promoting the use of efficient and locally adapted biocontrol agents.

Although faunistic surveys and EPN characterization studies date back several decades, they hold significant scientific relevance. Recent research highlights the ongoing need to explore native EPN populations and assess their biological and ecological features in order to identify strains with high commercial potential. These efforts broaden our understanding of EPN diversity and distribution and facilitate the selection of isolates with desirable traits such as heat tolerance, reproductive ability, and pathogenicity. Developing such robust, locally adapted biocontrol agents is essential for increasing the adoption of EPN-based approaches in sustainable pest management programs. Although various native EPN strains have been isolated and characterized worldwide, region-specific evaluations are still lacking, especially in underexplored areas such as Bilecik. The isolates used in this study were collected from Bilecik Province, located at the intersection of several major climatic zones in northwestern Türkiye. The region has a moderate elevation (~500 m) and receives approximately 450 mm of rainfall annually, mostly during the winter and spring months. Due to the presence of microclimatic zones along the Sakarya River, certain districts are suitable for intensive greenhouse agriculture. These conditions may contribute to the development of nematode strains with potential tolerance to variable soil moisture and temperature fluctuations. Identifying strains with enhanced ecological performance under local environmental conditions is essential for expanding the applicability of EPN-based pest control.

In this study, native EPN isolates collected from Bilecik and its districts were evaluated for their heat tolerance, dispersal capacity, and virulence against the great wax moth larvae. Their performance was compared with a commercial and hybrid strain.

The aim was to identify promising candidates for future use in biological control and to support the development of locally adapted strains in Türkiye.

2. MATERIALS AND METHODS

2.1. EPN Isolates

Entomopathogenic nematode isolates used in this study were obtained from soil samples collected between 2019 and 2022 in the city center and districts of Bilecik, Türkiye. All isolates were morphologically identified [26] (Unpublished data), and detailed information regarding their isolation is presented in Table 1. For comparison, a commercial strain of E-nema GmbH was supplied by the company Bioglobal A.Ş., along with the superior HBH hybrid strain [27] was included as a reference group.

2.2. *G. mellonella* Larvae Production

Last-instar larvae of the greater wax moth, *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera:Pyralidae), were used in the experiments. The larvae were reared on an artificial diet. The diet consisted of honey, glycerin, wheat bran, cornmeal, soybean flour, milk powder, and yeast. The mixture was prepared and then transferred into 1-liter glass jars. Filter paper containing *G. mellonella* eggs was placed on the surface of the cooled diet. The jars were covered with perforated wire mesh lids and incubated in a growth chamber at 28 ± 2°C. After hatching, the larvae fed on the artificial diet and reached the mature larval stage within approximately four weeks. The mature larvae were subsequently used for the production of EPNs.

2.3. Nematode Production

Before applying EPNs to *G. mellonella* larvae, a moist filter paper was placed at the bottom of sterile glass Petri dishes to maintain humidity. Then, IJs were applied to the larvae at a dose of 100 IJ/larva using a micropipette. Following the application, larval mortality was monitored daily, and dead individuals were transferred to the White traps [28]. Within two weeks of infection, IJs emerging from the nutrient-depleted cadavers migrated into the Ringer's solution. The collected IJs were then transferred into ventilated culture containers and stored at +10 °C for long-term preservation.

2.4. Determination of Dispersal

To observe the behavioral responses of EPNs, custom-designed olfactometers made of stainless steel were used. These olfactometers consisted of a central part and two lateral arms. The central part included an application hole through which IJs could be introduced into the system. The olfactometer had a diameter of approximately 2.5 cm, and the length from the central point to the end of each arm was about 10 cm. Before each trial, the olfactometers were filled with chemically inert, dust-free silica sand (particle size: 250 µm), moistened to 10% humidity, which is optimal for nematode movement [29].

Table 1. Details of EPN isolates

Isolate	Genus/Species	Latitude	Longitude	Altitude	Sampling Date	Crop
PY51	*Steinernema feltiae*	39,9858477	29,94950158	823	18.08.2020 17:15	Maize
ÇY6	*Heterorhabditis bacteriophora*	40,06836724	30,26193925	232	30.07.2021 17:24	Watermelon
GP7	*H. bacteriophora*	40,25212799	30,06849549	151	8.09.2021 17:01	Pepper
GP8	*H. bacteriophora*	40,25199756	30,06787971	146	8.09.2021 17:05	Persimmon
GP9	*H. bacteriophora*	40,25188	30,06781283	148	8.09.2021 17:06	Pepper
GP16	*H. bacteriophora*	40,24379435	30,07503532	149	8.09.2021 18:10	Pepper
GP27	*H. bacteriophora*	40,25450209	30,06558239	166	8.09.2021 16:33	Walnut
HBH	*H. bacteriophora*	-	-	-	-	-
Hb-Enema	*H. bacteriophora*	-	-	-	-	-

Before the experiments, the concentration of IJs in each culture was determined. Then, approximately 2,500 IJs were introduced into the central hole of the olfactometer using a pipette. After application, the hole was sealed with Parafilm, and the olfactometers were incubated at 24 °C for 24 hours.

At the end of the incubation period, the sand from the central part and lateral arms of the olfactometer was transferred into separate containers. The number of nematodes in each section was counted, and the dispersal rate for each isolate was calculated using the formula: "total number of nematodes in both arms / total number of nematodes in the olfactometer."

2.5. Determination of Heat Tolerance

To assess the effect of high temperatures, experiments were conducted at 30, 35, 38, 40, and 45 °C for each EPN isolate. Preliminary observations showed a sharp increase in nematode mortality between 35 °C and 40 °C; therefore, 38 °C was included as an additional test temperature to better capture the tolerance threshold. All treatments were carried out in 24-well cell culture plates (well diameter: 1.4 cm; well volume: 3 cm^3), with a total of four wells used per isolate across two different time points.

Prior to the experiments, isolates stored at +10 °C were removed from refrigeration and allowed to acclimate to room temperature for two hours. Approximately 250 infective juveniles (IJs), suspended in 30–50 µl of liquid, were added to each well. The final volume in each well was adjusted to 2 mL using sterile distilled water.

The plates were sealed with Parafilm and transferred into incubators pre-set to the target temperatures (60% ± 2% relative humidity) at least two hours in advance. The nematodes were exposed to the specified temperature conditions for 24 hours. After the exposure period, the plates were removed and left at room temperature for two additional hours to allow recovery from potential estivation caused by heat stress. Once the IJs resumed movement, the numbers of live and dead individuals were recorded.

2.6. Determination of Virulence

Virulence assays were conducted in 1.5 ml centrifuge tubes. To determine the lethal dose, *G. mellonella* larvae [30, 31] were exposed to 1, 5, 10, 25, 50, 100, and 200 IJ/larva doses. One last-instar larva was placed in each tube, and IJs suspended in 50 µl of water were applied using a micropipette. For each dose, 10 larvae were used. In the control group, 10 larvae received 50 µl of sterile water without nematodes. After application, the tubes were incubated at 24 ± 2 °C in darkness. The experiment was conducted in triplicate for each isolate, resulting in a total of 30 larvae per treatment. At the end of the four-day incubation period, larvae were removed from the tubes, dissected, and examined for the presence of nematodes. Mortality rates were calculated based on these observations and used for lethal dose (LD) estimations.

2.7. Statistical Analysis

The normality of the data obtained from the experiments was assessed using QQ plots. Homogeneity of variances between treatment groups was evaluated with Bartlett's test. One-way ANOVA was applied to compare the dispersal rates obtained from the olfactometer assays and the mortality rates from the heat tolerance tests, followed by Tukey's HSD test to determine differences among groups. Lethal dose (LD) values were estimated using probit analysis, for which the data were log_{10}-transformed. Significant differences among isolates were determined based on the non-overlapping 95% confidence intervals of the estimated lethal doses. All statistical analyses were performed at a 5% significance level. Statistical analyses and data visualization were conducted using R-Studio, GraphPad Prism v10.0, and SPSS v17.0 software.

3. RESULTS AND DISCUSSION

3.1. Dispersal

Dispersal capacity varied significantly among the tested EPN isolates ($F(8, 18) = 9.93$, $p < 0.0001$).

The isolate GP16 showed the highest average dispersal rate (56.0%), while the lowest was recorded for the hybrid strain HBH (34.7%). The commercial strain Hb-Enema exhibited an intermediate value (40.5%). These findings indicate that certain local isolates possess stronger dispersal abilities than both the hybrid and commercial reference strains under laboratory conditions (Figure 1).

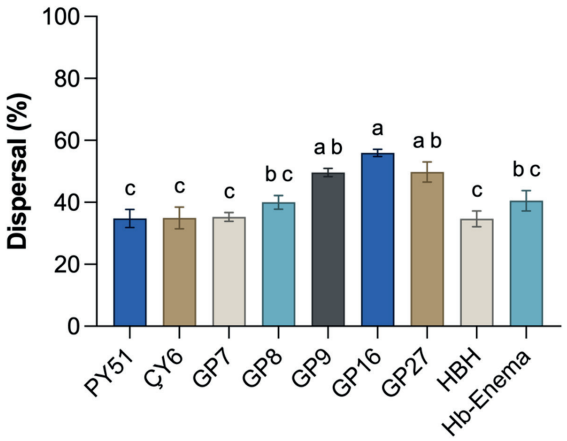

Figure 1. Dispersal ratios in the olfactometer after 24 hours. The assays were conducted at 24 °C, and the mean proportion of individuals found in the arms of the olfactometer is presented. Error bars represent the standard error of the mean. The bars marked with different letters are significantly different according to Tukey's HSD test ($p < 0.05$).

Entomopathogenic nematode dispersal is highly variable and can be significantly altered depending on the specific strain or isolate, with profound implications for other key traits like infectivity and reproduction [32]. Genetic selection studies on the ambush-foraging *S. carpocapsae*, for instance, have shown that dispersal can be substantially enhanced by selecting for "sprinters" in the absence of hosts, leading to a significant increase in dispersal distance over multiple generations [33]. This enhancement reflects the innate population variability and genetic basis of dispersal within a single species. Similarly, significant differences in vertical dispersal patterns and average displacement have been observed among various strains of *Steinernema* species, demonstrating strain-level variation in movement behavior [32]. Beyond intra-species variation, dispersal rates can also vary significantly at the genus level, with *Steinernema* species typically dispersing faster than *Heterorhabditis* species [34]. The observed variation among our isolates also supports that dispersal is a strain-dependent trait, consistent with previous studies highlighting the influence of genetic background and environmental factors on nematode movement [35, 36].

3.2. Heat Tolerance

The mortality of IJs significantly increased with rising temperature. While no significant differences were observed among isolates at 25 °C and 30 °C, mortality differences became statistically significant at 35 °C and above. At 35 °C, PY51 had the highest mortality (7.28 ± 1.31%), whereas GP8 showed the lowest (1.65 ± 0.49%). More pronounced differences were observed at 38 °C and 40 °C. At 38 °C, PY51 and ÇY6 exhibited the highest mortality rates (61.9% and 58.1%, respectively), while GP9, GP16, and HBH showed relatively lower mortality (33.7–36.1%). At 40 °C, GP16 demonstrated the greatest heat tolerance with the lowest mortality (57.4%), significantly lower than most other isolates, including the commercial strain Hb-Enema (71.0%) and the hybrid strain HBH (78.1%). At 45 °C, mortality reached 100% in all isolates (Table 2). These results suggest that although all isolates are highly sensitive to extreme heat, some native isolates, particularly GP16 and GP9, may possess comparatively higher tolerance to sub-lethal high-temperature conditions.

Mortality rates increased sharply beyond 35 °C, highlighting the strain-specific nature of heat tolerance. GP16 exhibited the lowest mortality at 40 °C, indicating better adaptation to heat stress than both the hybrid (HBH) and commercial (Hb-Enema) strains. These results are consistent with previous findings showing that some hybridized *H. bacteriophora* strains can exhibit enhanced thermal tolerance compared to commercial products [27]. Similarly, studies on Turkish *H. bacteriophora* populations have reported significant variability in heat tolerance, with certain native strains outperforming imported commercial strains [37]. Additionally, thermal and osmotic resistance have been documented in *S. carpocapsae* [25], and *S. siamkayai* has been identified as a thermophilic species with optimal activity between 30 and 35 °C [24]. The present findings indicate that

Table 2. Temperature-dependent mortality of the isolates

Isolates	25 °C	30 °C	35 °C	38 °C	40 °C	45 °C
PY51	3.48 ± 0.986 a*	4.93 ± 1.03 a	7.28 ± 1.31 a	61.9 ± 1.29 a	92.5 ± 0.935 a	100
ÇY6	3.71 ± 0.503 a	2.59 ± 0.868 a	2.03 ± 0.468 b	58.1 ± 1.38 a	84 ± 1.15 b	100
GP7	2.95 ± 0.565 a	2 ± 0.278 a	2.82 ± 0.604 b	43.9 ± 1.44 bc	79.9 ± 1.25 b	100
GP8	1.7 ± 0.353 a	2.63 ± 0.541 a	1.65 ± 0.487 b	49.5 ± 1.36 b	95.6 ± 0.972 a	100
GP9	2.53 ± 0.387 a	3.68 ± 0.696 a	3.24 ± 0.568 b	36 ± 1.19 d	83.8 ± 1.58 b	100
GP16	3.16 ± 0.568 a	3.88 ± 0.9 a	2.38 ± 0.271 b	36.1 ± 2.1 d	57.4 ± 2.83 d	100
GP27	1.83 ± 0.44 a	4.43 ± 0.542 a	2.59 ± 0.381 b	46.9 ± 2 b	84.4 ± 0.999 b	100
HBH	1.51 ± 0.394 a	2.93 ± 0.463 a	2.37 ± 0.78 b	33.7 ± 2.66 d	78.1 ± 1.69 b	100
Hb-Enema	1.48 ± 0.423 a	3.31 ± 0.284 a	2.6 ± 0.626 b	38.9 ± 1.45 cd	71 ± 1.73 c	100
ANOVA	F(8, 63) = 2.61	F(8, 63) = 1.98	F(8, 63) = 6.16	F(8, 63) = 34.2	F(8, 63) = 53.1	-
P	p = 0.0157	p = 0.0639	p < 0.0001	p < 0.0001	p < 0.0001	-

* Different letters within the same column indicate statistically significant differences among isolates according to Tukey's HSD test ($P < 0.05$).

native isolates such as GP16 may offer competitive thermal resilience. However, consistent with studies emphasizing isolate-level variation in abiotic stress responses [23], the results show that heat tolerance is not uniformly distributed even among local populations. These observations support the importance of strain-level screening for selecting EPNs suited to regions with elevated soil temperatures.

3.3. Virulence

The lethal dose (LD_{50}) values of the tested EPN isolates against *G. mellonella* larvae showed substantial variation. The least virulent isolate was PY51, with the highest LD_{50} (65.91 IJ/larva) and LD_{90} (245.08 IJ/larva), indicating low infectivity. In contrast, the commercial strain Hb-Enema and the hybrid strain HBH exhibited the lowest LD_{50} values (5.15 and 6.00 IJ/larva, respectively), along with relatively low LD_{90} values (47.11 and 34.95 IJ/larva, respectively), reflecting high virulence. Among local isolates, GP7 also showed high virulence with a low LD_{50} of 4.54 and LD_{90} of 70.34 IJ/larva (Table 3). For all isolates, the dose–mortality relationship was statistically significant ($p < 0.05$), indicating a consistent and reliable increase in mortality with increasing dose levels.

Virulence is one of the most decisive parameters in evaluating the efficacy of EPNs. In this study, significant differences in virulence were detected among isolates, as reflected in their LD_{50} and LD_{90} values. Isolate GP7 showed virulence levels comparable to those of the commercial (Hb-Enema) and hybrid (HBH) strains, while PY51 was the least

Table 3. Log-dose probit analysis and estimated LD_{50} and LD_{90} values of the isolates

Isolate	N (total)	Slope ± SE	LD_{50} (IJ/larva) (95% CL)	LD_{90} (IJ/larva) (95% CL)	$\chi 2$ (df)	P value
PY51	30	2.247 ± 0.300	65.913 (51.734-86.311)	245.076 (167.591-447.035)	4.295 (5)	<0.001
ÇY6	30	1.504 ± 0.184	14.664 (10.337-20.084)	104.337 (67.623-197.743)	6.241 (5)	<0.001
GP7	30	1.077 ± 0.153	4.540 (2.450-7.151)	70.342 (40.658-163.624)	5.188 (5)	<0.001
GP8	30	1.480 ± 0.177	9.715 (6.658-13.483)	71.389 (46.792-130.620)	6.624 (5)	<0.001
GP9	30	1.217 ± 0.157	11.902 (7.807-17.229)	134.396 (79.014-301.750)	5.238 (5)	<0.001
GP16	30	1.923 ± 0.230	17.756 (13.367-23.192)	82.341 (57.493-138.689)	9.077 (5)	<0.001
GP27	30	1.399 ± 0.170	17.337 (12.175-24.163)	142.853 (88.617-290.163)	3.360 (5)	<0.001
HBH	30	1.675 ± 0.206	6.001 (4.113-8.249)	34.951 (23.831-60.043)	3.144 (5)	<0.001
Hb-Enema	30	1.334 ± 0.173	5.154 (3.191-7.530)	47.111 (29.972-90.908)	1.184 (5)	<0.001

effective. These findings confirm that virulence is highly isolate-dependent, which is consistent with previous reports highlighting considerable intraspecific variation in pathogenicity among EPNs [17, 18, 22, 38-40]. Moreover, some studies have demonstrated that native isolates can outperform commercial strains under laboratory conditions [41, 42]. Nevertheless, laboratory-based virulence does not always translate to field performance, underscoring the importance of evaluating promising candidates under real-world conditions prior to commercialization.

Although the findings of the present study are not yet sufficient for definitive isolate selection, they provide an important foundation for future characterization efforts. The use of locally adapted isolates is considered crucial for improving the effectiveness and consistency of biological control applications under specific environmental conditions. Numerous researchers have also emphasized the value of identifying and characterizing native EPN populations to discover strains capable of high performance under local stress factors [17, 19, 22, 23]. Among various traits, temperature tolerance, virulence, and dispersal capacity are consistently highlighted as key indicators of field efficacy [22, 43]. Therefore, this study prioritized these fundamental characteristics to provide a preliminary assessment of the applied potential of the tested isolates.

4. CONCLUSION

This study highlights the biological and ecological diversity among EPN isolates collected from Bilecik, Türkiye. While certain local isolates, such as GP16, demonstrated promising characteristics others showed limitations in virulence or adaptability. These results reinforce that EPN performance is highly isolate-dependent and shaped by multiple interacting traits. Although the findings provide valuable initial insights, they are not sufficient for definitive isolate selection. Further research involving a broader range of biological and ecological traits, such as reproductive capacity, shelf life, and stress tolerance, is necessary. In addition, field-based evaluations under variable environmental conditions are essential to validate laboratory observations and assess practical performance. As the demand for environmentally friendly alternatives to chemical pesticides increases, the role of locally adapted EPNs in sustainable pest management is expected to grow. Integrating such isolates into biological or integrated pest management (IPM) programs could enhance control efficacy, reduce pesticide dependence, and support long-term agroecological sustainability.

Acknowledgements

The authors would like to thank Zahide Uygun and Merve Yazkan for their support.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Conceptualization, T.C.U. and F.R.Z.; Methodology, T.C.U., F.R.Z., A.T. and R.N.A.; Software, T.C.U.; Validation, T.C.U. and F.R.Z.; Formal analysis, T.C.U.; Investigation, T.C.U.; Resources, T.C.U. and F.R.Z.; Data curation, A.T. and R.N.A.; Writing—original draft preparation, T.C.U., A.T. and R.N.A.; Writing—review and editing, T.C.U. and F.R.Z.; Visualization, T.C.U.; Supervision, T.C.U. and F.R.Z.; Project administration, T.C.U.; Funding acquisition, T.C.U. All authors have read and agreed to the published version of the manuscript.

Source of funding

This study was supported by the Scientific Research Projects Unit of Bilecik Şeyh Edebali University under the grant no: 2019-01.BŞEÜ.06-02.

Conflict of interest

The authors declared that there is no conflict of interest.

REFERENCES

1. Campos-Herrera R, Vicente-Díez I, Blanco-Pérez R, Chelkha M, González-Trujillo MDM, Puelles M, Čepulitė R, Pou A. Positioning entomopathogenic nematodes for the future viticulture: Exploring their use against biotic threats and as bioindicators of soil health. Turk J Zool. (2021);45 (Special issue 1):335–346. https://doi.org/10.3906/ZOO-2106-40
2. Manochaya S, Udikeri S, Srinath BS, Sairam M, Bandlamori SV, Ramakrishna K. In vivo culturing of entomopathogenic nematodes for biological control of insect pests: A review. J Nat Pest Res (2022);1:100005. https://doi.org/10.1016/j.napere.2022.100005
3. Koppenhöfer AM, Shapiro-Ilan DI, Hiltpold I. Entomopathogenic nematodes in sustainable food production. Front Sustain Food Syst. (2020);4(125):1–14. https://doi.org/10.3389/fsufs.2020.00125
4. Poinar GO, Grewal P. History of entomopathogenic nematology. J Nematol. (2012);44(2):153–61. PMID: 23482453
5. Kaya HK, Gaugler R. Entomopathogenic nematodes. Ann Rev Entomol. (1993);38(1):181–206. https://doi.org/10.1146/annurev.en.38.010193.001145
6. Peters A. The natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact on insect populations. Biocontrol Sci Techn. (1996);6(3):389–402. https://doi.org/10.1080/09583159631361
7. Ehlers R-U. Mass production of entomopathogenic nematodes for plant protection. Appl Microbiol Biotechnol. (2001);56(5–6):623–633. https://doi.org/10.1007/s002530100711
8. Shapiro-Ilan DI, Han R, Dolinksi C. Entomopathogenic nematode production and application technology. J Nematol. (2012);44(2):206–217. PMID: 23482883
9. Dunn MD, Belur PD, Malan AP. A review of the in vitro liquid mass culture of entomopathogenic nematodes. Biocontrol Sci Techn. (2021);31(1):1–21. https://doi.org/10.1080/09583157.2020.1837072
10. Erdoğan H, Ünal H, Susurluk İA, Lewis EE. Precision application of the entomopathogenic nematode *Heterorhabditis bacteriophora* as a biological control agent through the Nemabot. Crop Prot. (2023);174:106429. https://doi.org/10.1016/j.cropro.2023.106429
11. Metwally HMS, Saleh MME, Abonaem M. Formulation for foliar and soil application of entomopathogenic nematodes for controlling the onion thrips *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). Egypt J Biological Pest Cont. (2025);35(1):4. https://doi.org/10.1186/s41938-025-00841-8
12. Askary TH, Abd-Elgawad MMM. Opportunities and challenges of entomopathogenic nematodes as biocontrol agents in their tripartite interactions. Egypt J Biological Pest Cont. (2021);31(1):42. https://doi.org/10.1186/s41938-021-00391-9
13. Peters A. Application and commercialization of nematodes. Appl Microbiol Biotechnol. (2013);97(14):6181–6188. https://doi.org/10.1007/s00253-013-4941-7
14. Kapranas A, Malone B, Quinn S, O'Tuama P, Peters A, Griffin CT. Optimizing the application method of entomopathogenic nematode suspension for biological control of large pine weevil *Hylobius abietis*. BioControl. (2017);62(5):659–667. https://doi.org/10.1007/s10526-017-9824-x
15. Nxitywa A, Malan AP. Formulation of *Steinernema yirgalemense* in gel for long-term storage at room temperature. J Plant Dis Prot. (2023);130(4):809–816. https://doi.org/10.1007/s41348-023-00764-2
16. Lalitha K, Nithya K, Bharathi BG, Venkatesan S, Shivakumar MS. Long-term storage does not affect the infectivity of entomopathogenic nematodes on insect hosts. Appl Microbiol Biotechnol. (2022);107(1):419-431. https://doi.org/10.1007/s00253-022-12309-y.
17. Flores P, Alvarado A, Lankin G, Lax P, Prodan S, Aballay E. Morphological, molecular and ecological characterization of a native isolate of *Steinernema feltiae* (Rhabditida: Steinernematidae) from southern Chile. Parasites and Vectors. (2021);14(1):45. https://doi.org/10.1186/s13071-020-04548-7
18. Bruno P, Machado RAR, Glauser G, Köhler A, Campos-Herrera R, Bernal J, Toepfer S, Erb M, Robert CAM, Arce CCM, Turlings TCJ. Entomopathogenic nematodes from Mexico that can overcome the resistance mechanisms of the western corn rootworm. Sci Rep. (2020);10(1):8257. https://doi.org/10.1038/s41598-020-64945-x
19. Susurluk A, Dix I, Stackebrandt E, Strauch O, Wyss U, Ehlers RU. Identification and ecological characterisation of three entomopathogenic nematode-bacterium complexes from Turkey. Nematology. (2001);3(8):833–841. https://doi.org/10.1163/156854101753625326
20. Hsieh FC, Tzeng CY, Tseng JT, Tsai YS, Meng M, Kao SS. Isolation and characterization of the native entomopathogenic nematode, *Heterorhabditis brevicaudis*, and its symbiotic bacteria from Taiwan. Curr Microbiol. (2009);58(6):564–570. https://doi.org/10.1007/s00284-009-9371-5
21. Morton A, García-del-Pino F. Ecological characterization of entomopathogenic nematodes isolated in stone fruit orchard soils of Mediterranean areas. J Invertebr Pathol. (2009);102(3):203–213. https://doi.org/10.1016/j.jip.2009.08.002
22. Yuksel E, Canhilal R. Isolation, identification, and pathogenicity of entomopathogenic nematodes occurring in Cappadocia region, central Turkey. Egypt J Biological Pest Cont. (2019);29(1):1–7. https://doi.org/10.1186/s41938-019-0141-9

23. Levy N, Faigenboim A, Salame L, Molina C, Ehlers RU, Glazer I, Ment D. Characterization of the phenotypic and genotypic tolerance to abiotic stresses of natural populations of *Heterorhabditis bacteriophora*. Sci Rep. (2020);10(1):1–16. https://doi.org/10.1038/s41598-020-67097-0

24. Raja RK, Sivaramakrishnan S, Hazir S. Ecological characterisation of *Steinernema siamkayai* (Rhabditida: Steinernematidae), a warm-adapted entomopathogenic nematode isolate from India. BioControl. (2011);56(5):789–798. https://doi.org/10.1007/s10526-011-9345-y

25. Glazer I, Salame L. Osmotic survival of the entomopathogenic nematode *Steinernema carpocapsae*. Biological Cont. (2000);18(3):251–257. https://doi.org/10.1006/bcon.2000.0814

26. Hunt DJ, Nguyen KB. Tabular keys to species of *Steinernema* and *Heterorhabditis*. In: Hunt DJ, Nguyen KB, editors. Advances in Entomopathogenic Nematode Taxonomy and Phylogeny. (2017).p. 59–109. Leiden, The Netherlands: Brill. https://doi.org/10.1163/9789004285347. ISBN: 978-90-04-28534-7

27. Susurluk IA, Ulu TC, Kongu Y. Tolerances of hybridized entomopathogenic nematode *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) strains to heat and desiccation. Turk J Entomol. (2013);37(2):221–228. https://doi.org/10.16970/ted.96639

28. White GF. A method for obtaining infective nematode larvae from cultures. Science. (1927);66(1709):302–303. https://doi.org/10.1126/science.66.1709.302.b

29. Rasmann S, Turlings TC. First insights into specificity of belowground tritrophic interactions. Oikos. (2008);117(3):362–369. https://doi.org/10.1111/j.2007.0030-1299.16204.x

30. Kour S, Khurma U, Brodie G. Ecological Characterisation of Native Isolates of *Heterorhabditis indica* from Viti Levu, Fiji Islands. J Nematol. (2021);53(1):1–20. https://doi.org/10.21307/jofnem-2021-085

31. Ulu TC, Susurluk IA. In vitro liquid culture production and post-production pathogenicity of the hybrid *Heterorhabditis bacteriophora* HBH strain. Crop Prot. (2024);175(106443):1–7. https://doi.org/10.1016/j.cropro.2023.106443

32. Yamanaka S, Tanabe H, Takeuchi K. Vertical Dispersal and Infectivity of *Steinernema glaseri*, *S. anomali* and *S. kushidai* (Nematoda: Steinernematidae). Nematol Res (Jap J Nematol). (1995);25(1):24–32. https://doi.org/10.3725/jjn1993.25.1_24

33. Bal HK, Michel AP, Grewal PS. Genetic selection of the ambush foraging entomopathogenic nematode, *Steinernema carpocapsae* for enhanced dispersal and its associated trade-offs. Evolutionary Ecology. (2014); 28(5):923–939. https://doi.org/10.1007/s10682-014-9706-y

34. Wu S-Y, Duncan LW. Entomopathogenic nematode species combinations alter rates of dispersal, host encounter and insecticidal efficiency. J Pest Sci. (2022); 95(3):1111–1119. https://doi.org/10.1007/s10340-021-01475-z

35. Bal HK, Grewal PS. Lateral Dispersal and Foraging Behavior of Entomopathogenic Nematodes in the Absence and Presence of Mobile and Non-Mobile Hosts. PLOS ONE. (2015); 10(6):e0129887. https://doi.org/10.1371/journal.pone.0129887

36. Rolston AN, Griffin CT, Downes MJ. Emergence and Dispersal Patterns of Two Isolates of the Entomopathogenic Nematode *Steinernema feltiae*. J Nematol. (2006); 38(2):221–228. PMID: 19259450

37. Ulu TC, Susurluk IA. Heat and desiccation tolerances of *Heterorhabditis bacteriophora* strains and relationships between their tolerances and some bioecological characteristics. Invertebr Surv J. (2014); 11(1):4–10. https://www.isj.unimore.it/index.php/ISJ/article/view/300/214. ISSN 1824-307X

38. Fallet P, De Gianni L, Machado RAR, Bruno P, Bernal JS, Karangwa P, Kajuga J, Waweru B, Bazagwira D, Degen T, Toepfer S, Turlings TCJ. Comparative Screening of Mexican, Rwandan and Commercial Entomopathogenic Nematodes to Be Used against Invasive Fall Armyworm, *Spodoptera frugiperda*. Insects. (2022); 13(2):205. https://doi.org/10.3390/insects13020205

39. Odendaal D, Addison MF, Malan AP. Entomopathogenic nematodes for the control of the codling moth (*Cydia pomonella* L.) in field and laboratory trials. J Helminth. (2016); 90(5):615–623. https://doi.org/10.1017/S0022149X15000887

40. Ferreira T, Malan AP. Potential of entomopathogenic nematodes for the control of the banded fruit weevil, *Phlyctinus callosus* (Schönherr) (Coleoptera: Curculionidae). J Helminth. (2014); 88(3):293–301. https://doi.org/10.1017/S0022149X13000175

41. Susurluk IA, Kumral NA, Peters A, Bilgili U, Açıkgöz E. Pathogenicity, reproduction and foraging behaviours of some entomopathogenic nematodes on a new turf pest, *Dorcadion pseudopreissi* (Coleoptera: Cerambycidae). Biocontrol Sci Techn. (2009); 19(6):585–594. https://doi.org/10.1080/09583150902957348

42. Özdemir E, Bayram Ş, Susurluk İA. First record of the entomopathogenic nematode *Steinernema litorale* (filipjev) (Rhabditida: Steinernematidae) and its symbiotic bacterium from Turkey, and its efficacy capability. Insects. (2020); 11(3):144. https://doi.org/10.3390/insects11030144

43. Wang J, Cao L, Huang Z, Gu X, Cui Y, Li J, Li Y, Xu C, Han R. Influence of the ascarosides on the recovery, yield and dispersal of entomopathogenic nematodes. J Invertebr Pathol. (2022); 188:107717. https://doi.org/10.1016/j.jip.2022.107717