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Biocontrol potential of native entomopathogenic nematodes against *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera, Chrysomelidae) in alfalfa

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Abstract

Background *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera: Chrysomelidae) inflicts substantial crop damage by consuming alfalfa leaves. The main aim of the study was to develop an efficient, sustainable control method against the pest, considering entomopathogenic nematodes (EPNs) as a potential control agent against soil-dwelling insect pests. This study investigated the impact of two native entomopathogenic nematodes (EPNs), *Heterorhabditis bacteriophora* (isolate Z-1) and *Steinernema feltiae* (isolate A-16), on mature larvae and adults of *G. fornicata*. Concentrations of 250, 500, and 1000 IJs/ml were used for adult and larval stages, and mortality rate was determined at the end of 144 h for larvae and 240 h for adults.

Results The highest effect was 94.7% at 120 h for isolate Z-1 and 91.0% at 168 h for isolate A-16 at a concentration of 1000 IJs/ml for adult stages. The highest effect on larval stage was 97.6% at 96 h for isolate Z-1 and 85.8% at 120 h for isolate A-16 at the concentration of 1000 IJs/ml. On the adult stage, isolate Z-1 showed 50.0–67.1% effect at concentrations of 250 and 500 IJs/ml, respectively, while isolate A-16 showed an effect ranging from 46.7 to 63.6%. In the larval stage, isolate Z-1 caused 39.7–77.6% mortality at 250 and 500 IJs/ml concentrations, respectively, while the rate was 32.9–63.9% for isolate A-16. In the trial, isolate Z-1 showed a high efficacy in both stages of *G. fornicata*.

Conclusions This study represents the first laboratory investigation encompassing both adult and mature larval stages of *G. fornicata*. The obtained data offer valuable insights for alternative control strategies targeting *G. fornicata*.

Keywords Alfalfa leaf beetle, Mortality, Biological control, Entomopathogenic nematodes, *Heterorhabditis bacteriophora*, *Steinernema feltiae*

Background

Alfalfa, the world's most cultivated forage crop, boasts a high feed value, abundant protein yield per unit area, and nutrient-rich green and dry grass that is not only delicious but also nutritious for a wide range of animals (Açıkgöz 2001). However, alfalfa, which holds a prominent position among forage crops in terms of production, is vulnerable to various diseases and harmful organisms during its cultivation. One such menace is the alfalfa leaf beetle, scientifically known as *Gonioctena fornicata* (Brüggemann) (Coleoptera: Chrysomelidae). The initial record of this pest's presence in Türkiye dates back to 1946 (Alkan 1946). Subsequently, it was correctly identified as the alfalfa leaf beetle, with a particular emphasis on its detrimental impact on alfalfa (Bodenheimer 1958).

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Extensive research has been conducted in various regions of Türkiye, including Western, Central, and Southeastern Anatolia, as well as the Black Sea region, focusing on the surveillance, morphology, and biology of this pest. These studies have consistently highlighted the alfalfa leaf beetle as a significant threat to alfalfa cultivation (Barış et al. 2021). Both the adult and larval stages of this species are destructive, leading to substantial crop losses in alfalfa (Barış et al. 2021). The pest is known to feed on alfalfa leaves, flowers, leaf buds, young shoots, and the tips of stems (Barış et al. 2021). In recent years, the alfalfa leaf beetle has attracted increasing attention, especially due to population increases and fluctuations triggered by climate change.

A review of existing research has revealed that this pest poses a potential threat to alfalfa fields, causing economic losses that vary from year to year and region to region. Consequently, there is a pressing need for research aimed at its effective control (Yücel 2021). In response to this necessity, it became evident that studies exploring alternative methods to manage the pest were essential, leading to the utilization of native entomopathogenic nematodes (EPNs). EPNs have become an attractive option in biological control due to their effectiveness against numerous pests, their capacity to seek out hosts, their compatibility with standard equipment for application, and their long-term efficacy (Ulu et al. 2016). The most prominent EPN species for biological control belong to the families Steinernematidae and Heterorhabditidae (Poinar 1979). In previous studies on EPNs in Türkiye, nine EPN species, including *Steinernema feltiae, S. bicornutum, S. carpocapsae, S. affine, S. anatoliense, S. weiseri, S. littorale, Heterorhabditis bacteriophora*, and *H. megidis*, have been identified so far (Yüksel et al. 2019). Members of these families are obligate generalist insect parasites, featuring a free-living infective stage known as infective juveniles (IJs) (Mazurkiewicz et al. 2019). In insects, infection is carried out by these juveniles, which are morphologically and physiologically adapted to long-term survival in soil (Adams and Nguyen 2002). EPNs release *Xenorhabdus* spp. and *Photorhabdus* spp. bacteria into the hemolymph of the host insect once they have entered it (Stock and Blair 2008). These bacteria play a crucial role in swiftly eliminating the insect by facilitating the development and colonization of the nematode's infective juveniles within the host insect (Dowds and Peters 2002). EPNs are capable of exterminating their hosts within 24–48 h after application (Forst and Clarke 2002). Subsequently, third instar EPNs larvae, also referred to as infective juveniles (IJs), emerge from the soil and actively search for new hosts (Kaya and Gaugler 1993). These nematodes possess the remarkable ability to actively locate their hosts, detect pest presence, and navigate toward their target (Susurluk and Toprak 2006).

The aim of this study was to evaluate the effect of two local isolates, *Heterorhabditis bacteriophora* (isolate Z-1) and *Steinernema feltiae* (isolate A-16), on *G. fornicata* adults, and determine the larvicidal effects of EPNs on the mature larval stage of *G. fornicata*, which has not been reported before.

Methods

Insect sources

The alfalfa plants used in this study were sourced from the experimental plots of the Plant Protection Central Research Institute, Ankara, Türkiye. Specimens of *G. fornicata* were collected from alfalfa fields located in the Sincan district of Ankara, at coordinates 40°04′09.1"N latitude and 32°28′30.9"E longitude, with an elevation of 967 m.

Gonioctena fornicata (Brüggeman) (Coleoptera, Chrysomelidae) Production

Adult *G. fornicata* specimens were collected from the field and then carefully transferred to laboratory growth cages, each measuring 20 × 10 × 7 cm in size. Fresh alfalfa plants were provided as a food source for the pests and were replaced when they began to wilt or dry out. To prevent adult beetles from escaping, alfalfa shoots were submerged in narrow-mouthed beakers filled with water, and the beakers were sealed with sterile cotton. Any eggs laid by the beetles on a daily basis were closely monitored and then transferred to cages containing fresh alfalfa plants. The first larva that hatched within the cage, which also contained fresh alfalfa plants, was carefully observed. Subsequently, as the larvae molted, these transitions were closely monitored at intervals of 6 h. Larvae were then separated into distinct rearing cages based on their developmental stages, with mature larvae at the fourth stage being selected for the experiments. The experiments were conducted under controlled laboratory conditions, maintaining a temperature of 25 ± 2 °C, relative humidity at 65 ± 2%, and a photoperiod of 16L: 8D hrs.

Following the fourth instar larvae, pupae were relocated at laboratory conditions characterized by a temperature of 25 ± 2 °C, RH at 65 ± 2%, and complete darkness. The pupae were placed in containers filled with sterile soil, approximately 10 cm deep, and covered with fine gauze. The process of pupation and the emergence of the first adults were systematically monitored. Newly emerged adults, appearing on a daily basis, were selected for use in the experiments.

Experiments

Plastic Petri dishes, with a diameter of 9 cm, were used for the experiment. A piece of filter paper (Whatman paper No.1, Whatman International, Maidstone, UK) was placed in each Petri dish. EPNs at concentrations of 250, 500, and 1000 IJs/ml were administered to each Petri dish, with a volume of 1 ml dispensed using a micropipette. For the control group, 1 ml of pure water without nematodes was used (Shahina and Salma 2010). Pipette tips were changed after each administration to maintain hygiene and prevent concentration variations. Within each Petri dish, five adults and five mature larvae were separately placed in accordance with their respective developmental stages. Fresh alfalfa leaves were provided in the Petri dishes as a food source for the insects and were replaced with fresh ones as needed to prevent drying. Counts were made at 24-h intervals and observations covered 144 h for mature larvae and 240 h for adults. Death insects were subjected to either dissection under a stereomicroscope or placed into White traps to facilitate the emergence of nematodes, thereby confirming their death due to EPNs (White 1927) (Fig. 1). Larval individuals were placed in separate Petri dishes containing sterile soil for pupation and kept in a completely dark climate chamber (Nüve, ID501, Türkiye) at 25 ± 2 °C, 65 ± 2% RH and complete darkness until adult emergence. Individuals reaching the adult stage were considered viable.

Source of nematode

In this research, the nematode *H. bacteriophora* (isolate Z-1), which was obtained from a soil sample collected from an alfalfa field located in the Zonguldak province of Türkiye and the other isolate *S. feltiae* (isolate A-16) was obtained from Department of Biology, Faculty of Arts and Sciences, Aydın Adnan Menderes University, Aydın,

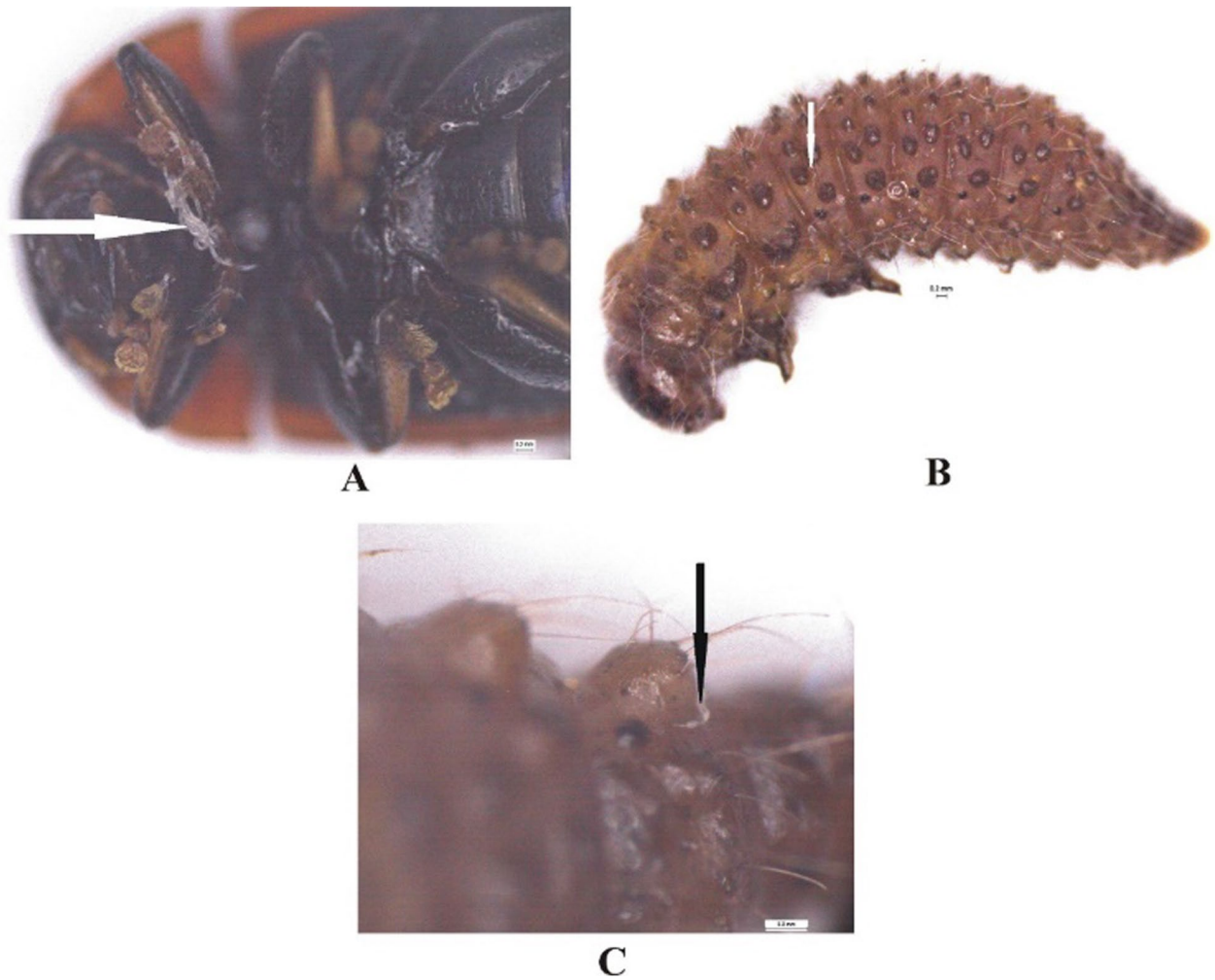

Fig. 1 Emerging infective juveniles of *Steinernema feltiae* (isolate A-16) (black arrow) and *Heterorhabditis bacteriophora* (isolate Z-1) (white arrows) from the body of *Gonioctena fornicata* (A: adult; B and C: larvae)

Türkiye, were utilized. The soil sample underwent nematode extraction using the wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) baiting method (Yağcı et al. 2023) as well as a white trap (White 1927). The pathogenicity of this isolated nematode was confirmed by inoculating healthy *G. mellonella* larvae. Furthermore, newly emerged infective juvenile nematodes (IJs) obtained from the white trap were stored at a temperature of 10 °C for subsequent investigations (Hazır et al. 2022). The nematode species were identified by Dr. Harun ÇİMEN (Department of Biology, Faculty of Arts and Sciences, Aydın Adnan Menderes University, Aydın, Türkiye) based on morphometric and molecular analyzes. In addition, isolate Z-1 underwent sequencing and has been deposited in GenBank with the accession number OQ130749.

Rearing of *Galleria mellonella* larvae

To mass production of *G. mellonella* larvae, one-liter bottles and provided those with a specialized diet consisting of the following ingredients was utilized: (Flour: 890 g, Milk powder: 445 g, Dry baker's yeast: 222 g, Honey: 500 g, Glycerin: 500 g, and Beeswax: 125 g). The preparation of this diet involved melting beeswax, honey, and glycerin, which were then mixed with the other components following the method described by Mohamed and Coppel (1983). Subsequently, wax moth eggs were deposited onto this nutrient-rich medium within one-liter glass jars. These jars were maintained in an incubator (Nüve, ID501, Türkiye) with a light-to-dark cycle of 16 h of light and 8 h of darkness, while the temperature was kept within the range of 23 ± 1 °C. This cultivation process spanned a duration of 40–45 days, during which sixth instar wax moth larvae were obtained and subsequently utilized for the large-scale cultivation of EPNs.

Rearing of entomopathogenic nematodes

Wax moth larvae served as the primary medium for mass cultivating the EPN isolate employed in the experiment. In this process, ten larvae within a 6-cm-diameter Petri dish containing filter paper (No. 1, Whatman International, Maidstone, UK) saturated with distilled water were placed. An inoculation of IJs was carefully applied to the wax moth larvae. Subsequently, the Petri dishes were securely sealed with parafilm and subjected to incubation at a controlled temperature ranging from 23 ± 1 °C. The daily monitoring of larval mortality was an integral part of this procedure. To extract EPN larvae from the infected wax moth larvae, the "White trap" method (White 1927) was utilized. Larvae infected by EPNs were then transferred into culture flasks and stored in a refrigerator maintained at a temperature of 10 °C to preserve the nematodes' activity. In order to ensure the continued vitality of the nematodes, this process every one to two months was repeated, employing fresh wax moth larvae as hosts. This systematic approach facilitated the periodic renewal of our nematode cultures at the Nematology Laboratory, Directorate of Plant Protection Central Research Institute in Ankara, Türkiye.

Data analysis

The initial step in analyzing the data from the concentration screening tests involved converting the results into percentage mortality. Subsequently, an arcsine transformation was applied, as described by (Warton and Hui 2011). To discern significant differences in means, Tukey's multiple range test was applied at a significance level of 5%. All statistical analyses were conducted using MINITAB. Release 18 statistical software, utilizing the general linear model to assess the statistical interactions between treatments, as outlined by literature (Mckenzie and Goldman 2004). Whole experiments were carried out with four replicates for each concentration and were repeated at two different time points.

Results

The efficacy of EPN isolates against *G. fornicata* was assessed for their potential as a biological control agent. The correlation between concentration and time, as well as the concentration and EPN interaction, concerning the *G. fornicata*, was found to be statistically significant ($P < 0.05$) (Tables 1 and 2).

Effect of EPNs to adults' stage

A statistically significant correlation ($P < 0.05$) was observed between the concentration of Z-1 and A-16 isolates and their effect on the adult stage. Furthermore, the effect of both EPN isolates displayed variability with increasing concentration and over time (Table 1). The highest entomopathogenicidal effect was of *H. bacteriophora* (isolate Z-1) on the adult stage of the *G. fornicata* (94.7%) at 120 h *(F*: 26.65; df: 3.23; $P < 0.05$) at the concentration of 1000 IJs/ml and the following time intervals. The initial impact of isolate Z-1 on the adult stage of the alfalfa beetle was observed at 48 h, a mortality of 7.0% was noted at the 1000 IJs concentration, which was significantly different from the other sub-concentrations (250 and 500 IJs) (*F*: 4.65; df: 3.23; $P < 0.05$). By the 72-h mark, the mortality for the 500 and 1000 IJs concentrations was determined to be 5.3 and 39.7%, respectively (*F*: 18.87; df: 3.23; $P < 0.05$). The highest concentration exhibited a significantly different effect compared to the lowest concentrations (*F*: 18.87; df: 3.23; $P < 0.05$). When assessing the impact of isolate Z-1 on the adult stage of the pest, it was observed that there was non-significant variation in the mortality between the 144th and 240th hrs (Table 1).

Table 1 Mortality (%) of *Heterorhabditis bacteriophora* (isolate Z-1) and *Steinernema feltiae* (isolate A-16) against *Gonioctena fornicata* adults in 240 h

HAT	***Heterorhabditis bacteriophora* (isolate Z-1)**				***Steinernema feltiae* (isolate A-16)**			
	Concentrations (IJs/adult)							
	250	**500**	**1000**	**0**	**250**	**500**	**1000**	**0**
24	0.0 A** c*	0.0 A d	0.0 A d	0.0 A a	0.0 A b	0.0 A c	0.0 A d	0.0 A a
48	0.0 B c	0.0 B d	7.0 A cd	0.0 B a	0.0 A b	0.0 A c	0.0 A d	0.0 A a
72	0.0 B c	5.3 B cd	39.7 A bc	0.0 B a	0.0 A b	0.0 A c	0.60 A cd	0.0 A a
96	16.9 B b	22.7 B bc	77.3 A ab	0.0 B a	5.3 A b	11.1 A b	29.2 A bc	0.0 A a
120	32.9 B ab	46.7 B ab	94.7 A a	0.0 C a	32.9 B a	36.1 B ba	57.0 A ac	0.0 C a
144	50.0 B a	67.1 B a	94.7 A a	0.0 C a	46.7 B a	63.6 AB a	86.1 A a	0.0 C a
168	50.0 B a	67.1 B a	94.7 A a	0.0 C a	46.7 B a	63.6 AB a	91.0 A a	0.0 C a
192	50.0 B a	67.1 B a	94.7 A a	0.0 C a	46.7 B a	63.6 AB a	91.0 A a	0.0 C a
216	50.0 B a	67.1 B a	94.7 A a	0.0 C a	46.7 B a	63.6 AB a	91.0 A a	0.0 C a
240	50.0 B a	67.1 B a	94.7 A a	0.0 C a	46.7 B a	63.6 AB a	91.0 A a	0.0 C a

HAT: Hours after treatment

*Means within column bearing the same letter are not significantly different (Tukey test, $P > 0.05$)

**Means within lines bearing the same letter are not significantly different (Tukey test, $P > 0.05$)

Table 2 Mortality (%) of *Heterorhabditis bacteriophora* (isolate Z-1) and *Steinernema feltiae* (isolate A-16) against *Gonioctena fornicata* mature larvae at 144 h

HAT	***Heterorhabditis bacteriophora* (isolate Z-1)**				***Steinernema feltiae* (isolate A-16)**			
	Concentrations (IJs/larvae)							
	250	**500**	**1000**	**0**	**250**	**500**	**1000**	**0**
24	0.0 A** b*	0.0 A c	0.0 A c	0.0 A a	0.0 A b	0.0 A c	0.0 A c	0.0 A a
48	0.0 B b	0.0 B c	19.7 A b	0.0 B a	0.0 A b	0.0 A c	3.6 A c	0.0 A a
72	0.6 B b	22.2 B b	77.3 A a	0.0 B a	0.6 B b	11.5 B b	43.0 A b	0.0 B a
96	26.2 C a	64.2 B a	97.6 A a	0.0 D a	26.2 B a	43.3 B a	83.1 A a	0.0 C a
120	39.7 C a	77.6 B a	97.6 A a	0.0 D a	32.9 C a	63.9 B a	85.8 A a	0.0 D a
144	39.7 C a	77.6 B a	97.6 A a	0.0 D a	32.9 C a	63.9 B a	85.8 A a	0.0 D a

HAT: Hours after treatment

*Means within column bearing the same letter are not significantly different (Tukey test, $P > 0.05$)

**Means within lines bearing the same letter are not significantly different (Tukey test, $P > 0.05$)

The highest effect of isolate *S. feltiae* (isolate A-16) on *G. fornicata* adults was recorded at 91.0% for the 1000 IJs concentration at the 168-h mark (F: 18.60; df: 3.23: P˃0.05). The initial effect was noted at 72 h, with a rate of 0.60% at the 1000 IJs concentration However, this effect was not statistically different from the other sub-concentrations (F: 0.67; df: 3.23; $P > 0.05$). By the 96-h mark, the effect for all three concentrations (250, 500, 1000) was 5.3, 11.1 and, 29.2%, respectively (F: 2.54; df: 3.23; $P > 0.05$). At the 144-h mark, the effect continued to increase with increasing concentration, resulting in values of 46.7, 63.6 and, 86.1%, respectively (F:16.87; df: 3.23: P˃0.05). Including 168 h, there was non-statistical significant difference in effect at other counting intervals thereafter (Table 1).

EPN effect on mature larvae stage

When assessing the impact of *H. bacteriophora* (isolate Z-1) and *S. feltiae* (isolate A-16) on the mature larval stage of the pest with increasing concentration, a significant correlation (concentration/EPN) was observed ($P < 0.05$). The effects of EPN isolates on the larval stage of the pest are detailed in Table 2.

The initial effect of *H. bacteriophora* (isolate Z-1) on mature larvae was noted at 48 h, with a mortality of 19.7% at the 1000 IJs concentration (F: 11.83; df: 3.23;

$P < 0.05$). However, the highest larval mortality was determined as 97.6% at 96 h at a concentration of 1000 IJs/ml (*F*: 40.97; df: 3.23; $P < 0.05$). Larval mortality was 39.7, 77.6, and 97.6%, respectively, depending on the increasing concentration at 120 h. After this application time, the effect remained constant and no statistical difference was recorded (*F*: 32.13; df: 3.23; $P < 0.05$). When evaluating the effect of each concentration of isolate Z-1on the larval period over time, a significant correlation (concentration/time) was observed ($P < 0.05$) (Table 2).

The effect of isolate A-16 on the larval stages was determined as 3.6% in 1000 IJs and was statistically in the same group with the other concentrations (*F*: 1.53; df: 3.23: $P > 0.05$). Nonetheless, the highest larval mortality was 85.8% at the end of 120th hrs with 1000 IJs/ml concentration (*F*: 35.77; df: 3.23: $P<0.05$). At 72 h, the effects were determined as 0.6, 11.5 and, 43.0% IJs, respectively, depending on the increasing concentrate, while 1000 IJs was in a different group from the other sub-concentrations (*F*: 9.86; df: 3.23: $P<0.05$). Depending on the increase in concentration, the effects at 120^{th} and 144^{th} hrs did not change and 32.9, 63.9, and 85.8% mortality was determined in the larval stage, respectively (Table 2).

Discussion

In this experiment, the insecticidal activity of two local EPNs, *H. bacteriophora* (isolate Z-1) and *S. feltiae* (isolate A-16), against adults and larvae of *G. fornicata* was investigated. EPNs have been reported by different researchers as a potential biological control agent for the control of different insect pest adults that overwinter in the soil and mature larvae that descend to the soil to pupate (Georgis et al. 2006). In terms of infectivity in the adult period, Z-1 isolate showed higher activity than A-16 isolate and caused death in a shorter time. In the larval stage, similarly, the mortality was high in Z-1 isolate.

Heterorhabditis bacteriophora (isolate Z-1) demonstrated a high impact on *G. fornicata* adults. Another study similar to these findings, the application of *H. bacteriophora* at a rate of 1000 IJs per *G. fornicata* adult resulted in a 100% mortality 3 days post-treatment in the laboratory (Majic et al. 2013). According to the results, *G. fornicata* adults were susceptible to all isolates tested in the study, while the lowest mortality was obtained from *S. feltiae* (isolate 09–31) with 94.7% at 2000 IJs/adult concentration. In other isolates, mortality ranged between 97.6 and 100% after 120 h of treatment (Çağlayan et al. 2021). It was determined that different isolates of the EPN species displayed varying activity levels against the target insect.

The other EPN, *S. feltiae* (isolate A-16), led to different mortality against *G. fornicata* adults. The highest effect was achieved at a concentration of 1000 IJs with 91.0% mortality. In addition, Trdan et al. (2006) reported that *S. feltiae* showed the highest efficacy in the management of *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) adult populations at 15 °C. Researchers found that a high efficacy of different strains and concentrations of *S. feltiae* on overwintering *L. decemlineata* adults (88 to 100%) (Wright et al. 1987). Atay and Kepenekci (2016) reported that Turkish isolates of (EPNs) (*Steinernema feltiae, S. carpocapsae* and *H. bacteriophora*) were tested against *Holotrichapion pullum* (Gyllenhal) (Coleoptera, Apionidae) under laboratory conditions at 500, 1000 and, 2000 IJs/ml and at 20 °C. *S. carpocapsae* caused 82.15% mortality at 2000 IJs/ml. In contrast, *S. feltiae* caused 35.21%, while *H. bacteriophora* caused less 30.55% mortality. It has been reported by researchers that EPNs can show different efficacy on the same pest as well and different isolates of the same species can show different efficacy on the same pest (Canhilal et al. 2007). The results of the present study were similar to previous studies and isolate Z-1 and isolate A-16 showed different efficacy on the same insect species for both adult and larval stages. Based on the obtained results, the mortality of insects exposed to EPNs fluctuates based on factors such as: virulence, nematode species and strains, insect species, biological stages of insects, EPN concentrations, exposure duration, and temperature (Yağcı et al. 2023) The observation that *G. fornicata* larvae achieved the highest efficacy in shorter time than both EPN types under investigation aligns with the aforementioned discoveries.

Since *G. fornicata* overwinters in the soil, and the mature larvae move into the soil for pupation, EPN isolates may enhance the chances of success in pest control. EPNs are more efficient against larvae and other pre-imagine stages of insects because they can enter their bodies more easily (LeBeck et al. 1993). According to the results obtained, isolate Z-1 exhibited the highest efficacy against fourth-stage larvae at a concentration of 1000 IJs, reaching 97.6% mortality at the 96th hrs, while isolate A-16 showed the highest efficacy at 120th hrs, with 85.8% mortality. It has been known that EPNs harbor symbiotic bacteria (Salvadori 2012), which multiply and release various virulence factors, including toxin complexes, hydrolytic enzymes, hemolysins, and antimicrobial compounds. These factors typically lead to the death of the insect host within 48 h (Eleftherianos et al. 2010). In both isolates, similar to previous experiments the first effect on *G. fornicata* mature larvae started at 48 h and at a concentration of 1000 IJs/ml and increased over time. So far, no other study has been found where it was possible to successfully control *G. fornicata* larvae with EPNs. In this study, the efficacy of EPN isolates against the mature larval stage of the pest was demonstrated for the first

time. Since there are no studies in the literature on the larval stage of *G. fornicata*, some studies were evaluated. The efficacy of several EPN isolates against *L. decemlineata* larvae, another economic pest in the Chrysomelidae family, has been determined (Kepenekci et al. 2015). Researchers found high efficacy of different strains and concentrations of *S. feltiae* on larvae (80–90%) (Wright 1987). *S. feltiae* caused 96% mortality and *H. bacteriophora* resulted in 75% mortality. As evident from other studies, there are a number of factors that contribute to variations in EPN pathogenicity. Among these, the ability to encounter, find and penetrate hosts is thought to influence pathogenicity.

Conclusions

Two local EPN isolates, *H. bacteriophora* (isolate Z-1) and *S. feltiae* (isolate A-16), demonstrated efficacy against both larval and adult stages of *G. fornicata*. Biological measures are notably eco-friendly. Exploring locally available nematode strains is essential, as they are better suited to adapt to regional climatic conditions compared to commercial alternatives, potentially yielding superior outcomes. Therefore, environmentally sound pest control activities need alternatives. Nonetheless, conducting field assessments of these EPN species and strains will yield more comprehensive insights into their potential. For sustainable pest control, it is very important to search for locally available nematode species that are adapted to regional climatic conditions and have the potential to give superior results compared to other control methods.

Abbreviations

EPNs	Entomopathogenic nematodes
IJs	Infective juveniles
G. fornicata	*Gonioctena fornicata*
S. feltiae	*Steinernema feltiae*
H. bacteriophora	*Heterorhabditis bacteriophora*
G. mellonella	*Galleria mellonella*

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Author contributions

AB executed the laboratory study, conceived the idea, supervised the work, and wrote the original MS.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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