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Efficacy of some native entomopathogenic nematodes against the alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), and the lucerne beetle, *Gonioctena fornicata* (Brüggemann) (Coleoptera: Chrysomelidae), adults under laboratory conditions

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Abstract

Background: Entomopathogenic nematodes (EPNs) have more important role in biological control of economic insect pests. The effect of native EPNs on adults of the lucerne beetle, *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera: Chrysomelidae), and the alfalfa weevil, *Hypera postica* (Gyllenhal, 1813) (Coleoptera: Curculionidae), which are important alfalfa pests in Turkey and around the world, was investigated.

Results: Dose-mortality assays were carried out with 5 isolates [*Steinernema carpocapsae* (Weiser, 1955) (Nematoda: Steinernematidae) (Black sea isolate), *S. feltiae* Filipjev, 1934 (isolate 09-31), *Heterorhabditis bacteriophora* Poinar, 1976 (Nematoda: Heterorhabditidae) (isolate 09-43), *H. bacteriophora* Tokat-Songut, and *S. carpocapsae* Tokat-Ulas] using doses of 500, 1000, and 2000 IJs ml^{-1} under the laboratory conditions. Studies showed that all isolates had an effect 90% and more at 2000 IJs ml^{-1} and at the end of 112 h [except, *H. bacteriophora* (isolate 09-43) and *H. bacteriophora* Tokat-Songut isolates against *H. postica*]. In addition, LT_{30}, LT_{50}, and LT_{90} values at 1000 IJs ml^{-1} were determined.

Conclusions: According to the results, *G. fornicata* adults were susceptible to all isolates tested in the study and *H. postica* adults were susceptible to the isolates *S. carpocapsae* (Black sea isolate), *S. feltiae* (isolate 09-31), and *S. carpocapsae* Tokat-Ulas. This is the first study conducted in Turkey for the virulence of EPNs against *G. fornicata* and *H. postica*.

Keywords: Entomopathogenic nematodes, Virulence, *Hypera postica*, *Gonioctena fornicata*

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Background

Alfalfa [*Medicago sativa* L. (Fabaceae)] plant is a forage plant that provides a high-value feed and has a very dense production area in Turkey (Karakurt and Fırıncıoğlu 2003). The alfalfa weevil [*Hypera postica* (Gyllenhal, 1813) (Coleoptera: Curculionidae)] and the lucerne beetle [*Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera: Chrysomelidae)] are among the alfalfa pests, spread to almost all grown areas of alfalfa in Turkey and cause significant crop losses if no control is made (Efe and Özgökçe 2014; Efil 2018). Larvae and adults of both species feed on leaves, young shoots, and the tips of the stems. As a result of feeding, the plant turns yellow and withers, and then the leaves dry completely (Atanasova and Semerdjieva 2009; Majić et al. 2013).

Frequent cuttings are the most effective cultural method of the pest in the alfalfa fields. Gözüaçık and İreç (2019) reported that cutting or grazing in the fall will reduce the spring larval population of alfalfa weevil. Chemical control against *H. postica* is recommended in Turkey, but no chemical pesticides have been registered for the control of *G. fornicata*. Therefore, pest control activities that are environmentally friendly are needed.

Entomopathogens nematodes (EPNs) are highly virulent. They have recently become one of the most emphasized groups due to these features. Studies have indicated that more than 30 nematode families are in contact with insects (Kaya and Stock 1997). EPNs have a variety of distinct and advantageous characteristics, such as high virulence and the ability to actively seek out hosts, making them promising chemical alternatives (Gulcu et al. 2017). EPNs are now used in many countries to control a wide range of economically important insect pests (Odendaal et al. 2015; Belien 2018). EPNs belong to the Steinernematidae and Heterorhabditidae families and live in the soil and are obligatory insect pathogens. They are one of the most frequently used groups in microbial control of pests (Glazer and Lewis 2000).

Studies using EPNs against *G. fornicata* and *H. postica* are limited (Falahi et al. 2011; Roodaki et al. 2011). Therefore, the aim of this study was to investigate the virulence of EPNs against *G. fornicata* and *H. postica* adults under laboratory conditions.

Methods

Nematode sources

Native Turkish EPNs, *Steinernema feltiae* (isolate 09-31) from a vegetable garden in Aydin, *S. carpocapsae* (Black sea isolate) from grassland in Rize, and *Heterorhabditis bacteriophora* (isolate 09-43) from peach orchard in Aydın, Turkey, were obtained and supplied by Prof. Dr. Selçuk HAZIR (Aydın Adnan Menderes University, Faculty of Arts and Sciences, Department of Biology, Aydın, Turkey). *S. carpocapsae* Tokat-Ulas and *H. bacteriophora* Tokat-Songut were isolated from alfalfa cultivated areas of Tokat province (Turkey) (Çağlayan et al. 2020). The nematodes were cultured in last instar larvae of wax moth, *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) at room temperature (23–24 °C) using methods described by Kaya and Stock (1997). The harvested infective juveniles (IJs) were used within 2 weeks after emergence for the experiments.

Insect sources

The lucerne beetle and alfalfa weevil adults were obtained from alfalfa fields in Emirseyit town of Tokat, Turkey (40° 2′ 13″ N, 36° 23′ 59″ E, 554 m).

Bioassays

Experiments were carried out in Petri-dishes (9 cm diameter). In the bottom of each Petri dish, filter paper was placed and 1 ml of prepared EPNs concentrations was poured onto the filter paper. For the control group, 1 ml of no-nematode water was used (Shahina and Salma 2010). In each Petri dish, 5 adult insects were released using the camel's hair brush. Each EPN species was applied at 3 concentrations 500, 1000, and 2000 IJs ml^{-1}. A micropipette was used to make the applications. After each treatment, the pipette tips were replaced. Alfalfa leaves were placed in Petri dishes to feed the insects. Experiments were carried at 4 replicates and 2 different times for each concentration. The data for mortality were recorded at 8-h intervals and the counts continued until to 120th hour. Dead insects were either dissected under a stereomicroscope or placed into White traps for nematode emergence to ensure that they were killed with EPNs (White 1927).

Statistical analysis

Data was analyzed using analysis of variance (ANOVA), and the means were compared by Tukey's multiple comparison test. All statistical analyses were carried out using the MINITAB Release 16 packet program. LT_{30}, LT_{50}, and LT_{90} values were calculated by using the Probit analysis.

Results

Mortality rates caused by EPNs isolates varied according to the test insect, time, isolates, and concentration. There was a positive correlation between nematode concentration and exposure time and mortality. *S. carpocapsae* (Black sea isolate) started to show a significant effect at all doses against *G.*

fornicata from the 32nd hour (F:21.67, DF: 3.28, $P < 0.05$), and this effect increased over 90% at the 64th hour (F:103.29, DF: 3.28, $P < 0.05$) (Table 1). *S. feltiae* (isolate 09-31) was 92% effective in 64 h at a dose of 2000 IJs ml^{-1}. Other doses caused a mortality rate of over 50% at the same time period (F:30.51, DF: 3.27, $P < 0.05$). By the 112th hour, all doses produced over 90% death (F:68.59, DF: 3.27, $P < 0.05$) (Table 1). Similar to the *S. carpocapsae* (Black sea isolate) isolate, *S. carpocapsae* Tokat-Ulas showed a significant efficacy after 48 h and caused more than 90% mortality at all doses (F:148.74, DF: 3.26, $P < 0.05$) (Table 1). *H. bacteriophora* (isolate 09-43) caused an effect of 98% at the 120th hour at a dose of 2000 IJs ml^{-1}, while this effect did not exceed 70% at other doses (F:34.25, DF: 3.21, $P < 0.05$) (Table 2). *H. bacteriophora* Tokat-Songut revealed a mortality rate of over 90% at doses of 1000 and 2000 IJs ml^{-1} at 72 h (F:54.81, DF: 3.28, $P < 0.05$). At the 112th hour, the effect of all doses was over 90% (F:91.73, DF: 3.28, $P < 0.05$) (Table 2).

When the LT_{30} rates of the isolates against *G. fornicata* were examined, the most effective isolate was *S. carpocapsae* Tokat-Ulas. This was followed by *S. carpocapsae* (Black sea isolate), *H. bacteriophora* Tokat-Songut, *S. feltiae* (isolate 09-31), and *H. bacteriophora* (isolate 09-43). The order formed by the isolates for LT_{30} value is valid for LT_{50} and LT_{90} values (Table 3).

Steinernema carpocapsae (Black sea isolate) showed a mortality rate of over 90% against *H. postica* from 72 h onwards at all doses (F:57.55, DF: 3.26, $P < 0.05$) (Table 4). Similarly, *S. carpocapsae* Tokat-Ulas caused a significant mortality rate against *H. postica* by more

Table 1 Mortality rates of *Gonioctena fornicata* adults exposed to *Steinernema carpocapsae* (Black sea isolate), *S. feltiae* (isolate 09-31), and *S. carpocapsae* Tokat-Ulas and controls over 120 h from treatment

	Mortality ± SEM*(%)							
HAT**	***Steinernema carpocapsae* (Black sea isolate)**				***S. feltiae* (isolate 09-31)**			
	500 IJs	**1000**	**2000**	**Control**	**500 IJs**	**1000**	**2000**	**Control**
32	26.6 ± 2.4b***	50.3 ± 4.7ab	60.5 ± 0.9a	0.0 ± 0.0c	15.0 ± 1.1a[1]	15.4 ± 2.5a	33.7 ± 3.20a	0.0 ± 0.0b
40	55.3 ± 6.5b	68.7 ± 3.7ab	92.1 ± 3.8a	0.0 ± 0.0c	30.4 ± 2.8a	31.4 ± 3.7a	46.7 ± 3.76a	0.0 ± 0.0b
48	85.0 ± 4.3b	88.6 ± 3.1b	100.0 ± 0.0a	0.0 ± 0.0c	35.8 ± 3.6a	53.3 ± 4.6a	60.2 ± 1.38a	0.0 ± 0.0b
56	85.0 ± 4.3b	92.0 ± 3.5ab	100.0 ± 0.0a	0.0 ± 0.0c	45.4 ± 2.0a	61.0 ± 3.8a	76.1 ± 4.69a	0.0 ± 0.0b
64	90.3 ± 2.6b	96.0 ± 2.8ab	100.0 ± 0.0a	0.0 ± 0.0c	51.4 ± 1.5b	76.7 ± 6.0ab	92.0 ± 3.46a	0.0 ± 0.0c
72	93.4 ± 2.8b	97.0 ± 2.0ab	100.0 ± 0.0a	0.0 ± 0.0c	57.7 ± 2.3b	83.1 ± 5.0ab	94.7 ± 2.13a	0.0 ± 0.0c
80	98.0 ± 2.6a	97.0 ± 2.0a	100.0 ± 0.0a	0.0 ± 0.0b	57.7 ± 2.3b	83.1 ± 5.0ab	94.7 ± 2.13a	0.0 ± 0.0c
88	98.0 ± 2.6a	97.0 ± 2.0a	100.0 ± 0.0a	0.0 ± 0.0b	57.7 ± 2.3b	83.1 ± 5.0ab	94.7 ± 2.13a	0.0 ± 0.0c
96	98.0 ± 2.6a	99.7 ± 0.9a	100.0 ± 0.0a	0.0 ± 0.0b	77.9 ± 2.5a	85.2 ± 4.6a	94.7 ± 2.13a	0.0 ± 0.0b
104	98.0 ± 2.6a	99.7 ± 0.9a	100.0 ± 0.0a	0.0 ± 0.0b	82.7 ± 1.5a	88.6 ± 3.1a	94.7 ± 2.13a	0.0 ± 0.0b
112	98.0 ± 2.6a	99.7 ± 0.9a	100.0 ± 0.0a	0.0 ± 0.0b	91.5 ± 2.9a	94.7 ± 2.1a	94.7 ± 2.13a	0.0 ± 0.0b
120	99.3 ± 2.0a	99.7 ± 0.9a	100.0 ± 0.0a	0.0 ± 0.0b	91.5 ± 2.9a	97.0 ± 2.0a	97.0 ± 2.00a	0.0 ± 0.0b
	***S. carpocapsae* Tokat-Ulas**							
	500 IJs		**1000**		**2000**		**Control**	
32	57.0 ± 0.9b		66.1 ± 2.8ab		88.6 ± 3.1a		0.0 ± 0.0c	
40	85.8 ± 1.3a		84.6 ± 2.5a		97.0 ± 2.0a		0.0 ± 0.0b	
48	94.7 ± 2.2a		97.0 ± 2.0a		100.0 ± 0.0a		0.0 ± 0.0b	
56	97.6 ± 2.0a		97.0 ± 2.0a		100.0 ± 0.0a		0.0 ± 0.0b	
64	99.4 ± 1.3a		98.7 ± 1.6a		100.0 ± 0.0a		0.0 ± 0.0b	
72	99.4 ± 1.3a		98.7 ± 1.6a		100.0 ± 0.0a		0.0 ± 0.0b	
80	99.4 ± 1.3a		98.7 ± 1.6a		100.0 ± 0.0a		0.0 ± 0.0b	
88	100.0 ± 0.0a		100.0 ± 0.0a		100.0 ± 0.0a		0.0 ± 0.0b	
120	100.0 ± 0.0a		100.0 ± 0.0a		100.0 ± 0.0a		0.0 ± 0.0b	

*SEM: standard error of the mean
**HAT: hours after treatment
***Means in a line followed by the same letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

Table 2 Mortality rates of *Gonioctena fornicata* adults exposed to *Heterorhabditis bacteriophora* (isolate 09-43) and *H. bacteriophora* Tokat-Songut and controls over 120 h from treatment

	Mortality ± SEM*(%)							
HAT**	*Heterorhabditis bacteriophora* (isolate 09-43)				*H. bacteriophora* Tokat-Songut			
	500 IJs	1000	2000	Control	500 IJs	1000	2000	Control
32	0.0 ± 0.0a***	0.0 ± 0.0a	2.4 ± 2.0a	0.0 ± 0.0a	5.3 ± 2.1ab[1]	8.2 ± 2.0ab	16.9 ± 5.0a	0.0 ± 0.0b
40	0.9 ± 1.5a	0.6 ± 1.3a	9.0 ± 4.0a	0.0 ± 0.0a	34.2 ± 2.2a	33.9 ± 3.7a	58.3 ± 1.5a	0.0 ± 0.0b
48	0.9 ± 1.5ab	7.0 ± 3.2ab	16.4 ± 4.4a	0.0 ± 0.0b	47.2 ± 1.8a	78.4 ± 9.0a	80.6 ± 3.5a	0.0 ± 0.0b
56	0.9 ± 1.5ab	13.7 ± 3.9ab	16.4 ± 4.4a	0.0 ± 0.0b	52.8 ± 1.8a	83.0 ± 5.5a	86.9 ± 3.9a	0.0 ± 0.0b
64	0.9 ± 1.5ab	19.2 ± 5.9a	25.6 ± 3.2a	0.0 ± 0.0b	63.4 ± 1.5b	85.0 ± 5.2ab	94.8 ± 3.6a	0.0 ± 0.0c
72	12.6 ± 4.7ab	35.5 ± 6.1a	50.0 ± 9.4a	0.0 ± 0.0b	68.9 ± 3.3b	94.7 ± 2.1a	96.0 ± 2.8a	0.0 ± 0.0c
80	26.4 ± 4.6a	46.7 ± 2.9a	72.0 ± 7.5a	0.0 ± 0.0b	71.2 ± 2.9b	94.7 ± 2.1a	96.0 ± 2.8a	0.0 ± 0.0c
88	30.4 ± 6.1a	46.7 ± 2.9a	78.1 ± 5.6a	0.0 ± 0.0b	73.7 ± 2.8b	98.7 ± 1.6a	97.0 ± 2.0a	0.0 ± 0.0c
96	52.4 ± 1.2a	46.7 ± 2.9a	81.0 ± 5.3a	0.0 ± 0.0b	75.8 ± 2.3b	98.7 ± 1.6a	97.0 ± 2.0a	0.0 ± 0.0c
104	60.4 ± 0.8a	57.7 ± 6.1a	93.3 ± 5.6a	0.0 ± 0.0b	82.8 ± 3.2b	98.7 ± 1.6a	97.0 ± 2.0ab	0.0 ± 0.0c
112	64.7 ± 1.1a	68.7 ± 7.5a	93.3 ± 5.6a	0.0 ± 0.0b	90.6 ± 4.4a	99.7 ± 0.9a	99.7 ± 0.9a	0.0 ± 0.0b
120	64.7 ± 1.1b	68.7 ± 7.5b	97.6 ± 2.0a	0.0 ± 0.0c	90.6 ± 4.4b	99.7 ± 0.9ab	100.0 ± 0.0a	0.0 ± 0.0c

*SEM: standard error of the mean
**HAT: hours after treatment
***Means in a line followed by the same letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

than 85% from the 64th hour at all doses (F:31.87, DF: 3.27, $P < 0.05$) (Table 4). *S. feltiae* (isolate 09-31) reached 90% effect only at 2000 IJs ml^{-1} doses and at the end of 112th hour (F:27.80, DF: 3.26, $P < 0.05$) (Table 4). The rate of effect of *H. bacteriophora* (isolate 09-43) remained quite low than other isolates and did not exceed 26% at all doses at the end of 120th hour (F:8.41, DF: 3.24, $P < 0.05$) (Table 5). At 120 h, the effect of *H. bacteriophora* Tokat-Songut was only 37% at the highest dose and its effect remained quite low, similar to that of *H. bacteriophora* (isolate 09-43) (F:9.28, DF: 3.25, $P < 0.05$) (Table 5).

When the LT rates of the isolates against *H. postica* were examined, the order formed by LT_{30} and LT_{50} values was *S. carpocapsae* (Black sea isolate), *S. carpocapsae* Tokat-Ulas, *S. feltiae* (isolate 09-31), *H. bacteriophora* Tokat-Songut, and *H. bacteriophora* (isolate 09-43). This ranking was *S. carpocapsae* Tokat-Ulas, *S. carpocapsae* (Black sea isolate), *S. feltiae* (isolate 09-31), *H. bacteriophora* Tokat-Songut, and *H. bacteriophora* (isolate 09-43) at LT_{90} value (Table 6).

Discussion

The results showed that *S. carpocapsae* (Black sea isolate) and *S. carpocapsae* Tokat-Ulas were more effective against both *H. postica* and *G. fornicata* adults than other isolates. Similarly, Atay and Kepenekci (2016) reported that the *S. carpocapsae* (Black sea isolate) had an effect of 80, 83, and 82% on another alfalfa pest, *Holotrichapion pullum* (Gyllenhal, 1833) (Coleoptera: Apionidae) adults, at 20 degrees and at 3 different concentrations (500, 1000, 5000 IJs ml^{-1}), respectively. In addition, Kepenekci et al. (2018) stated that *S. carpocapsae* (Black sea isolate) showed 99% efficacy in adults of *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae) at a dose of 1000 IJs ml^{-1}. Brivio and Mastore (2018) emphasized that symbiotic

Table 3 Lethal time (LT_{30}, LT_{50}, and LT_{90}) values of treated isolates against adults of *Gonioctena fornicata* (hour)

Isolates	Slope ± SE	LT_{30} (95% fiducial limit)	LT_{50} (95% fiducial limit)	LT_{90} (95% fiducial limit)	Heterogeneity
Steinernema carpocapsae (Black sea isolate)	5.024 ± 0.376	29.584 (25.789–32.900)	37.621 (33.943–41.041)	67.688 (61.707–75.841)	1.39
S. feltiae (isolate 09-31)	4.060 ± 0.318	40.000 (35.517–43.952)	53.853 (49.528–58.083)	111.391 (100.005–128.141)	1.12
Heterorhabditis bacteriophora (isolate 09-43)	4.181 ± 0.383	58.684 (51.365–64.913)	78.331 (71.189–86.845)	158.650 (133.011–210.174)	1.96
H. bacteriophora Tokat-Songut	5.729 ± 0.424	37.263 (33.590–40.478)	46.006 (42.547–49.264)	77.002 (71.236–84.735)	1.21
S. carpocapsae Tokat-Ulas	5.999 ± 0.502	26.030 (23.358–28.372)	31.833 (29.295–34.212)	52.060 (48.186–57.158)	0.91

Table 4 Mortality rates of *Hypera postica* adults exposed to *Steinernema carpocapsae* (Black sea isolate), *S. feltiae* (isolate 09-31), and *S. carpocapsae* Tokat-Ulas and controls over 120 h from treatment

	Mortality ± SEM*(%)							
HAT**	***Steinernema carpocapsae* (Black sea isolate)**				***S. feltiae* (isolate 09-31)**			
	500 IJs	**1000**	**2000**	**Control**	**500 IJs**	**1000**	**2000**	**Control**
32	1.3 ± 1.6b***	31.1 ± 3.3a	54.3 ± 2.5a	0.0 ± 0.0b	6.3 ± 5.4ab[1]	26.6 ± 2.4a	25.3 ± 0.4a	0.0 ± 0.0b
40	11.3 ± 4.5b	57.8 ± 1.6a	72.2 ± 1.0a	0.0 ± 0.0c	9.4 ± 5.2ab	31.4 ± 2.9a	31.0 ± 0.5a	0.0 ± 0.0b
48	53.0 ± 5.1a	83.0 ± 5.5a	85.4 ± 3.3a	0.0 ± 0.0b	19.0 ± 6.1ab	39.3 ± 1.3a	52.3 ± 4.3a	0.0 ± 0.0b
56	71.2 ± 2.9a	88.9 ± 5.7a	93.1 ± 2.1a	0.0 ± 0.0b	23.7 ± 5.1a	44.5 ± 2.1a	63.9 ± 3.1a	0.0 ± 0.0b
64	78.6 ± 4.0a	90.6 ± 5.2a	93.1 ± 2.1a	0.0 ± 0.0b	23.7 ± 5.1b	47.2 ± 1.8ab	66.7 ± 2.7a	0.0 ± 0.0b
72	90.4 ± 4.0a	98.7 ± 1.6a	96.1 ± 2.1a	0.0 ± 0.0b	33.9 ± 3.7a	50.0 ± 1.3a	66.7 ± 2.7a	0.0 ± 0.0b
80	90.4 ± 4.0a	98.7 ± 1.6a	96.1 ± 2.1a	0.0 ± 0.0b	42.0 ± 2.0a	50.0 ± 1.3a	66.7 ± 2.7a	0.0 ± 0.0b
88	90.4 ± 4.0a	98.7 ± 1.6a	98.3 ± 1.8a	0.0 ± 0.0b	47.8 ± 4.7a	50.0 ± 1.3a	69.6 ± 2.8a	0.0 ± 0.0b
96	92.0 ± 3.5a	98.7 ± 1.6a	98.3 ± 1.8a	0.0 ± 0.0b	53.3 ± 4.6a	55.3 ± 0.8a	80.2 ± 1.8a	0.0 ± 0.0b
104	92.0 ± 3.5a	98.7 ± 1.6a	98.3 ± 1.8a	0.0 ± 0.0b	66.1 ± 3.7a	63.7 ± 3.1a	85.2 ± 2.8a	0.0 ± 0.0b
112	92.0 ± 3.5a	98.7 ± 1.6a	98.3 ± 1.8a	0.0 ± 0.0b	66.1 ± 3.7a	66.1 ± 2.8a	89.6 ± 3.5a	0.0 ± 0.0b
120	93.4 ± 2.8a	98.7 ± 1.6a	98.3 ± 1.8a	0.0 ± 0.0b	66.1 ± 3.7a	66.1 ± 2.8a	89.6 ± 3.5a	0.0 ± 0.0b
	***S. carpocapsae* Tokat-Ulas**							
	500 IJs	**1000**	**2000**	**Control**				
32	16.9 ± 10.2ab	26.1 ± 5.17ab	52.8 ± 8.2a	0.0 ± 0.0b				
40	36.8 ± 6.8a	33.4 ± 5.54a	76.3 ± 4.2a	0.0 ± 0.0b				
48	61.0 ± 9.9a	76.3 ± 4.21a	88.7 ± 3.5a	0.0 ± 0.0b				
56	83.4 ± 7.2a	88.6 ± 3.09a	93.4 ± 2.8a	0.0 ± 0.0b				
64	85.4 ± 6.9a	91.8 ± 2.00a	93.4 ± 2.8a	0.0 ± 0.0b				
72	85.4 ± 6.9a	97.0 ± 2.00a	97.0 ± 2.0a	0.0 ± 0.0b				
80	90.6 ± 5.2a	98.7 ± 1.60a	98.7 ± 1.6a	0.0 ± 0.0b				
88	92.3 ± 6.1a	98.7 ± 1.60a	98.7 ± 1.6a	0.0 ± 0.0b				
96	93.7 ± 5.5a	100.0 ± 0.0a	100.0 ± 0.0a	0.0 ± 0.0b				
104	95.0 ± 3.9a	100.0 ± 0.0a	100.0 ± 0.0a	0.0 ± 0.0b				
112	95.0 ± 3.9a	100.0 ± 0.0a	100.0 ± 0.0a	0.0 ± 0.0b				
120	95.0 ± 3.9a	100.0 ± 0.0a	100.0 ± 0.0a	0.0 ± 0.0b				

*SEM: standard error of the mean
**HAT: hours after treatment
***Means in a line followed by the same letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

bacteria within the nematodes affect its virulence. The other possibility of variation in pathogenicity of EPN could be the host finding and penetration capability of nematodes. H. bacteriophora (isolate 09-43) and H. bacteriophora Tokat-Songut showed 98 and 100% effect on *G. fornicata* at 2000 IJs ml^{-1} doses in 120 h, respectively, while this effect was determined as 26 and 37% for *H. postica* at the same time and dose. Difference in the susceptibility of host insect species may be related with their immune system and the physical structure.

Kim et al. (2007) investigated the efficiency of 4 Korean EPNs against late larvae of alfalfa weevil and reported that *S. glaseri* (Steiner, 1929) Dongrae strain and *Heterorhabditis* sp. Gyeongsan strain were more effective against *H. postica* larva than *S. carpocapsae* GSN1 strain and *H. bacteriophora* Hamyang strain. Shah et al. (2011) stated that application of *H. indica* Poinar, Karunakar & David, 1992 and *S. carpocapase* at the rate 1 billion IJs/acre can decrease 72 and 50% population of alfalfa weevil, *H. postica* grubs, respectively. Falahi et al. (2011) tested *S. carpocapsae* against *H. postica* adults under laboratory conditions at doses of 250, 500, 1000, and 2000 IJs ml^{-1} and determined the highest effect as 97% at the dose of 2000 IJs ml^{-1} at the 72nd hour as our study.

Majić et al. (2013) showed that *H. bacteriophora* application in rate of 1000 IJs per adult of *G. fornicata*

Table 5 Mortality rates of *Hypera postica* adults exposed to *Heterorhabditis bacteriophora* (isolate 09-43) and *H. bacteriophora* Tokat-Songut and controls over 120 h from treatment

	Mortality ± SEM*(%)							
HAT**	*Heterorhabditis bacteriophora* (isolate 09-43)				*H. bacteriophora* Tokat-Songut			
	500 IJs	1000	2000	Control	500 IJs	1000	2000	Control
32	0.0 ± 0.0a***	0.0 ± 0.0a	0.6 ± 1.3a	0.0 ± 0.0a	0.0 ± 0.0	0.00 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
40	0.0 ± 0.0a	0.0 ± 0.0a	1.3 ± 2.7a	0.0 ± 0.0a	0.0 ± 0.0a[1]	0.00 ± 0.0a	1.7 ± 1.8a	0.0 ± 0.0a
48	0.0 ± 0.0a	0.0 ± 0.0a	1.3 ± 2.7a	0.0 ± 0.0a	0.3 ± 0.9a	0.4 ± 1.1a	1.7 ± 1.8a	0.0 ± 0.0a
56	0.0 ± 0.0a	1.7 ± 1.8a	5.1 ± 4.2a	0.0 ± 0.0a	0.3 ± 0.9ab	0.4 ± 1.1ab	8.5 ± 2.9a	0.0 ± 0.0b
64	0.0 ± 0.0a	1.7 ± 1.8a	5.1 ± 4.2a	0.0 ± 0.0a	0.7 ± 2.0ab	1.7 ± 1.8ab	12.6 ± 2.4a	0.0 ± 0.0b
72	0.0 ± 0.0b	6.9 ± 2.1ab	9.0 ± 4.0a	0.0 ± 00ab	2.0 ± 2.6ab	1.7 ± 1.8ab	12.6 ± 2.4a	0.0 ± 0.0b
80	0.0 ± 0.0b	6.9 ± 2.1ab	16.4 ± 4.4a	0.0 ± 0.0b	4.0 ± 2.8ab	6.9 ± 2.1ab	19.8 ± 1.8a	0.0 ± 0.0b
88	3.0 ± 2.0ab	6.9 ± 2.1ab	16.4 ± 4.4a	0.0 ± 0.0b	4.0 ± 2.8ab	10.6 ± 1.8a	19.8 ± 1.8a	0.0 ± 0.0b
96	3.0 ± 2.0ab	6.9 ± 2.1ab	22.5 ± 3.0a	0.0 ± 0.0b	4.0 ± 2.8ab	12.6 ± 2.4a	19.8 ± 1.8a	0.0 ± 0.0b
104	8.0 ± 3.5ab	10.6 ± 1.8ab	22.5 ± 3.0a	0.0 ± 0.0b	6.6 ± 2.8bc	17.3 ± 1.5ab	30.7 ± 1.0a	0.0 ± 0.0c
112	8.0 ± 3.5ab	17.3 ± 1.5a	22.5 ± 3.0a	0.0 ± 0.0b	7.9 ± 3.8bc	17.3 ± 1.5ab	33.6 ± 0.9a	0.0 ± 0.0c
120	11.4 ± 3.1a	22.6 ± 0.3a	25.6 ± 3.2a	0.0 ± 0.0b	13.1 ± 4.8a	17.3 ± 1.5a	36.7 ± 0.8a	0.0 ± 0.0b

*SEM: standard error of the mean
**HAT: hours after treatment
***Means in a line followed by the same letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

caused 100% mortality rate on 3 day post-treatment in the laboratory. Similarly, in the present study, *H. bacteriophora* isolates had a significant effect on adult of *G. fornicata*. On other chrysomelids, mortality studies have been carried out with success using a variety of steinernematid and heterorhabditid. Trdan et al. (2008) assessed virulence of EPNs *S. feltiae*, *S. carpocapsae*, *H. bacteriophora*, and *H. megidis* against adult flea beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae) under laboratory conditions and revealed that *S. feltiae* was the most effective nematode. Also, Trdan et al. (2009) stated that *S. feltiae* showed the highest efficacy in controlling adults of *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) at 15 °C. Similarly, in the present study, *S. feltiae* (isolate 09-31) was more effective on *G. fornicata* than *H. postica*. Unlike these studies, Laznik et al. (2010) determined that *S. carpocapsae* C101 caused more mortality than *S. feltiae* B30 and *H. bacteriophora* D54 against *Oulema melanopus* (Linnaeus, 1758) (Coleoptera: Chrysomelidae) adults.

Conclusions

This study highlighted the first analysis of EPNs' efficacy against *H. postica* and *G. fornicata* in Turkey. *G. fornicata* adults were susceptible to all isolates tested, while *H. postica* adults were susceptible to *S. carpocapsae* (Black Sea isolate), *S. feltiae* (isolate 09-31), and *S. carpocapsae* Tokat-Ulas isolates. Therefore, these native EPNs can be used as bio-control agents against the adults of these 2 important alfalfa pests. Further studies should be conducted under field conditions.

Abbreviations

EPNs: Entomopathogenic nematodes; IJs: Infective juveniles; LT: Lethal time

Table 6 Lethal time (LT_{30}, LT_{50}, and LT_{90}) values of treated isolates against adults of *Hypera postica* (hour)

Isolates	Slope ± SE	LT_{30} (95% fiducial limit)	LT_{50} (95% fiducial limit)	LT_{90} (95% fiducial limit)	Heterogeneity
Steinernema carpocapsae (Black sea isolate)	3.869 ± 0.283	25.916 (22.380–29.113)	35.407 (31.790–38.848)	75.909 (68.501–86.031)	1.14
S. feltiae (isolate 09-31)	2.381 ± 0.249	45.810 (39.570–51.478)	76.074 (68.288–85.999)	262.756 (202.900–384.894)	0.77
Heterorhabditis bacteriophora (isolate 09-43)	4.424 ± 0.809	121.745 (109.212–147.749)	159.954 (135.437–222.873)	311.667 (223.473–624.159)	0.43
H. bacteriophora Tokat-Songut	4.341 ± 0.683	108.901 (99.625–124.271)	143.823 (125.686–182.167)	283.818 (213.812–481.143)	0.87
S. carpocapsae Tokat-Ulas	5.073 ± 0.376	29.840 (26.880–32.501)	37.858 (34.967–40.629)	67.729 (62.496–74.601)	0.95

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Authors' contributions
AÇ, TA, and İK conceived and designed the research. AÇ conducted the experiments. TA analyzed the data and wrote the manuscript. İK corrected and revised the manuscript, corrected language mistakes and translation, and corrected references. All authors read and approved the final manuscript.

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Availability of data and materials
The dataset(s) supporting the conclusions of this article is (are) included within the article (and its additional file(s)).

Declarations

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