Journal of Plant Diseases and Protection (2023) 130:85–92
https://doi.org/10.1007/s41348-022-00686-5

ORIGINAL ARTICLE

Pathogenicity of four native isolates of entomopathogenic nematodes against *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae)

Mürşide Yağcı[1] · Fatma Dolunay Erdoğuş[1] · Tuğba Akdeniz Fırat[1] · Sait Ertürk[1]

Received: 24 September 2022 / Accepted: 17 November 2022 / Published online: 22 November 2022

Abstract

The confused flour beetle, *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae) is a major pest on stored grains. Chemical pesticides are usually used to control stored grain pests but insecticides residue and other negative effects on human and environmental health are the constrain ones in this concern. The biological control agents such as entomopathogenic nematodes (EPNs), which are used to control different harmful organisms, get attention in the alternative control methods. The aim of this study was carried out to determine biocontrol potential of local entomopathogenic nematode isolates, *Steinernema carpocapsae* (Tokat-Bakışlı05), *S. feltiae* (Tokat-Emir), *Heterorhabditis bacteriophora* (TOK-20), and *H. bacteriophora* (11KG) against adult beetles of confused flour beetle under controlled conditions. EPNs isolates were applied at four different concentrations *i.e.,* 0.0, 25, 50 and 100 IJs/beetle in 1 ml of distilled water at 15 and 25 ± 1 °C temperatures. The highest mortality rates for *H. bacteriophora* (11KG) and *H. bacteriophora* (TOK-20) isolates at 25 ± 1 ºC were determined as 91.0 and 81.2%, respectively. *H. bacteriophora* (11KG) and *H. bacteriophora* (TOK-20) were found to be the most effective EPNs at a concentration of 100 IJs/beetle at 15 ºC with a mortality of 57.7 and 55.6%, respectively. Mortality rates for the adult confused flour beetle were increased with an increasing the concentration of all EPNs species and also the degrees of temperature. Results showed that local EPNs isolates may use as an alternative biological control agent for *T. confusum.* Efforts should be made to develop new formulations that will allow nematodes to survive until they find their hosts, and they also need to be tested under field conditions.

Keywords Biological control · Entomopathogenic nematodes · *Steinernema carpocapsae* · *S. feltiae* · *Heterorhabditis bacteriophora* · *Tribolium confusum*

Introduction

The confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), is one of the most harmful pests of stored grain beetle, native to Africa, but usually found all over the world (Hagstrum and Subramanyam 2009). As a secondary pest, the confused flour beetle primarily prefers infested or mechanically damaged grains. Both adults and larvae are responsible for severe post-harvest losses, particularly in stored products, such as wheat, maize, barley, sorghum, oilseeds, spices, bran, and dried herbs. (Boyer et al. 2012; Hagstrum et al. 2013). Post-harvest losses in developed countries are estimated that 5–10%, whereas this rate rises to 75% in developing countries (Boxall 2001; Hodges et al. 2011; Mason and McDonough 2012).

Chemical insecticides are considered the primary control method for insect pests. However, the high frequency and widespread use of chemical insecticides stimulates the development of resistance in insects and causes environmental pollution as they cannot be broken down easily. The demand for pesticide-free products in society, and environmental problems caused by the use of synthetic chemical pesticides against such pests, encourage scientists to search for different control options (Lu and Wu 2010; Alkan 2020; Ertürk et al. 2020).

The EPNs are widely used in many countries and have attracted interest as potential biocontrol agents. They can be preferred in the control of harmful insects due to their wide

✉ Sait Ertürk
saiterturk@gmail.com

[1] Plant Protection Central Research Institute, Yenimahalle, Ankara, Turkey

host range, active host seeking, easy and low cost mass production, long-term efficacy, easy application, compatibility with most chemicals and environmentally safe for humans and other non-target organisms (Kaya 1990; Shields et al. 1999; Rumbos and Athanassiou 2017; Vitta et al. 2017; Belien 2018; Yuksel et al. 2019; Yağci et al. 2021a, b). EPNs are obligate parasites and they have the ability to actively search for their host. A number of stimuli factors such as the body temperature of the host insect, the CO_2 released by the host, the host's feces, and kairomones are affecting the effectiveness of infective juveniles (IJs) (Athanassiou et al. 2008; Rasmann and Turlings 2008; Rumbos and Athanassiou 2012; Tülek et al. 2015; Yağcı et al. 2021c). The IJs, the only free life stage of the EPNs, enter the host through natural openings or penetrate into the insect cuticle. The toxins produced by the symbiotic bacteria, *Xenorhabdus* spp. (Enterobacterales: Morganellaceae) and *Photorhabdus* spp. (Enterobacterales: Morganellaceae) associated with EPNs sacrifice the host within between 24 and 48 h (Woodring and Kaya 1988; Shapiro and Lewis 1999; Forst and Clarke 2002; Tülek et al. 2015; Canhilal 2016; Alotaibi et al. 2022).

EPNs are promising alternatives to chemical pesticide that are widely used in agricultural biological control programs (Ffrench-Constant et al. 2007). Steinernematidae and Heterorhabditidae are well known and utilized endoparasites for numerous agricultural insect pests. However, the pathogenicity of EPNs on insect pests of stored commodities are limited despite that EPNs have the potential for control of coleopteran pests (Duncan and McCoy 1996; Laznik et al. 2010; Javed et al. 2020). EPNs have received little attention in post-harvest protection except for *Steinernema carpocapsae* and *S. feltiae* that so far are the most commonly tested steinernematid nematode species against stored product pests (Rumbos and Athanassiou 2017).

It is clear that in vitro experiments are primary and essential for the success of field experiments. Therefore, the present study aimed to determine the pathogenicity, virulence, and biocontrol potential of different native EPNs isolates from Türkiye of *S. carpocapsae* (Tokat-Bakışlı05) (Weiser) (Rhabditita: Steinernematidae) and *S. feltiae* (Tokat-Emir) (Filipjev) (Rhabditita: Steinernematidae), *Heterorhabditis bacteriophora* (TOK-20) Poinar (Rhabditita: Heterorhabditidae), *H. bacteriophora* (11KG) Poinar (Rhabditita: Heterorhabditidae) at different concentrations and temperature on the mortality of *T. confusum* adults under laboratory conditions.

Materials and methods

Insect rearing

Tribolium confusum

Adults of *T. confusum* were grown on cracked wheat-containing 5% brewing yeast (w/w), aged between 7 and 28 days (Athanassiou et al. 2016) in a 1000 ml capacity glass jar. The insect culture was raised in continuous darkness in a climate chamber at 25 ± 1 °C and 60 ± 5% relative humidity (R.H.) (Nüve ID 501, Ankara, Türkiye). The culture has been rearing since 2010 in the Entomology Laboratory of the Plant Protection Central Research Institute, Ankara, Türkiye.

Galleria mellonella

Wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae, which are known susceptible to infection of entomopathogenic nematode species, were used in the experiments (Barrón-Bravo et al. 2021). For this purpose, the original stock culture of *G. mellonella* was received from the Department of Plant Protection, Faculty of Agriculture, Tokat Gaziosmanpaşa University, Türkiye. All EPNs cultures were raised in the Nematology Laboratory of the Plant Protection Central Research Institute, Ankara, Türkiye since 2018. The larvae of *G. mellonella* were fed on a diet containing glycerin (500 g), honey (500 g), wheat flour (890 g), dry baker's yeast (222 g), milk powder (445 g), and beeswax (125 g). Beeswax and dry baker's yeast were melted and then mixed with other materials. The prepared artificial diet has been stored in the refrigerator for the intended use.

The last instar larvae of *G. mellonella* were used, according to Mohamed and Coppel (1983). Wax moth eggs were kept in the diet in one liter glass jar under 16:8 h light and dark under 23–24 ± 1 °C degree (Nüve ID 501, Ankara, Türkiye). After about 30 days, the wax moth's last larvae were obtained for mass rearing.

Entomopathogenic nematodes culture

Steinernema carpocapsae (Tokat-Bakışlı05), *S. feltiae* (Tokat-Emir), *Heterorhabditis bacteriophora* (TOK-20), and *H. bacteriophora* (11KG) were obtained from the Plant Protection Department of Tokat Gaziosmanpaşa University, Türkiye. The nematode species were identified by Dr. Selçuk HAZIR and Dr. Harun ÇİMEN (Department of Biology, Faculty of Arts and Sciences, Aydin Adnan Menderes University, Aydin, Turkey) based on morphometric and molecular analyzes (Kepenekci et al. 2017, 2018; Çağlayan et al. 2020). EPNs

were gathered on the last stage larvae of the greater wax moth at 25 ± 1 °C and 60 ± 5% R.H. under laboratory conditions according to Kaya and Stock (1997). For mass rearing of EPNs isolates; ten instar larvae of *G. mellonella* were placed in six cm diameter Petri-dishes lined with Whatman paper (No.1, Whatman International, Maidstone, UK), and wetted with distilled water. Suspension of juveniles of each nematode isolate were prepared, and then applied on the greater wax moth larvae. Petri-dishes were sealed with a parafilm, and then placed in the incubator at 25 ± 1 °C and 60 ± 5% R.H. IJs were collected from infected larvae by using the "White trap" method (White 1927).

Biossays

Experiments were conducted in 6 cm Petri-dishes under controlled conditions (15, 25 °C and at constant 65% R.H.). The dishes lined with Whatman paper (No.1 Whatman International, Maidstone, UK) and 0, 25, 50, and 100 IJs/larva concentrations were prepared. The determined nematode concentrations were added to the filter paper with a pipette in 1 ml of distilled water to each Petri-dish. Ten *T. confusum* adults were introduced into Petri-dishes containing 5 g sterilized cracked wheat. For control treatments, only distilled water was used. Petri-dishes were sealed and then wrapped with Parafilm to prevent insects from escaping. The Petri-dishes were put in a rearing chamber. Mortality of beetles was checked at 48, 72, and 96 h after the inoculation. Dead beetles were counted at each time interval (Yuksel et al. 2019; Yağci et al. 2021a, b, c). The whole experiment was repeated three times with different dates. Each treatment was replicated five times and 10 individuals were used in each replicate.

Data analysis

The data obtained from the concentrations screening tests were first converted to percentage mortality, and then arcsine transformation was carried out (Zar 1999; Warton and Hui 2011). To separate the mean differences, Tukey's multiple range test was used at 5% significance levels. All statistical analyses were performed using MINITAB Release 18 statistical software with the general linear model in order to determine the statistical interactions between treatments (Mckenzie and Goldman 2005).

Results

The insecticidal activity of EPNs isolates against *T. confusum* was determined, in order to be used as biological control agents. All tested EPNs isolates caused varying levels of death and were found significant ($P < 0.05$). Also, the mortality rate increased with increasing concentrations, temperature, and exposure times (Tables 1, 2). The adults of *T. confusum* were found susceptible to all EPNs isolates. The results show that all EPNs isolates increased the mortality of *T. confusum* ($P < 0.05$) when compared with the control group, which recorded a maximum mortality of 0.7% at all temperatures, exposure times, and concentrations.

Mortalities of confused flour beetle varied strongly at 15 °C (Table 1). *H. bacteriophora* (TOK-20), recorded the highest mortality rate with 57.7% at a concentration of 100 IJs/adult after 96 h of exposure. *H. bacteriophora* (TOK-20) isolate was the most effective, which caused 17.4% mortality at 50 IJs/adult concentration after 96 h of exposure. *S. carpocapsae* (Tokat-Bakışlı05) showed the highest efficacy of 36.5% at the concentration of 100 IJs/adult after 96 h of exposure.The highest mortality rate for *S. feltiae* (Tokat-Emir) was 36.6% at the concentration of 100 IJs/adult after 96 h of exposure. However, the lowest effect at the concentration 25 IJs/adult was recorded with 1.6% mortality after 48 h. The highest mortality rate of 55.6% was resulted at a concentration of 100 IJs/ml from *H. bacteriophora* (11KG) isolate at 15 °C after 96 h of exposure.

The results showed that the highest mortality rate was 91% for *H. bacteriophora* (11KG) at 100 IJs/adults concentration after 96 h post treatment at 25 °C (Table 2). This mortality rate of *H. bacteriophora* (TOK-20), *S. feltiae* (Tokat-Emir), *S. carpocapsae* (Tokat-Bakışlı05) depicted 81.2, 74.1, 56.1%, respectively. The lowest mortality rate in all isolates at 25 IJs/adult were less at 25 °C temperature and all exposure period. It was found that the mortality rate was increased proportionally as exposure time passed from the inoculation.

Discussion

This research studies showed that the virulence and biocontrol potantial of different EPNs isolates against *T. confusum* is dependent on ambient temperature and nematode concentration. Rumbos and Athanassiou (2012) found that *S. carpocapsae* caused low mortality rate (15.2 and 22.4%) on *T. confusum* after 4 and 8 days of exposure at 27 °C and 70% R.H. Alikhan et al. (1985) noted that the effect of *S. carpocapsae* against *Tribolium confusum* at two different concentration and calculated 48–56 h after death, and they caused low mortality levels. In the previous studies, Majić et al. (2021), studied the pathogenicity of different strains of *S. feltiae* at different concentrations of 0, 300, and 700 IJs insect^{-1} at 15 and 25 °C temperatures against *T. castaneum*. They found that the activity did not exceed than 82.0% depending on the insect biological stage, temperature, and EPNs concentration. Furthermore, Kepenekci et al. (2015) tested the virulence of *H. bacteriophora, S. carpocapsae,*

Table 1 Efficacy of entomopathogenic nematode isolates on mortality rate of confused flour beetle at 15 °C

EPNs Isolates	Concentrations (IJs/adult)	Mortality rate after (h)			
		48	72	96	
S. carpocapsae (Tokat-Bakışlı05)	25	1.9 ± 3.2bc♦A♦♦	3.6 ± 3.6bcA	3.6 ± 3.6cA	*F* = 0.39 df = 2, 42, P > 0.05
	50	6.1 ± 4.7abA	9.1 ± 5.4bA	12.2 ± 4.2bA	*F* = 0.88 df = 2, 42, P > 0.05
	100	12.2 ± 4.2aC	22.3 ± 0.7acC	36.5 ± 0.6aA	*F* = 17.43 df = 2, 42, *P* < 0.05
	0	0.2 ± 1.3cA	0.2 ± 1.3A	0.2 ± 1.3cA	*F* = 0.0 df = 2, 42, P > 0.05
		F = 8.23 df = 3, 56 *P* < 0.05	*F* = 19.48 df = 3, 56 *P* < 0.05	*F* = 41.60 df = 3, 56 *P* < 0.05	
S. feltiae (Tokat-Emir)	25	1.6 ± 2.6bA	2.2 ± 2.7cA	4.5 ± 2.4cA	*F* = 1.14 df = 2, 42, P > 0.05
	50	1.1 ± 2.4bB	10.4 ± 1.2bA	12.6 ± 1.9bA	*F* = 15.58 df = 2, 42, *P* < 0.05
	100	16.3 ± 6.5aB	30.1 ± 3.3aAB	36.3 ± 3.5aA	*F* = 4.72 df = 2, 42, *P* < 0.05
	0	0.0 ± 0.0bA	0.0 ± 0.7cA	0.2 ± 1.3bA	*F* = 1.05 df = 2, 42, P > 0.05
		F = 16.15 df = 3, 56 *P* < 0.05	*F* = 44.48 df = 3, 56 *P* < 0.05	*F* = 42.32 df = 3, 56 *P* < 0.05	
H. bacteriophora (TOK-20)	25	0.4 ± 1.8bB	0.7 ± 2.2cAB	4.0 ± 3.0cA	*F* = 3.55 df = 2, 42, *P* < 0.05
	50	1.1 ± 2.4bB	10.2 ± 2.4bA	17.4 ± 1.0bA	*F* = 20.73 df = 2, 42, *P* < 0.05
	100	22.4 ± 1.8aC	43.9 ± 1.3aC	57.7 ± 2.4aA	*F* = 28.22 df = 2, 42 *P* < 0.05
	0	0.0 ± 0.7bA	0.0 ± 0.7cA	0.0 ± 0.7dA	*F* = 0.0; df = 2, 42 P > 0.05
		F = 41.98 df = 3, 56 *P* < 0.05	*F* = 92.57 df = 3,56 *P* < 0.05	*F* = 109.93 df = 3, 56 *P* < 0.05	
H. bacteriophora (11KG)	25	0.4 ± 1.8bA	0.7 ± 2.2bcA	0.7 ± 2.2bA	*F* = 0.11 df = 2, 42 P > 0.05
	50	3.6 ± 3.6bA	4.8 ± 3.7bA	5.2 ± 4.1bA	*F* = 0.17 df = 2, 42 P > 0.05
	100	44.9 ± 3.0aA	49.3 ± 2.0aA	55.6 ± 2.6aA	*F* = 0.0 df = 2, 42 P > 0.05
	0	0.2 ± 1.3bA	0.2 ± 1.3c	0.7 ± 2.2bA	*F* = 0.58 df = 2, 42 P > 0.05
		F = 65.32 df = 3, 56 *P* < 0.05	*F* = 65.32 df = 3, 56 *P* < 0.05	*F* = 70.32 df = 3, 56 *P* < 0.05	

♦ Means with the same lower-case letter in a column do not differ significantly (Anova $P < 0.05$. Tukey test)

♦♦ Means with the same upper-case letter in a row do not differ significantly (Anova $P < 0.05$. Tukey test)

and *S. feltiae* against the *T. castaneum* and *T. confusum* adults under three different temperatures (15, 20, and 25 °C) and found that *S. carpocapsae* was the most effective to the *T. confusum* (85.35%) and *T. castaneum* (86.47%) adults and other isolates were less effective. Javed et al. (2020) reported that different concentrations of *S. pakistanenses* (LM07), *S. bifurcatum* (LM-30) depicted higher mortality rates against *T. confusum*, at 30 ºC than at 20 ºC. They added that the same EPNs species were found to increase mortality rates with an increase in temperatures. Also, Laznik et al. (2010) reported that three different isolates of *S. feltia* showed varying efficacy against *S. oryzae* adults, and this difference is

Table 2 Efficacy of entomopathogenic nematode isolates on mortality rate of confused flour beetle 25 °C

EPNs Isolates	Concentrations (IJs/adult)	Mortality rate after (h)			
		24	48	72	
S. carpocapsae (Tokat-Bakışlı05)	25	3.7 ± 2.6c♦A♦♦	7.3 ± 3.3cA	8.8 ± 4.1aA	$F = 1.40$ df = 2, 42, P > 0.05
	50	18.1 ± 0.9bB	34.4 ± 1.1bA	41.1 ± 1.3bA	$F = 23.8$ df = 2, 42; $P < 0.05$
	100	36.6 ± 2.8aB	48.0 ± 1.0aAB	56.1 ± 1.3aA	$F = 8.57$ df = 2, 42, $P < 0.05$
	0	0.0 ± 0.0dA	0.0 ± 0.7dA	0.0 ± 0.7dcA	$F = 0.5$ df = 2, 42, P > 0.05
		$F = 75.21$ df = 3, 56 $P < 0.05$	$F = 112.24$ df = 3, 56 $P < 0.05$	$F = 113.99$ df = 3, 56 $P < 0.05$	
S. feltiae (Tokat-Emir)	25	13.7 ± 0.5cB	18.8 ± 0.8cAB	22.9 ± 0.8bA	$F = 7.53$ df = 2, 42, $P < 0.05$
	50	27.1 ± 0.6bC	38.4 ± 0.9bB	46.0 ± 0.4aA	$F = 22.93$ df = 2, 42, $P < 0.05$
	100	39.9 ± 0.6aC	57.6 ± 1.5aB	74.1 ± 3.9dA	$F = 23.31$ df = 2, 42, $P < 0.05$
	0	0.4 ± 1.8dA	0.7 ± 2.2dA	0.7 ± 2.2cA	$F = 0.11$ df = 2, 42, P > 0.05
		$F = 121.53$ df = 3, 56 $P < 0.05$	$F = 123.08$ df = 3, 56 $P < 0.05$	$F = 132.01$ df = 3, 56 $P < 0.05$	
H. bacteriophora (TOK-20)	25	10.0 ± 3.4cB	19.6 ± 0.7cA	21.6 ± 0.7bA	$F = 7.01$ df = 2, 42, $P < 0.05$
	50	21.3 ± 3.2bB	32.3 ± 1.2bA	37.8 ± 1.0aA	$F = 7.22$ df = 2, 42, $P < 0.05$
	100	46.6 ± 1.1aB	61.4 ± 2.5aB	81.2 ± 6.9dA	$F = 14.65$ df = 2, 42, $P < 0.05$
	0	0.0 ± 0.0dA	0.2 ± 1.3dA	0.2 ± 1.3A	$F = 1.08$ df = 2, 42, P > 0.05
		$F = 76.07$ df = 3, 56 $P < 0.05$	$F = 133.82$ df = 3, 56 $P < 0.05$	$F = 119.45$ df = 3, 56 $P < 0.05$	
H. bacteriophora (11KG)	25	7.5 ± 4.8cB	20.5 ± 3.8cA	32.3 ± 1.1bA	$F = 12.43$ df = 2, 42, $P < 0.05$
	50	22.4 ± 2.5bB	36.3 ± 1.2bA	40.4 ± 1.6bA	$F = 8.90$ df = 2, 42, $P < 0.05$
	100	59.0 ± 1.6aB	80.5 ± 4.8aA	91.0 ± 5.6aA	$F = 14.39$ df = 2, 42, $P < 0.05$
	0	0.0 ± 0.7dA	0.4 ± 1.8dA	0.4 ± 1.8cA	$F = 0.658$ df = 2, 42, P > 0.05
		$F = 81.31$ df = 3, 56 $P < 0.05$	$F = 95.89$ df = 3, 56 $P < 0.05$	$F = 143.79$ df = 3, 56 $P < 0.05$	

♦ Means with the same lower-case letter in a column do not differ significantly (Anova $P < 0.05$. Tukey test)

♦♦ Means with the same upper-case letter in a row do not differ significantly (Anova $P < 0.05$. Tukey test)

due to the origin of the isolates. It was concluded that different isolates of the same EPNs species showed different activity for the target insect.

In the present study, especially in the *Heterorhabditis* species were observed of weak mortality rates even at the highest concentrations, time, and temperature. On the contrary, Eldefrawy and Raheem (2017) found 100% mortality of *T. confusum* at variable doses of 1000, 2000, and 3000 IJs of *H. bacteriophora*.

Another comment to be made on this subject is that the smaller the host's biomass, the lower the infection density by EPNs (Wójcik 1986). *T. confusum* adults are small-sized

insects. Due to the low biomass of this insect, it is thought that nematodes cannot produce new generations in high numbers within the insect body and this causes low mortality rates. As it is known, the bacteria that carry by the nematodes transform the insect body mass into a nutrient medium to complete the nematode life cycle. According to the obtained results, the mortality occurring in the exposed to EPNs insects varies depending on virulency, nematode species and strains, insect species, insect biological stages, EPNs concentrations, exposure time, and temperature. Unlike synthetic pesticides, EPNs like other insect pathogens, cause slow mortality in insects (Moore et al. 2000). Although the insect biological stage is considered an essential parameter in insect individual's rapid or slow death, the larval stage is out of the scope of this study. Athanassiou et al. (2008) revealed that *T. confusum* larvae are more sensitive than adults to EPNs. They added that *S. feltiae* caused larval stage mortality rates ranging from 79 to 100% after 7 to 14 days of nematode exposure at the highest dose, respectively. One could say that an adult insect body was assessed to be less likely to be invaded by IJs due to the dense sclerotized integument, which presents a significant barrier to penetration of EPNs, when compared to a larval body structure.

Conclusions

The present study indicated that the *S. feltiae* (Tokat-Emir), *S. carpocapsae* (Tokat-Bakışlı05), *H. bacteriophora* (11KG), and *H. bacteriophora* (TOK-20) isolates can be used for control of *T. confusum* adults under controlled conditions. *H. bacteriophora* (11KG) was the most effective species against the confused flour beetle at 25 °C. EPNs are considered to have a high potential for biological control agents in storage facilities. Besides that, a few species have also been reported as commercially available (Grewal 2002; Georgis et al. 2006). However, there may be some difficulties in the effective use of nematodes as biocontrol agents under storage conditions. In order for nematodes to survive in these conditions, adequate humidity levels must be provided (Doberski 1981). These difficulties require efforts to develop new formulations that will enable nematodes to survive until they find their hosts and need to be tested under field conditions.

Author contribution

MY: Supervision, Conceptualization, Methodology, Investigation, Writing—original draft, SE: Conceptualization, Investigation, Writing—original draft, Supervision, Data curation, FDE: Methodology, Writing—review and editing, TAF: Methodology, Writing—review and editing.

Acknowledgements We would like to thank Professor İlker KEPENEKCİ for providing local EPNs isolates.

Funding Not applicable.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no confict of interest.

Consent for publication All the authors have their own contribution in writing of this review article.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Alikhan MA, Bednarek A, Grabiec S (1985) The physiological and-morphological characteristics of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae) in two insect hosts. J Invertebr Pathol 45:168–173. https://doi.org/10.1016/0022-2011(85)90006-0

Alkan M (2020) Chemical composition and insecticidal potential of different *Origanum* spp. (Lamiaceae) essential oils against four stored product pests. Turkish J Entomol 44(2):149–163. https://doi.org/10.16970/entoted.620387

Alotaibi SS, Darwish H, Zaynab M, Alharthi S, Alghamdi A, Al-Barty A, Asif M, Wahdan RH, Baazeem A, Noureldeen A (2022) Isolation, identification, and biocontrol potential of entomopathogenic nematodes and associated bacteria against *Virachola livia* (Lepidoptera: Lycaenidae) and *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae). Biology 11(2):295. https://doi.org/10.3390/biology11020295

Athanassiou CG, Palyvos NV, Kakouli-Duarte T (2008) Insecticidal effect of *Steinernema feltiae* (Filipjev) (Nematoda: Steinernematidae) against *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) and *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) in stored wheat. J Stored Prod Res 44:52–57. https://doi.org/10.1016/j.jspr.2007.04.002

Athanassiou CG, Kavallieratos NG, Chiriloaie A, Vassilakos TN, Fatu V, Drosu S, Dudoiu R, Ciobanu M, Dudoiu R (2016) Insecticidal efficacy of natural diatomaceous earth deposits from Greece and Romania against four stored grain beetles: the effect of temperature and relative humidity. B Insectol 69(1):25–34

Barrón-Bravo O, Montiel-Maya I, Cruz-Avalos A, Avila-Ramos F, Ochoa JM, Angel-Sahagún C (2021) Entomopathogenic nematodes: Biological model of studies with Anthelmintics. In: Cristiano C , Kaspary TE (eds) Nematodes—recent advances, management and new perspectives. IntechOpen. https://doi.org/10.5772/intechopen.99663

Belien T (2018) Entomopathogenic nematodes as biocontrol agents of insect pests in orchards. CAB Rev 13(058):1–11. https://doi.org/10.1079/PAVSNNR201813058

Boxall RA (2001) Post-harvest losses to insects—a world overview. Int Biodeterior Biodegrad 48(1–4):137–152. https://doi.org/10.1016/S0964-8305(01)00076-2

Boyer S, Zhang H, Lempérière G (2012) A review of control methods and resistance mechanisms in stored-product insects. B Entomol Res 102(2):213–229. https://doi.org/10.1017/S0007485311000654

Çağlayan A, Atay T, Kepenekci İ (2020) Entomopathogenic nematodes occurring in alfalfa fields, Tokat, Turkey. Plant Protect Bull 60(4):41–47. https://doi.org/10.16955/bitkorb.749288

Canhilal R (2016) The use of entomopathogens in the controlling of insect pests of stored product. Sci Pap Ser A Agron LIX59:235–240

Doberski JW (1981) Comparative laboratory studies on three fungal pathogens of the elm bark beetle Scolytus scolytus: effect of temperature and humidity on infection by *Beauveria bassiana, Metarhizium anisopliae,* and *Paecilomyces farinosus*. J Invertebr Pathol 37(2):195–200. https://doi.org/10.1016/0022-2011(81)90075-6

Duncan LW, McCoy CW (1996) Vertical distribution in soil persistence and efficacy against citrus root weevil (Coleoptera: Curculionidae) of two species of entomogenous nematodes (Rhabditida: Steinernematidae; Heterorhabditidae). Environ Entomol 25(1):174–178. https://doi.org/10.1093/ee/25.1.174

Eldefrawy BM, Raheem AM (2017) Insects infesting stored grain crops and their biological control using entomopathogenic nematodes. Menoufia J Plant Protect 2(3):213–222

Ertürk S, Atay T, Toprak U, Alkan M (2020) The efficacy of different surface applications of wettable powder formulation of Detech® diatomaceous earth against the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). J Stored Prod Res 89:101725. https://doi.org/10.1016/j.jspr.2020.101725

Ffrench-Constant RH, Eleftherianos I, Reynolds SE (2007) A nematode symbiont sheds light on invertebrate immunity. Trends Parasitol 23(11):514–517. https://doi.org/10.1016/j.pt.2007.08.021

Forst S, Clarke D (2002) Bacteria-nematode symbiosis. In: Gaulger R (ed) Entomopathogenic nematology. CABI Publishing, Wallingford, pp 57–77

Georgis R, Koppenhöfer AM, Lacey LA et al (2006) Success and failures in the use of parasitic nematodes for pest control. Biol Control 38:103–123. https://doi.org/10.1016/j.biocontrol.2005.11.005

Grewal PS (2002) Formulation and application technology. In: Gaugler P (ed) Entomopathogenic nematology. CABI Publishing, Wallingford, pp 265–287

Hagstrum DW, Subramanyam B (2009) Stored product insect resource. AACC International, Saint Paul, MN, USA

Hagstrum DW, Klejdysz T, Subramanyam BH, Nawrot J (2013) Atlas of stored-product insects and mites. AACC International, St. Paul, MN, USA

Hodges RJ, Buzby JC, Bennett B (2011) Postharvest losses and waste in developed and less developed countries: opportunities to improve resource use. J Agric Sci 149:37–45. https://doi.org/10.1017/S0021859610000936

Javed S, Tabassum AK, Khan S (2020) Biocontrol potential of entomopathogenic nematode species against *Tribolium confusum* (Jac.) (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae) under laboratory conditions. Egypt J Biol Pest Co 30(5):1–6. https://doi.org/10.1186/s41938-020-0206-9

Kaya HK (1990) Soil ecology. In: Gaugler R, Kaya HK (eds) Entomopathogenic nematodes in biological control, 1st edn. CRC Press, Boca Raton, pp 93–115. https://doi.org/10.1201/9781351071741

Kaya HK, Stock SP (1997) Techniques in insect nematology. In: Lacey L (ed) Manual of techniques in insect pathology. Academic Press, San Diego, pp 281–324. https://doi.org/10.1016/B978-0-12-432555-5.X5000-3

Kepenekci İ, Ertürk S, Tülek A, Atay T (2015) Efficacy of native entomopathogenic nematodes against flour beetles *Tribolium castaneum* (Herbst) and *T. confusum* (Jacquelin du Val) (Coleoptera: Tenebrionidae). 5th Entomopathogens and Microbial Control Congress, 9–11 September, Ankara, Turkey.

Kepenekci İ, Çimen H, Akın A, Hazır S (2017) First report of entomopathogenic nematodes (Nematoda) in Kyrgyzstan. In: 6th Entomopathogens and Microbial Control Congress, 14–16 September 2017, Gaziosmanpaşa University, Tokat, Turkey, pp 85

Kepenekci İ, Atay T, Çimen H, Akın A, Keleş G, Hazır S (2018) Entomopathogenic nematodes (Nematoda) in Tokat province (Turkey). In: 6th ASM International congress of agriculture and environment, 11–13 October 2018 Antalya / Turkey, Proceeding Book, pp 273–285

Laznik Ž, Tóth T, Lakatos T, Vidrih M, Trdan S (2010) The activity of three new strains of *Steinernema feltiae* against adults of *Sitophilus oryzae* under laboratory conditions. J Food Agric Environ 8(1):150–154

Lu J, Wu S (2010) Bioactivity of essential oil from *Ailanthus altissima* bark against 4 major stored-grain insects. Afr J Microbiol Res 4(3):154–157. https://doi.org/10.5897/AJMR.9000127

Majić I, Sarajlić A, Veselovac D, Dorić D, Tambolaš A, Mađar M, Ereš H, Kanižai Šarić G, Liška A (2021) Mortality of the red flour beetle (*Tribolium castaneum*) when exposed to croatian strain of entomopathogenic nematodes *Steinernema feltiae*. In: Proceedings of the 1st international electronic conference on entomology, 1–15 July 2021, MDPI: Basel, Switzerland, https://doi.org/10.3390/IECE-10361

Mason LJ, McDonough M (2012) Biology, behavior, and ecology of stored grain and legume insects. In: Hagstrum DW, Phillips TW, Cuperus G (eds) Stored product protection. Kansas State University, Manhattan, pp 7–20

Mckenzie JD, Goldman R (2005) The Student guide to MINITAB release 14 manual. Pearson Education, Boston

Moore D, Lord JC, Smith S (2000) Pathogens. In: Subramanyam Bh, Hagstrum DW (eds) Alternatives to pesticides in stored product IPM. Kluwer Academic Publishers, Dordrecht, pp 193–227

Rasmann S, Turlings TC (2008) First insights into specificity of belowground tritrophic interactions. Oikos 117(3):362–369

Rumbos CI, Athanassiou CG (2012) Insecticidal effect of six entomopathogenic nematode strains against *Lasioderma sericorne* (F.) (Coleoptera: Anobiidae) and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). J Stored Prod Res 50:21–26. https://doi.org/10.1016/j.jspr.2012.02.003

Rumbos CI, Athanassiou CG (2017) The use of entomopathogenic nematodes in the control of stored-product insects. J Pest Sci 90:39–49

Shapiro DI, Lewis EE (1999) Comparison of entomopathogenic nematode infectivity from infected hosts versus aqueous suspension. Environ Entomol 28:907–911. https://doi.org/10.1093/ee/28.5.907

Shields EJ, Testa A, Miller JM, Flanders KL (1999) Field efficacy and persistence of the entomopathogenic nematodes *Heterorhabditis bacteriophora* 'Oswego' and *H. bacteriophora* 'NC' on Alfalfa snout beetle larvae (Coleoptera: Curculionidae). Environ Entomol 28(1):128–136. https://doi.org/10.1093/ee/28.1.128

Tülek A, Erturk S, Kepenekci İ, Atay T (2015) Efficacy of native entomopathogenic nematodes against the stored grain insect Pest, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) Adults. Egypt J Biol Pest Co 25(1):251–254

Vitta A, Fukruksa C, Yimthin T, Deelue K, Sarai C, Polseela R, Thanwisai A (2017) Preliminary survey of entomopathogenic nematodes in upper northern Thailand. Southeast Asian J Trop Med Public Health 48(1):18–26

Warton DI, Hui FKC (2011) The arcsine is asinine: the analysis of proportions in ecology. Ecology 92:3–10. https://doi.org/10.1890/10-0340.1

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

Wójcik WF (1986) Influence of the size of host on the growth of the *Neoplectana carpocapsae* Weiser, 1955 nematodes. Annals of Warsaw Agricultural University SGGW-AR Animal. Science 20:75–85

Woodring JL, Kaya HK (1988) Steinernematid and Heterorhabditid nematodes: a handbook of biology and techniques. Southern cooperative series bulletin 331, Arkansas Agricultural Experimental station Fayetteville, Arkansas, pp 30

Yağci M, Yücel C, Erdoğuş FD, Akbulut FM (2021a) Determination of efficiency of entomopathogenic nematode *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) on the potato tuber moth (*Phthorimaea operculella* (Zeller)) (Lepidoptera: Gelechiidae) under controlled conditions. Anadolu J Aegean Agric Res Institute 31(2):170–174. https://doi.org/10.18615/anadolu.1030016

Yağci M, AkdenizFırat T, Erdoğuş FD, Şahin M (2021b) Virulence of four entomopathogenic nematode against different stages of the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). Egypt J Biol Pest Co 31(1):1–5. https://doi.org/10.1186/s41938-021-00472-9

Yağci M, Özdem A, Erdoğuş FD, Ayan E (2021c) Efficiency of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) on the codling moth (*Cydia pomonella* L.) (Lepidoptera: Tortricidae) under controlled conditions. Egypt J Biol Pest Co 31(1):1–5. https://doi.org/10.1186/s41938-021-00399-1

Yuksel E, Canhilal R, Imren M (2019) Potential of four Turkish isolates of entomopathogenic nematodes against three major stored products insect pests. J Stored Prod Res 83:317–321. https://doi.org/10.1016/j.jspr.2019.08.003

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice Hall, Upper Saddle River