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Insecticidal effect of entomopathogenic nematodes and the cell-free supernatants from their symbiotic bacteria against different larval instars of *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae)

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Abstract

Background: Entomopathogenic nematodes (EPNs) (Steinernematidae and Heterorhabditidae) and their symbiotic bacteria are pathogenic for a wide range of insect pests and have been used successfully as a biological control agent. Although EPNs are well studied against many agricultural insect pests, the efficacy of their symbiotic bacteria still remains unclear for many insect pests of agricultural importance. In the present study, the virulence of native EPN isolates and their cell-free supernatants of symbiotic bacteria were tested against the 3rd and 4th larval instars of *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae) under laboratory conditions (25 ± 1 °C and R.H. 60%).

Results: The 4th instar larvae were more susceptible to infective juveniles (IJs) and mortalities over (95%) were achieved by all tested EPN isolates at the concentration of 100 IJs/cm^2 after 72 hrs of exposure. The cell-free supernatants were more effective against the 3rd instar larvae and the highest mortalities were recorded as 42 and 60% in the contact and leaf disc bioassays, respectively.

Conclusion: The results indicated that the cell-free supernatants can be an ideal application for young larval stages of *A. segetum*. However, further studies are required to test the effectiveness of both EPNs and the cell-free supernatants of their symbiotic bacteria in field conditions.

Keywords: Biological control, *Steinernema feltiae*, *Heterorhabditis bacteriophora*, *Xenorhabdus* sp., *Photorhabdus* sp., *Agrotis segetum*

Background

The turnip moth, *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae) is a polyphagous pest occurring throughout Europe, Asia, and Africa (EPPO 2021). The larvae of *A. segetum* attack a variety of economically important crops including corn, potato, and sugar beet (Chandel et al. 2019). Starting from the 3rd instar *A. segetum* larvae, they generally feed at night or during overcast days and stay hidden under the ground during daylight hours (Capinera 2001). The monitoring of *A. segetum* larvae is challenging due to their nocturnal feeding habits and the presence of larvae is often detected when the seedlings of the plants are already severely damaged (Chandel et al. 2019).

Chemical insecticides used against this pest generally failed to satisfy the expectation of growers because of the soil-dwelling habits of the larvae (Chandel et al. 2008).

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In addition, indiscriminate use of insecticides leads to various environmental problems as well as development of resistance and human health problems (Ansari et al. 2014). Therefore, recently, many researchers are in search of finding environmentally-friendly control methods for the management of the larvae of *A. segetum* (Goudarzi et al. 2015).

Among the various entomopathogenic organisms, entomopathogenic nematodes (EPNs) (Steinernematidae and Heterorhabditidae) hold particular importance for the control of soil-dwelling insect pests (Hazır et al. 2004). The Infective juveniles (IJs) of EPNs are soil-adapted and obligate parasites of insects (Kaya and Gaugler 1993). Once IJs locate a possible host in the soil environment, they gravitate to their target and penetrate the host hemolymph through natural openings such as the mouth and anus (Lewis et al. 2006). Then, IJs release their symbiotic bacteria (*Xenorhabdus* spp. in *Steinernema* spp., *Photorhabdus* spp. in *Heterorhabditis* spp.) into the host hemolymph which serves as a nutrition source for both symbiotic bacteria and IJs (Boemare et al. 1996). During the multiplication of the symbiotic bacteria in the host hemolymph, they secrete a wide range of toxins, killer proteins and antimicrobial compounds that lead to the death of the host and the inhibition of the growth of other microorganisms (Vicente-Díez et al. 2021). The death of the host generally occurs 48–72 h after infection due to the joint effects of bacterial multiplication and excretion/secretion products of infective juveniles (Shapiro-Ilan et al. 2017). After a few generations with the depletion of the food resources, the IJs emerge from the cadaver of the host into the soil environment to search for a new potential target (Adams et al. 2006).

Recently, the cell-free supernatants of *Xenorhabdus* sp. and *Photorhabdus* sp. bacteria have aroused the interest of many researchers due to their biocontrol potential against a variety of agriculturally important pests (Cevizci et al. 2020). These studies revealed that the cell-free supernatants of *Xenorhabdus* sp. and *Photorhabdus* bacteria were able to induce substantial mortality against the tested pests. Earlier studies focused on the efficacy of different EPN species on the larvae of *A. segetum*, however, no study has been conducted about the effect of the cell-free supernatants of *Xenorhabdus* sp. and *Photorhabdus* sp. bacteria against *A. segetum* (Goudarzi et al. 2015). The pathogenicity of EPN species/isolates and their symbiotic bacteria showed a great variation depending on many factors such as; foraging strategies and adaptation capabilities of IJs, environmental extremes, and secondary metabolites produced by their symbiotic bacteria (Vicente-Díez et al. 2021). Therefore, this study was conducted to determine the efficacy of local EPN species/isolates and their cell-free supernatants of symbiotic bacteria against different larval instars of *A. segetum.*

Methods

Source of entomopathogenic nematodes and insects

Three EPN strains previously recovered from the Cappadocia Region of Turkey were used in this study (Table 1) (Yuksel and Canhilal 2019). The EPNs were reared in vivo on the last instar larvae of *Galleria mellonella* (L.) Lepidoptera: Pyralidae) under laboratory conditions (25 ± 2 °C, 60% RH) and stored in 50-ml distilled water at 9 °C. The larvae of *G. mellonella* were obtained from the Entomology laboratory of Erciyes University and cultured in glass jars (1 L) at 28 ± 2 °C, 60% RH, with an artificial diet described by Metwally et al. (2012)

To obtain the late larval instars of *A. segetum*, corn and sugar beet fields infested with *A. segetum* were searched in the Anatolia region of Turkey during May and June of 2021 by digging around the roots of the plants. The collected larvae were placed individually into plastic containers (63 × 80 mm, 180 ml capacity) with a surface area of 27 cm^2 to avoid cannibalism and brought to the Entomology laboratory of Erciyes University. Pesticide-free fresh lettuce leaves and boiled beans were provided daily for the larvae as food. The larvae were observed until the emergence of the adults under controlled conditions (25 ± 2 °C, 60% RH), and the healthy adults were selected to establish a new laboratory culture. The adults were placed into insect rearing cages (60 × 60 × 60 cm)

Table 1 Entomopathogenic nematode (EPN) and symbiotic bacterial species used in the pathogenicity bioassays against the different larval instars of *Agrotis segetum*

EPN Species	Strain	Location	GenBank Accession Number	Symbiotic Bacteria	GenBank Accession Number
Steinernema feltiae	ÜTP-5	38°30′8 N 34°56′5 E	MG602331	*Xenorhabdus bovienii*	MZ688375
S. feltiae	MÇB-8	38°40′40 N 34°29′5 E	MG602334	*Xenorhabdus bovienii*	MZ688377
Heterorhabditis bacteriophora	AVB-15	8°43′43 N 34°52′1 E	MG602341	*Photorhabdus luminescens* subsp. *kayaii*	MZ688380

in small groups (each consisting of 10 male and female adults) and a cotton pad soaked in 10% honey solution was added to each cage to promote the egg-laying. Several blotting papers were placed in each cage for the adults to cling to and lay eggs. The eggs on blotting papers were collected using a camel hairbrush and put into Petri dishes in groups of 50. Petri dishes were incubated at 25 ± 2 °C, 60% RH, and hatching 1st instar larvae were transferred individually to plastic containers. The larvae were fed on lettuce during their development. The 3rd and 4th larval instars were determined based on their head capsule size and larval body length measurements (Manjula and Kotikal 2018). The larval and adult specimens of *A. segetum* were shipped to an entomologist, Professor Dr. Halil KÜTÜK (Abant Izzet Baysal University) to confirm the identification.

Isolation of symbiotic bacteria and preparation of cell-free supernatants

To isolate the symbiotic bacteria of the EPN strains, newly harvested IJs were surface sterilized with 10% w/v sodium hypochlorite for 5 min and rinsed with sterile water several times. Nearly 500 IJs in 100 µl of distilled water were mashed thoroughly using a mini hand-held homogenizer in 1 ml of sterile PBS buffer without Mg^{2+} and Ca^{2+} salts. 10 µl of this suspension was seeded onto nutrient bromothymol blue triphenyl tetrazolium chloride agar (NBTA medium) consisting of 37 g nutrient agar, 0.025 g bromothymol blue and 0.004 g triphenyl tetrazolium chloride in 100 ml distilled water (Boemare and Akhurst 2006). Then, Petri dishes were sealed with a parafilm and incubated for 24 h under controlled conditions (28 °C, 20% RH, in the dark). The blue-colored bacterial colonies were sub-cultured on NBTA medium until pure colonies were obtained (Lacey 1997). A single bacterial colony was selected for each Petri dish and inoculated into 100 ml Luria–Bertani (LB) broth in a 250-ml Erlenmeyer flask. Subsequently, the flasks were put into a shaking incubator at 150 rpm for 144 h (28 °C, 20% RH in the dark) (Eroglu et al. 2019). To extract the cell-free supernatant, the bacterial culture in the broth suspension was centrifuged at 20,000 rpm for 15 min at 4 °C in 50 ml Falcon tubes. The centrifuged supernatant solution was separated from the bacterial cells by passing through a 0.22 µm millipore filter. The filtrated solution was checked for the presence of bacterial cells by streaking onto NBTA agar (Hazir et al. 2016).

Pathogenicity Bioassays of Entomopathogenic nematodes

The pathogenicity screening studies were evaluated against 3rd and 4th larval instars of *A. segetum* in plastic containers (Ø 63 × 80 mm) including approximately 20 g of autoclaved sterilized soil (application surface area 27 cm^2). The moisture content of the soil was adjusted to 10% (w/w) by spraying distilled water before the inoculation of the IJs. The IJs of the EPN strains were inoculated to plastic containers at the concentrations of 25, 50, and 100 IJs/cm^2 in 1 ml of distilled water (corresponding to 675, 1350, and 2700 IJs per plastic containers or larva, respectively). The 3rd and 4th larval instars of *A. segetum* were placed individually into each plastic container. To feed the larvae, a piece of lettuce (approximately 2 cm^2) was provided daily. Subsequently, the containers were sealed with a perforated lid to allow airflow and maintained under controlled conditions [25 ± 1 °C, 60% RH, and 16:8 h of L/D) for 3 days. In the control treatments, only distilled water was applied to plastic containers. There were 10 larvae in each replication and each concentration of EPN strains were tested against 40 larvae (10 larvae × 4 replicates). The mortalities of the larvae were checked and recorded daily. To confirm nematode infection, the cadavers of the dead larvae were collected and dissected under a stereomicroscope.

Pathogenicity bioassays of cell-free supernatants

The contact and leaf disc bioassays were conducted in Petri dishes (60 mm diameter) lined with a filter paper disc to evaluate the insecticidal efficacy of cell-free supernatants. In the contact efficacy bioassay, the 3rd and 4th larval instars of *A. segetum* were transferred individually to each Petri dish. Then, 500 µl of cell-free supernatant were sprayed to the larvae and a piece of lettuce (approximately 2 cm^2) was added to each Petri dish. In the leaf disc bioassay, 500 µl of cell-free supernatant were sprayed to both upper and lower surfaces of lettuce (approximately 2 cm^2) and placed into Petri dishes containing only one 3rd or 4th larval instars. The Petri dishes were maintained at 25 ± 1 °C, 60% RH, and 16:8 h of L/D after sealing with parafilm. In control groups, Petri dishes were treated with only Nutrient Broth. The mortalities of the larvae were recorded daily for 3 days. Each treatment consisted of 10 Petri dishes and all the bioassays (EPNs, contact, and leaf disc) were carried out twice with 4 replicates. Only one-week-old supernatants and IJs were used in the bioassays, and they were kept at 9 °C until their use in the bioassays.

Statistical analyses

Prior to analyses, the data were arcsine-transformed to stabilize the variance of means and analyzed using IBM SPSS statistics version 20.0 for Windows (SPSS Inc., Chicago, IL, USA) statistical software package. Significant differences between treatments were determined by factorial repeated measures ANOVA using a General Linear Model. The mean differences were carried out using Tukey's multiple range tests ($P \leq 0.05$). Since no mortality

occurred in control treatments, all the data from the two repeats were pooled for each experiment.

Results

Efficacy of entomopathogenic nematodes

The 3rd and 4th larval instars of *A. segetum* were tested for their susceptibility to native EPN isolates under laboratory conditions. All the isolates were able to effectively infect both larval instars. Mortality rate of the 3rd and 4th larval instars of *A. segetum* was affected by all main factors (Table 2). In general, increasing concentrations of IJs and exposure time led to high mortalities on both larval instars. *Steinernema feltiae* UTP-5 isolate was generally was more virulent to the 3rd instar larvae of *A. segetum* and caused the highest mortality (82%) at the 100 IJs/cm^2 72 h post-inoculation. The mortality rates in the 4th instar larvae were remarkably high at 100 IJs/cm^2 concentration 72 h post-inoculation compared to the 3rd instar larvae and mortalities over 90% were achieved by all EPN isolates during the same period (Table 3).

Efficacy of cell-free supernatants from the symbiotic bacteria

Contact efficacy

Application of cell-free supernatants from the symbiotic bacteria of different EPNs resulted in varying larval mortalities. The mortality rates of the 3rd and 4th larval instars were significantly affected by the larval stage, exposure time, and their associated interactions (Table 4).

There was non-significant difference in the mortality rates of the 3rd and 4th larval instars among the cell-free supernatants of different EPN isolates (Table 4). The 3rd instar larvae of *A. segetum* was more susceptible to the cell-free supernatants and the highest mortalities were 42.5 and 15% for the 3rd and 4th larval instars of *A. segetum*, respectively (Table 5).

Mortality rates increased notably with increasing exposure time. The 3rd instar larvae of *A. segetum* exhibited higher larval mortality than the 4th instar larvae and the highest mortalities were recorded as 60 and 32% for the 3rd and 4th larval instars, respectively (Tables 6 and 7).

Table 2 ANOVA parameters for the main effects and associated interactions for the mortality levels of 3rd and 4th instar larvae of *Agrotis segetum*

Sources	df	*F*-value	*P*-value
Nematode species (N)	2	5.392	**0.007**
Concentration (C)	2	240.486	**< 0.001**
Larval stage (L)	1	221.405	**< 0.001**
N*C	4	0.993	0.419
N*L	2	38.932	**< 0.001**
C*L	2	3.622	**0.033**
N*C*L	4	0.845	0.503
Error 1	54		
Exposure time (t)	2	2661.161	**< 0.001**
t*N	4	15.214	**< 0.001**
t*C	4	25.661	**< 0.001**
t*L	2	53.375	**< 0.001**
t*N*C	8	1.085	0.379
t*N*L	4	14.911	**< 0.001**
t*C*L	4	4.089	**0.004**
t*N*C*L	8	1.665	0.115
Error 2	108		

Bold numbers indicate statistical significance at level alpha = 0.05

Table 3 The efficacy of infective juveniles of different entomopathogenic nematodes against 3rd and 4th instar *Agrotis segetum* larvae at the 1st, 2nd, and 3rd day after treatment (DAT) under laboratory conditions (25 ± 1 °C, R.H. 60%)

Stage	Concentrations	Time (hrs)	EPNs*		
			ÜTP-5	MÇB-8	AVB-15
L3	10 IJs/cm^2	24	2.5A[a]a[b]*A*[c]	2.5Aa*A*	2.5Aa*A*
		48	30.0Ba*A*	17.5Bb*A*	20.0Bb*A*
		72	62.5Ca*A*	40.0Cb*A*	45.0Cb*A*
	50 IJs/cm^2	24	7.5Aa*A*	5.0Aa*A*	5.0Aa*A*
		48	37.5Ba*A*	27.5Ba*A*	32.5Ba*AB*
		72	77.5Ca*B*	52.5Cb*AB*	52.5Cb*A*
	100 IJs/cm^2	24	12.5Aa*A*	10.0Aa*A*	10.0Aa*A*
		48	50.0Ba*B*	52.5Ba*B*	52.5Ba*B*
		72	82.5Ca*B*	62.5Cb*B*	60.0Cb*A*
L4	10 IJs/cm^2	24	5.0Aa*A*	2.5Aa*A*	2.5Aa*A*
		48	25.0Ba*A*	32.5Bab*A*	45.0Bb*A*
		72	62.5Ca*A*	65.0Ca*A*	67.5Ca*A*
	50 IJs/cm^2	24	12.5Aa*A*	7.5Aa*A*	10.0Aa*A*
		48	37.5Ba*A*	52.5Bb*B*	57.5Bb*B*
		72	75.0Ca*A*	77.5Ca*A*	82.5Ca*B*
	100 IJs/cm^2	24	15.0Aa*A*	12.5Aa*A*	15.0Aa*A*
		48	55.0Ba*B*	70.0Bb*C*	62.5Bab*B*
		72	95.0Ca*B*	95.0Ca*B*	97.5Ca*B*

ÜTP-5 Steinernema feltiae ÜTP-5 strain; *MÇB-8 Steinernema feltiae* MÇB-8 strain; *AVB-15 Heterorhabditis bacteriophora* AVB-15 strain; *L3* Third larval instar of *Agrotis segetum; L4* Fourth larval instar of *A. segetum*

*EPNs Entomopathogenic nematodes

[a] Different capital letters show statistically significant differences among exposure times for each concentration (Tukey, $P \leq 0.05$)

[b] Different lowercase letters show statistically significant differences among nematode strains for each exposure time (Tukey, $P \leq 0.05$)

[c] Different italic capital letters show statistically significant differences among concentrations for the same nematode strain and exposure time (Tukey, $P \leq 0.05$)

Table 4 ANOVA results for the main effects and associated interactions for the mortality levels of 3rd and 4th larval instars of *Agrotis segetum* for the contact efficacy of cell-free supernatants

Source	df	*F*	*P*
Supernatants (S)	2	1.729	0.206
Larval stage (L)	1	188.082	**< 0.001**
S*L	2	1.518	0.246
Error 1	18		
Exposure time (t)	2	108.457	**< 0.001**
t*S	4	0.163	0.956
t*L	2	17.674	**< 0.001**
t*S*L	4	1.337	0.275
Error 2	36		

Bold numbers indicate statistical significance at level alpha = 0.05

Table 5 The effect of cell-free supernatants from the symbiotic bacteria of different entomopathogenic on the mortality (%) of different larval instars of *Agrotis segetum* for 1, 2 and 3-day post application under laboratory conditions (25 ± 1 °C, R.H. 60%)

Stage	Time (hrs)	Cell-free supernatant*		
		ÜTP-5	MÇB-8	AVB-15
L3	24	7.5A[a]a[b]	15.0Aa	17.5Aa
	48	25.0Ba	25.0Aa	27.5Aa
	72	37.5Ba	42.5Ba	42.5Ba
L4	24	2.5Aa	0.0Ab	0.0Ab
	48	5.0Ab	5.0Ab	7.5Bb
	72	12.5Ab	10.0Ab	15.0Bb

*ÜTP-5: *Xenorhabdus bovienii*, MÇB-8: *X. bovienii*, AVB-15: *Photorhabdus luminescens* subsp. *kayaii*

*L3: Third larval instar of *Agrotis segetum;* L4: Fourth larval instar of *A. segetum*

[a] Different capital letters show statistically significant differences among exposure times for each cell-free supernatant of EPN (Tukey, $P \leq 0.05$)

[b] Different lowercase letters show statistically significant differences between 3rd and 4th larval stages for each cell-free supernatant of EPN (Tukey, $P \leq 0.05$)

Table 6 ANOVA results for the main effects and associated interactions for the mortality levels of 3rd and 4th larval instars of *Agrotis segetum* for the contact efficacy of cell-free supernatants

Source	df	*F*	*P*
Supernatant (S)	2	5.953	**0.010**
Larval stage (L)	1	168.750	**< 0.001**
S*L	2	1.828	0.189
Error 1	18		
Exposure time (t)	2	820.548	**< 0.001**
t*S	4	3.871	**0.010**
t*L	2	49.645	**< 0.001**
t*S*L	4	0.290	0.882
Error 2	36		

Bold numbers indicate statistical significance at level alpha = 0.05

Table 7 The oral effect of supernatants of symbiotic bacteria of different entomopathogenic on the mortality (%) of different larval instars of *Agrotis segetum* for 1, 2 and 3-day post application under laboratory conditions (25 ± 1 °C, R.H. 60%)

Stage	Time (hrs)	Supernatant*		
		ÜTP-5	MÇB-8	AVB-15
L3	24	2.5A[a]a[b]*A*[c]	5.0Aa*A*	5.0Aa*A*
	48	37.5Ba*A*	52.0Bb*B*	45.0Bab*A*
	72	52.5Ca	60.0Ba*A*	57.5Ba*A*
L4	24	0.0Aa*A*	0.0Aa*A*	0.0Aa*A*
	48	20.0Ba*B*	27.5Ba*A*	22.5Ba*B*
	72	32.5CaB	32.5Ba*B*	32.5Ba*B*

*ÜTP-5: *Xenorhabdus bovienii*, MÇB-8: *X. bovienii*, AVB-15: *Photorhabdus luminescens* subsp. *kayaii*

*L3: Third larval instar of *Agrotis segetum;* L4: Fourth larval instar of *A. segetum*

[a] Different capital letters show statistically significant differences among exposure times for each cell-free supernatant of EPN (Tukey, $P \leq 0.05$)

[b] Different lowercase letters show statistically significant differences among cell-free supernatant of EPN isolates for each exposure time (Tukey, $P \leq 0.05$)

[c] Different italic uppercase letters show statistically significant differences between 3rd and 4th larval stages for each cell-free supernatant of EPN (Tukey, $P \leq 0.05$)

Leaf disc bioassay

The mortality rates of the 3rd and 4th larval instars were significantly affected by all main factors and their interactions with each other (except for Supernatant*Larval Stage) and the interaction between cell-free supernatants and larval stage (S*L). Three-way interaction of the main factors (t*S*L) had non-significant effect on the mortality rates (Table 6).

Discussions

Although many studies were conducted to evaluate the pathogenicity of IJs of different EPN species/isolates against the larvae of *A. segetum*, no study has been reported about the control potential of cell-free supernatants from the symbiotic bacteria of EPNs to date (Devi 2020).

In the present study, the effectiveness of native EPNs and cell-free supernatants from their symbiotic bacteria were studied on the different larval instars of *A. segetum*. In the pathogenicity bioassays of IJs, the trend of increasing mortality with increasing IJs concentration and exposure time was observed. In the pathogenicity bioassays of IJs, the trend of increasing mortality with increasing IJs concentrations and exposure time was observed. The same trend was also reported by Yoshida (2010) against different larval instars of *A. segetum* and mortalities over 70% were obtained by different EPNs species in these studies. In another study conducted by Goudarzi et al. (2015), a slight decrease in the mortality

rates was also observed at the highest concentration (200 IJs/Petri), while there was a continuous increase in the mortality rates with increasing exposure times. To a certain extent, there is a tendency for mortality rates to increase at higher concentrations of IJs under favorable conditions since high concentrations enhance the probability of target hosts getting infected by IJs and the number of IJs penetrating the host body. However, high concentrations outside of the optimum range may also influence unfavorably the survival and penetration capability of IJs (Yüksel et al. 2019). Goudarzi et al. (2015) also reported that the 5th instar larvae were more susceptible to EPNs tested. Ebssa and Koppenhöfer (2012) investigated the pathogenicity of EPN species against different larval instars of another cutworm species, *A. ipsilon* and reported that the 4th and 5th larval instars were the most susceptible development stages against EPNs tested. Host size is a significant factor in the pathogenicity process of IJs as it affects the penetration into host body and host location capability of IJs (Bastidas et al. 2014). In the present study, the 4th instar larvae of *A. segetum* was found more susceptible to IJs in parallel with the study conducted by Ebssa and Koppenhöfer (2012). However, this was not the case in the study conducted by Chandel et al. (2010). Temperature plays a key role, together with other environmental factors, in the pathogenicity and survival of IJs and the adaptation ability of different EPN species/isolates to temperature shows great variation (Hummel et al. 2002). Chandel et al. (2010) carried out their study at room temperature ranging between 21 and 30 °C. This may be the reason behind the low mortalities with increasing larval instars. The fluctuation in the temperature may also have lowered the mobility of both IJs and the larval instars.

To our knowledge, this is the first study evaluating the control potential of cell-free supernatants recovered from *Xenorhabdus* and *Photorhabdus* species on different larval instars of *A. segetum*. The results showed that the lowest mortalities were obtained from the application of cell-free supernatants compared to the application of IJs of the same EPN species. These results may indicate that in the absence of a nematode vector, the efficacy of cell-free supernatants may remain limited since they naturally function in the host hemolymph (Ruiu et al. 2017). Obtained results also showed that 3rd instar larvae exhibited a higher mortality than the 4th instar larvae when exposed to cell-free supernatants. This might be explained by the higher immune ability of the 4th instar larvae (Abdolmaleki et al. 2017). In earlier studies, the insecticidal effect of cell-free supernatants of *Xenorhabdus* and *Photorhabdus* was tested against different lepidopteran pests and different levels of pathogenicity were obtained against the tested insects (Ruiu et al. 2017). Mahar et al. (2008) reported 95% larval mortality of *Spodoptera exigua* (Hübner) (Noctuidae: Lepidoptera) after 72 h of exposure to cell-free supernatants of *X. nematophila*. In another study, Adithya et al. (2020) tested the cell-free supernatants of *X. nematophila* and *P. luminescens* against the larvae of *Earias vittella* (Lepidoptera: Noctuidae) and mortality rates ranged between 65 and 70%, 72 h after treatment. Here in this study, the highest mortalities in the contact efficacy studies were 42% for the 3rd larval instars of *A. segetum*. The differences in the mortality rates can be attributed to the production of diverse bacterial toxins and secondary metabolites by different symbiotic bacteria species and strains (Eroglu et al. 2019). In the present study, although mortalities in both contact and leaf disc efficacy bioassays with cell-free supernatants were quite similar, the highest efficacies were generally obtained in case of the leaf disc efficacy bioassay. Both larval instars were more susceptible to cell-free supernatants of *Xenorhabdus* and *Photorhabdus* bacteria by oral ingestion. This may suggest that cell-free supernatant may be more efficacious on the intestine of the target host as indicated in earlier studies (da Silva et al. 2020). Many studies revealed that cell-free supernatants of *Xenorhabdus* and *Photorhabdus* have insecticidal effects on the different insect groups with varying degrees (Vicente-Díez et al. 2021). Differences in mortality rates may be associated with the symbiotic bacteria species/strains producing different amounts and types of toxin complexes (Wenski et al. 2020).

Conclusions

The results of this study demonstrated that EPNs species/isolates and their cell-free supernatants had the potential of controlling the *A. segetum* larvae. Although contact efficacy bioassays of cell-free supernatants achieved limited mortality in this study, oral digestion of cell-free supernatants showed more potential to control the 3rd instar larvae of *A. segetum*. However, further studies are required to reveal the field potential of both IJs and their cell-free supernatants on the larvae of *A. segetum*.

Abbreviations

EPNs: Entomopathogenic nematodes; IJs: Infective juveniles.

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Author contributions

EY and EÖ designed the project, performed the laboratory work, and wrote the paper with full support of Mİ, RB and RC. All authors read and accept the final manuscript.

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Availability of data and materials
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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
This study does not contain any individual person's data.

Competing interests
The authors have no competing interests.

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