*International Journal of Agriculture and Wildlife Science (IJAWS) 2021, 7(3): 429 - 437*
*doi: 10.24180/ijaws.981190*

International Journal of Agriculture and Wildlife Science

http://dergipark.org.tr/ijaws

Research Article

# Field Performance of Entomopathogenic Nematodes against the Larvae of *Zabrus* spp. Clairville, 1806 (Coleoptera: Carabidae)**

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Received: 10.08.2021 Accepted: 19.10.2021

**Keywords:**
*Heterorhabditis* sp., *Steinernema* sp., wheat, cereal ground beetle

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**Abstract.** The ground beetles, *Zabrus* spp. Clairville, 1806 (Coleoptera: Carabidae) is one of the major pests of wheat plants across the world, and the control of this pest is a challenging issue. In the present study, the control potential of two local entomopathogenic nematode (EPN) species [*Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) and *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae)] against the larvae of *Zabrus* spp. was evaluated with two trials under field conditions in 2015. EPNs were applied at the concentration of 1 x $10^6$ IJs $m^{-2}$ to the soil surface in mid-April and the number of alive larvae was assessed 14 days after treatment (AT). In addition, *Zabrus* spp.-damaged wheat plants were counted to establish the efficacy of EPNs on the larvae of *Zabrus* spp. in short (14 days AT) and long term (6 months AT). EPNs reduced the number of alive *Zabrus* spp. larvae by at least 50% as compared to the control treatments in both trials. Altgough there was a remarkable decrease in the number of *Zabrus* spp.-damaged wheat plants to which EPNs were applied, this decrease did not produce a significant effect. Present findings indicate that EPNs tested have a good potential for sustainable management of *Zabrus* spp.

# Entomopatojen Nematodların Arazi Koşullarında *Zabrus* spp. Clairville, 1806 (Coleoptera: Carabidae) Larvalarına Karşı Etkinliği

**Anahtar kelimeler:**
*Heterorhabditis* sp., *Steinernema* sp., buğday, ekin kambur böceği

**Özet.** Ekin Kambur böcekleri, *Zabrus* spp. Clairville, 1806 (Coleoptera: Carabidae) buğday bitkilerinin dünya çapında ana zararlılarından biridir ve bu zararlının kontrolü oldukça zordur. Bu çalışmada, yerel iki entomopatojen nematod (EPN) türünün [*Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) ve *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae)] *Zabrus* spp. larvalarına karşı kontrol potansiyeli 2015 yılında arazi koşullarında kurulan iki deneme ile değerlendirilmiştir. EPN'lar, nisan ayı ortasında toprak yüzeyine 1 x $10^6$ IJs $m^{-2}$ konsantrasyonunda uygulanmıştır ve uygulamadan 14 gün sonra (AT) canlı larva sayısı değerlendirilmiştir. Bunun yanısıra, EPN'lerin *Zabrus* spp. larvaları üzerindeki etkinliğini belirlemek için *Zabrus* spp.-zarar görmüş buğday bitkileri uygulamadan sonraki kısa (14 gün AT) ve uzun vadede (6 ay AT) sayılmıştır. Test edilen EPN'lerin, canlı *Zabrus* spp. larvalarının sayısını her iki denemede de kontrol uygulamasına kıyasla en az %50 oranında azalttığı belirlenmiştir. EPN uygulamasıyla *Zabrus* spp.-hasarlı buğday bitkilerinin sayısında önemli bir azalma olmasına rağmen bu azalma önemli bir etki yaratmamıştır. Mevcut bulgular, test edilen EPN'lerin *Zabrus* spp.'lerin sürdürülebilir mücadelesinde iyi bir potansiyele sahip olduğunu göstermektedir.

## INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most common staple crop in the world and provides over 20% of the global food requirement (Shiferaw *et al.,* 2013; FAOSTAT, 2020). The cereal ground beetles, *Zabrus* spp. Clairv. (Coleoptera: Carabidae) are a group of the dangerous pests of wheat crops worldwide among various biotic and abiotic factors contributing to the decrease in the crop yield of wheat (Georgescu *et al.,* 2017). *Zabrus* genus has nearly 100 known species in the world and 37 of them were found in mixed groups in cereal cultivation areas of Turkey. *Zabrus tenebrioides* Goeze, *Z. spinipes* Fabr., *Z. femoratus* Dej., *Z. rotundicollis* Menetr., *Z. graecus* Dej., *Z. asiaticus* Cast., *Z. corpulentus* Schaum., *Z. iconiensis* Ganglb., and *Z. melancholicus* Schaum. are the most damaging and encountered ones among the other species in Turkey. The adults and larvae of *Zabrus* spp. can induce considerable damage on wheat plants. New generation adults start damaging the ears of wheat in early summer by climbing stems and feeding upon seeds nocturnally until harvest. The adults and larvae resume feeding after an aestivation period, which they go through during the elevated temperatures. The larvae reside in the burrows that are adjacent to wheat plants and feed nocturnally on the wheat leaves until temperatures drop to below 0°C. They overwinter in soil beneath the host plants generally as the 2$^{nd}$ or 3$^{rd}$ larval stages and continue nourishing with wheat plants in spring (Küçükkayki *et al.,* 2008; Georgescu *et al.,* 2017). In high population densities, the larval and adult stages can strip the wheat fields down to bare soil (Georgescu *et al.,* 2017). The degree of injury done by *Zabrus* spp. varies according to the climate conditions and heavy infestations occur particularly in mild weather conditions at the beginning of the winter. Studies showed that over 70% of crop losses can occur due to these infestations. The control of *Zabrus* spp. is quite challenging due to the nocturnal feeding and soil-dwelling habits of these species. Seed and surface chemical applications are the most preferred control methods, however, these methods mostly yield unsatisfactory results and increase the cost of production as the adults can survive on other germinating cereal seeds under the soil and the larval stages continue feeding on leaves by climbing stems or pulling leaves of others cereals into the soil (Lodos, 2007; Georgescu *et al.,* 2017). The inactive stages of these species such as the aestivation and overwintering period may also yield misleading results to growers about the presence of *Zabrus* spp. as the scouting/monitoring of *Zabrus* spp. is challenging. In addition, some of the currently used synthetic insecticides may soon be removed from the market due to their harmful effects on non-target organisms and promoting the evolution of resistance in target pests (Collins and Schlipalius, 2018).

Entomopathogenic nematodes (EPNs) from the genera *Steinernema* and *Heterorhabditis* are soil-born organisms and lethal endoparasites of many arthropods. The genus *Xenorhabdus* and *Photorhabdus* are symbiotic bacteria that live in the intestine of *Steinernema* and *Heterorhabditis* species, respectively. They play a critical role in the infection process of EPNs. Infective juveniles (IJs), the only life stage of EPNs that exist in the soil environment, inoculate the symbiotic bacteria of the genus *Xenorhabdus* (*Steinernema* sp.) and *Photorhabdus* (*Heterorhabditis* sp.) into the hemolymph of the host after penetration into host body through natural body openings and thin cuticle between body segments of the insects (Poinar and Grewal, 2012; Özdemir and Bayram, 2017). The bacteria use the insect hemolymph as a nutrition environment and release toxic substances as they develop and reproduce. This process generally results in the death of host insects within a short time. Finally, the new generations of IJs in large numbers leave the host cadaver and begin to search for a new potential insect host once the host cadaver is depleted (Vashisth *et al*., 2013).

EPNs can play a key role in the suppression of soil-dwelling pest populations such as *Zabrus* spp. since the soil environment is the natural habitat of both organisms. Entomopathogenic nematodes are soil adapted organisms and capable of infecting pests that live in cryptic habitats with the active host-seeking ability of IJs (Lacey and Georgis, 2012). EPNs may also provide long-term control especially for those pests in the soil environment by settling into the application area and keep the pest population naturally below the economic threshold hazard levels depending on the environmental conditions (Poinar and Grewal, 2012; Azizoglu *et al.,* 2016; Acharya *et al.,* 2019; Mokrini *et al.,* 2020). The laboratory efficacy of EPNs is an important phase of pathogenicity screening studies and is well studied by many researchers around the world (Azizoglu *et al.,* 2016; Özdemir and Evlice, 2020; Acharya *et al.,* 2020; Mokrini *et al.,* 2020). However, the field effectiveness studies of EPNs against many agricultural pests are still limited and essential to uncover the potential of EPNs as biological control agents. Soil-borne pests are the first target of many EPNs as they cohabit in the same environment. *Zabrus* spp. spend most of their life cycle underground which makes them a perfect target for EPNs. The aim of this study was conducted to assess the field efficacy of local EPNs on the larvae of *Zabrus* species and determine the most pathogenic EPN isolates.

## MATERIAL AND METHOD

### *Source and Production of Nematodes*

Field experiments were carried out with two EPN species (*Steinernema feltiae* KCS-4S and *Heterorhabditis bacteriophora* FLH-4H) recovered from the same geographical region in the earlier studies (Canhilal *et al*., 2014; 2015). The production of the IJs was carried out on the last instars of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) in a Petri dish arena lined with two filter papers at the concentrations of 100 IJs $ml^{-1}$ per larvae under controlled conditions (25±1°C and 60% R.H.). The dead larvae were gathered after a 48-96 h incubation period and placed individually onto White traps. The emerged IJs from the cadaver were harvested for up to 10 days and stored at 7°C for a week after rinsing three times with tap water. *Galleria mellonella* was obtained from the Plant Protection Department of Agricultural Faculty, Ankara University. The larvae (i.e., waxworm) of *G. mellonella* were reared in dark (32±1°C, R.H 60%) using an artificial diet including wheat flour, corn flour, milk powder, baking yeast powder, honey, and glycerin (Metwally *et al*., 2012).

### *Preparation of EPN Species for the Experiment*

The IJ stock suspensions of EPN species were kept at room temperature (20–24 °C) for half an hour to acclimatize prior to their use in the bioassays. The alive and dead IJs of EPN species were checked using a stereomicroscope. The quality of the IJs taken from backpack sprayer was evaluated before the field application by conducting a Petri dish (9 cm diameter) experiment including sterilized air-dried soil with ten *G. mellonella* larvae (Van and Malan, 2015). The IJs in tap water were applied to Petri dishes at the concentration of 1000 IJs $ml^{-1}$ using a micropipette. Then, Petri dishes were maintained for two days at 25 ± 1 °C, 60% RH. The experiment replicated 4 times. Mortality rates of *G. mellonella* larvae were recorded 2 days after treatment and compared their pathogenicity with the IJs from the stock culture kept for two weeks at 7°C (Canhilal *et al*., 2017).

### *Experimental Setup*

Field experiments were conducted in two different wheat cultivation areas (Trial 1 and 2) (Approximately 0,5 hectare) 2 km away from each other in the same geographical region, which were heavily infested with *Zabrus* species. The experiments were conducted in 2014 two years after the last insecticide treatment. In order to establish the initial population density of the larvae of *Zabrus* spp. in the experiment field, a wood frame (50x50 cm) was used. The frame was thrown randomly 8 times for each plot. Then, *Zabrus* spp.-damaged plants remaining in each frame were checked and recorded. Twenty soil samples (Approximately 1 kg) were taken randomly using a shovel from a depth of up to 25 cm from each plot of the field and examined to determine the existence of EPNs in the application area before the application of the IJs. The samples were placed in a cool box at 10-15°C and transported to the laboratory. The soil samples were slightly moisturized and placed into plastic boxes. Then, ten *G. mellonella* larvae were buried in the boxes. The boxes were covered by perforated lids and shaken up daily to facilitate the movement of the *Galleria* larvae. The viability of the larvae was checked daily after 3 days of incubation period in the dark at 25 °C for ten days. Wheat seeds (Bayraktar 2000) (Approximately 2 kg/hectare) were planted in the first week of October after treated against *Tilletia* sp. diseases (Lamardor®).

The IJs of *S. feltiae* and *H. bacteriophora* were put in a cool box at 10–15 °C and taken to the application area directly. After arrival at the application area, 5 sub-samples from the IJs of each species were taken and the number of alive IJs was checked. Before the application, the IJs were kept in a shadowy place to acclimatize to ambient temperature for half an hour. The application area was irrigated to supply an adequate amount of moisture for the IJs before the field trials were conducted (Susurluk, 2007; Salame and Glazer, 2015; Shapiro-Ilan *et al.,* 2017; Malan *et al.,* 2018).

The applications of IJs were carried out in the second week of April when the soil temperature was above 10 °C and the larvae were mobile (Table 1). The evaluations of the applications were made two weeks (Short-term efficacy) and six months (Long-term efficacy) after the applications of IJs. The assessment of the short-term efficacy of EPNs was made by counting both alive *Zabrus* spp.-larvae and *Zabrus* spp.-damaged plants in the plots while the long-term efficacy was evaluated only by counting the *Zabrus* spp.-damaged plants. The alive *Zabrus* spp. larvae were searched by digging out the soil up to 15 cm with a shovel (Glazer and Nikaido, 2007). *Zabrus* spp.-damaged plants were evaluated as the percentage of skeletonized leaves that near ground and pulled into the soil which is the indication of the presence of larvae in the field. The IJs were put into the tanks filled with tap water and mixed thoroughly. The application was made by using a backpack sprayer with a 0.5 mm nozzle diameter. The concentrations of IJs were adjusted to $1x10^6$ IJs $m^{-2}$ and were sprayed to the soil surface of the plots at the sunset of a non-windy day to avoid the damaging effects of sunlight. The suspension of tap water and IJs in the tank was stirred often with the help of a wood stick during the application to ensure a homogeneous

mixture. Only tap water equal to the amount of water applied to the nematode plots was sprayed to the surface of control plots. One meter of security line was left among the plots and only two weeks old IJs were used in the field experiments. The trials were set up with 4 replications according to randomized blocks design and each plot comprised of a 4m x 6m area.

In order to establish the persistence of IJs in the application area, ten soil samples were taken at the depth of 10–15 cm monthly from each plot for one year after the application of EPNs and processed with five *Galleria* larvae as described above. The cadavers of the dead larvae were observed for the nematode infection.

**Table 1.** The ranges of air and soil temperatures (°C) and soil moisture (%) during the short-term efficacy studies (From mid-April to early May of 2014).

*Çizelge 1. Kısa süreli etkinlik çalışmaları sırasında hava ve toprak sıcaklıkları (°C) ve toprak nemi (%) değerleri (2014 yılı Nisan ortasından Mayıs başına kadar).*

| Location and climate conditions | Trial 1 | Trial 2 |
|---|---|---|
| GPS Coordinates | 38° 57' 37'' N, 35° 04' 41'' E | 38° 58' 01" N, 35° 04' 26" E |
| Altitude (m) | 1194 | 1203 |
| Air temperature (°C) | 18.4-23.8 | 16.8-24.7 |
| Soil temperature (°C) | 10.3-14.8 | 10.8-13.7 |
| Soil moisture (%) | 22.6- 26.9 | 21.7-25.5 |

### *Data Analysis*

After the assessment of the normality of data, the needed transformation (Arcsine) (Rangaswamy, 2010) was performed to obtain normal distribution and meet the assumptions of ANOVA. The *Zabrus* spp.-damaged plants were analyzed using the repeated measures analysis of variance (RMANOVA) while the alive larvae of *Zabrus* spp. were subjected to one-way ANOVA. Tukey's multiple range test ($P \leq 0.05$) was used to analyze differences among the treatments. The SPSS-software (Version 11.0) was used to analyze all statistics.

## RESULTS AND DISCUSSION

Climate conditions were around seasonal normals during the short-term efficacy studies (Table 1). No difference was found between the pathogenicity of IJs taken from the sprayer and stock suspensions of IJs and both samples of IJs caused 100% mortality on *G. mellonella* larvae two days after treatment. The number of *Zabrus* spp.-damaged plants previous to field studies was found as 4.6 $m^{-2}$ for trial 1 and 2.1 $m^{-2}$ for trial 2.

**Table 2.** The number of alive larvae of Zabrus spp. 14 days after treatment in the field application of different entomopathogenic nematode species at the concentration of $1x10^6$ IJs $m^{-2}$.

*Çizelge 2. Saha çalışmalarında farklı entomopatojen nematod türlerinin $1x10^6$ IJs $m^{-2}$ konsantrasyonunda uygulanmasından 14 gün sonraki canlı Zabrus spp. larva sayısı.*

| Treatments | Alive larvae 14 days AT* (Mean ± St. error) | |
|---|---|---|
| | Trial 1 | Trial 2 |
| *Steinernema feltiae* | 3.3±0.3 a | 2.0±0.5a |
| *Heterorhabditis bacteriophora* | 2.3±1.1 a | 2.0±0.5a |
| Control | 7.0±1.0 b | 4.3±2.0a |

*AT: After treatment. Mean values followed by different lowercase letters in the same column are significantly different according to Tukey's test ($P \leq 0.05$).

### *Zabrus spp. Clairville, 1806 (Coleoptera: Carabidae)-Damaged Plants*

The number of damaged plants was not different among treatments and only the time periods between the short-term and long-term studies differed significantly in both trials (Table 3). The number of *Zabrus* spp.-damaged plants decreased with time and was the lowest in the plots where S. feltiae was applied in the long-term efficacy studies in both trials. However, *H. bacteriophora* yielded the least number of *Zabrus* spp.-damaged plants in the short-term efficacy. Similar results were obtained from the long-term efficacy studies in both trials although the population densities of larvae of *Zabrus* spp. were different in trials 1 and 2. The number of *Zabrus* spp.-damaged plants in the plots where EPN species were sprayed was generally lower than control plots. Although there were remarkable differences among the treatments as compared to the number of *Zabrus* spp.-damaged plants in the control plots, these differences were not significant and all treatments were clustered in the same group in both trials (Table 4).

**Table 3.** RMANOVA parameters for the main effects and associated interactions for the *Zabrus* spp.-damaged plants in Trial 1 and 2.

*Çizelge 3. Deneme 1 ve deneme 2 sahalarındaki Zabrus spp.-hasarlı bitkilere ait ana faktörler ve interaksiyonlarının (RMANOVA) ANOVA parametereleri.*

| Source* | Trial 1 | | | Trial 2 | | |
|---|---|---|---|---|---|---|
| | df | F | P | df | F | P |
| t | 1 | 106.606 | 0.000 | 1 | 9.627 | 0.021 |
| N | 1 | 2.619 | 0.152 | 1 | 1.255 | 0.350 |
| N*t | 1 | 2.704 | 0.145 | 1 | 1.386 | 0.320 |

*t: Time, N: Nematode species ($P \leq 0.05$), df: the degree of freedom, F: F-statistic, and P: Significance level.

**Table 4.** The number of *Zabrus* spp.-damaged plants after the field application of different entomopathogenic nematode species at the concentration of $1x10^6$ IJs $m^{-2}$.

*Çizelge 4. Saha çalışmalarında farklı entomopatojen nematod türlerinin $1x10^6$ IJs $m^{-2}$ konsantrasyonunda uygulandıktan 14 gün sonraki canlı Zabrus spp. larva sayısı.*

| Treatments | ***Zabrus* spp.-damaged plants (Mean ± St. error)** | |
|---|---|---|
| | **Trial 1** | |
| | Short-Term Efficacy (14 days AT*) | Long-Term Efficacy (6 months AT) |
| *Steinernema feltiae* | 10.3±1.5a | 0.6±0.5a |
| *Heterorhabditis bacteriophora* | 9.0±3.6a | 2.0±2.0a |
| Control | 17.0±5.1a | 4.6±5.5a |
| | **Trial 2** | |
| | Short-Term Efficacy (14 days AT*) | Long-Term Efficacy (6 months AT) |
| *Steinernema feltiae* | 4.6±2.3a | 0.6±0.5a |
| *Heterorhabditis bacteriophora* | 2.6±1.5a | 1.0±1.0a |
| Control | 10.6±6.5a | 3.3±2.4a |

*AT: After treatment. Mean values followed by different lowercase letters in the same column are significantly different according to Tukey's test ($P \leq 0.05$).

***The Efficacy of EPNs on the Larvae of Zabrus spp. Clairville, 1806 (Coleoptera: Carabidae)***

A significant variation in the number of larval mortality of *Zabrus* spp. was detected among treatments in Trial 1 (df:2; F:11.643; P:0.009), with the two EPN species treatments having significantly fewer *Zabrus* spp. than the nontreated control. The number of *Zabrus* spp. was not statistically different between the two EPNs species. Although no statistical differences were found in trial 2 (df:2; F:1.14; P:0.381) compared to the control treatment, the number of alive larvae in EPNs-applied plots was less than half of the number of alive larvae in the control group. The same number of alive larvae was found in trial 2 for both EPN species while *H. bacteriophora* performed better than *S. feltiae* in trial 1 (Table 2).

To date, the pathogenicity of EPN species has been studied against a variety of insect pests of agricultural importance under controlled conditions and promising results have been reported (Karabörklü *et al*., 2015; Yuksel and Canhilal, 2018; Acharya *et al.,* 2019; Mokrini *et al.,* 2020; Öğretmen *et al.,* 2020; Özdemir *et al.,* 2021). However field studies on the efficacy of EPNs against many agricultural pests are still limited and the performance of EPNs in field conditions may vary remarkably as compared to laboratory bioassays. The effectiveness of *H. bacteriophora*, *H. indica*, *S. feltiae* (local and commercial), *S. bicornortum*, and *S. carpocapsae* species on the larvae of *Zabrus* spp. was evaluated previously at the concentrations of 50, 100, and 200 IJs $cm^{-2}$ at different temperatures (15, 20, and 25°C) in a laboratory study (Canhilal *et al*., 2016; Marianelli *et al*., 2017). The results of this study showed that the rising temperatures led mortality rates to increase.

The lowest mortality rates of *Zabrus* spp. larvae at the concentration of 100 IJs $cm^{-2}$ (Corresponding to $1 \times 10^6$ IJs $m^{-2}$) were 30%, 35%, and 55% at 15°C ten days after treatment for *S. feltiae*-commercial, *S. feltiae*-local, and *H. bacteriophora*, respectively. The mortality rates of *Zabrus* spp. larvae were 50%, 37.5%, and 65% at 25°C for the same nematodes, respectively. In present study, evaluation of the EPNs was made based on the alive *Zabrus* spp.-larvae found in the plots as finding the dead cadavers of *Zabrus* spp. larvae is very difficult in field conditions. Similar efficacies were achieved in both studies despite the differences in the evaluation methods and experiment environment. In both studies, the number of alive larvae generally decreased by half after EPNs application compared to control treatments.

There are many factors influencing the effectiveness of EPNs against insect hosts in nature such as insect host species, host finding behavior of EPNs, climate and soil conditions, and application methods. The tolerance and adaptation capability of EPN species and isolates to these factors varies considerably (Shapiro-Ilan *et al.,* 2006;

Lacey *et al.,* 2015; Shapiro-Ilan *et al.,* 2015). Both studies were conducted with the same EPN isolates on the same host populations and the air temperatures during the field study were also similar to this laboratory study. These factors may have contributed to attaining similar performance of EPNs. Local EPN species are known to be more adaptive to environmental conditions where they are isolated (Bhat *et al.,* 2020; Gulzar *et al.,* 2020). The EPNs tested were recovered from the same geographical region and this may be another factor affecting the results.

In laboratory studies, the soil is generally used after autoclaved/sterilized, and by this way, biotic limitation factors of IJs such as predation and pathogens (Predatory mites and nematodes, bacteria, and fungi, etc.) are removed. In field conditions, other environmental extremes such as exposure to ultraviolet light, temperature, and lack of soil moisture suppress the activity of IJs along with these biotic factors (Baimey *et al.,* 2015). In present study, the field was irrigated with surface irrigation systems as soil moisture is crucial for both movement and survival of IJs. In addition, the application of IJs was carried out at the sunset to protect the IJs from exposure to UV and desiccation. These practices play a critical role in the survival, persistence and performance of IJs in the application area (Griffin, 2015; Gulzar *et al.,* 2020). Soil structure affects the movement of water and IJs in soil and this can lead to higher or lower mortality rates depending on the survival and host contact of IJs. In present field study, the soil structure of the field was clay-loam consisting of 45% sand, 30% silt, and 25% clay. Clay-loam soil texture was reported to be unfavorable for EPNs infection in earlier studies and this may have constrained the performance of EPNs along with other limiting factors. In long-term efficacy studies, *Zabrus* spp.-damaged plants were the lowest in the plots where *S. feltiae* applied in both trials. This result may be attributed to host-searching behavior and the symbiotic bacteria (*Xenorhabdus* sp.) of *S. feltiae*, and the temperatures which were mostly within the optimal range for *S. feltiae* during field studies. Furthermore, *S. feltiae* has been reported to be more competitive at different temperatures than *H. bacteriophora* in earlier studies (Susurluk, 2007). Contrarily, *H. bacteriophora* was more efficient in the short-term efficacy and provided better control against *Zabrus* spp. larvae. Earlier studies also stated that the IJs of *H. bacteriophora* tend to take place more uniformly in soil than IJs of *S. feltiae*. Additionally, the IJs of *H. bacteriophora* have been reported to have a cruiser foraging strategy that moves actively in search of a suitable host in the soil while the IJs of *S. feltiae* adopting an intermediate host searching strategy (Ambusher and cruiser). This could be explained by the mobility, vertical movement, and dispersal pattern of EPN species in the application area since these factors have an impact on the infection process and the depth of soil that EPNs penetrated into.

In field studies, patchy distribution of target hosts with other variables leads to variations in data and such a case could complicate the statistical analysis of the results (Abd-Elgawad, 2021). In the present study, although *Zabrus* spp.-damaged plants and the number of alive *Zabrus* spp. larvae were generally lower than the control plots, they formed a homogeneous statistical subset based on Tukey analysis. Likewise, McGraw (*et al*.,2010) experienced the same difficulty in detecting the effects of EPNs treatments against the annual bluegrass weevil, *Listronotus maculicollis* (Coleoptera: Curculionidae). In this study, proportionately similar results were observed in both trials which indicate that EPN isolates tested could exhibit similar performance under particular conditions.

All of the *Galleria* larvae processed with the soil samples collected from the field after the application of IJs were dead which indicates that IJs were able to settle in the application area at least for one year. Earlier studies illustrated that some Heterorhabditid and Steinernematid species were capable of persisting in the application area for at least three years which is in line with the present study (Dillon *et al.,* 2008). This could be explained by the presence of soil-dwelling insect hosts in the application area and the adaptation capability of EPNs.

The host immune system and physical body structure play a key role in the infection process of EPNs and can prolong the penetration and mortality rates and duration. Coleopteran species possess a hard exoskeleton that may adversely influence the penetration of EPNs and this may have played a role in the efficacy of EPNs tested (Müller *et al.,* 2008; Beckage, 2011; Noh *et al.,* 2016).

## CONCLUSION

To our knowledge, this is the first field evaluation of EPNs against the larvae of *Zabrus* spp. The tested EPNs were successful in reducing the number of larvae of *Zabrus* spp. and the number of *Zabrus* spp.-damaged plants in field conditions. Present findings indicate that tested EPNs alone could successfully be used in the biological control of the larvae of *Zabrus* spp. and provide a sustainable control on the *Zabrus* spp. larvae at least for one year after application. However, further examination of the efficacy of different EPN species and isolates is required to identify the most suitable EPN species and isolates against the larvae of *Zabrus* spp. in different locations.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## DECLARATION OF AUTHOR CONTRIBUTION

YET and RC conceived and designed research. RC and YET conducted the experiments, EY and RC analysed data and wrote the manuscript. All authors read and approved the manuscript.

## ACKNOWLEDGMENT

This study was funded by TÜBİTAK (The Scientific and Technological Research Council of Turkey) (Project No. 111O786). The funders had no role in the design of the study and collection, analysis, interpretation of data, and writing the manuscript.

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