

## Original article (Orijinal araştırma)

# Determination of the efficacy of some entomopathogenic nematodes on *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) in laboratory conditions<sup>1</sup>

Bazı entomopatojen nematodların *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae)'e etkinliklerinin laboratuvar koşullarında belirlenmesi

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## Abstract

*Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) is a polyfag pest causing losses all over the world. Entomopathogenic nematodes (EPN) have a regulatory role on insect populations in the soil ecosystem. The aim of this study was to determine the effects of commercial biopreparations of some EPNs on fifth instar larvae of *S. littoralis* at different doses and days. Biopreparations containing *Heterorhabditis bacteriophora* (Poinar, 1975) (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* (Weiser, 1955) and *Steinernema feltiae* (Filipjev, 1934) (Rhabditida: Steinernematidae) were used in the study. These biopreparations were adjusted at doses of 50, 100, 200 and 400 IJs/5ml of tap water and applied to 50 g of sterilized sandy soil in a plastic container, and one individual of *S. littoralis* larvae was released on it. The study was conducted in 2022 at 27±2°C under laboratory conditions for 5 days. According to the results of the study, the highest average mortality rates were 100 and 95% in the *S. carpocapsae* preparation application at 400 and 200 IJs doses on the 5<sup>th</sup> day, respectively. In the application of *S. feltiae* preparation, 90% mortality rate on average was observed at 200 and 400 IJs doses on the 5<sup>th</sup> day. *Heterorhabditis bacteriophora* preparation treatment showed the highest mean mortality rate of 75% at 400 IJs dose and on the 5<sup>th</sup> day. This study is an acceptable step in determining the possibilities of using EPNs in the control of *S. littoralis*.

**Keywords:** *Heterorhabditis bacteriophora*, *Spodoptera littoralis*, *Steinernema carpocapsae*, *Steinernema feltiae*

## Öz

Pamuk yaprak kurdu, *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) tüm dünyada kayıplara sebep olan polifag bir zararlıdır. Entomopatojen nematodlar (EPN), toprak ekosistemi içinde böcek popülasyonlarını düzenleyici role sahiptirler. Bu çalışmanın amacı, bazı EPN'lerin ticari biyopreparatlarının, farklı dozlarda ve günlerde *S. littoralis*'in beşinci dönem larvaları üzerindeki etkilerinin belirlenmesidir. Çalışmada, *Heterorhabditis bacteriophora* (Poinar, 1975) (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* (Weiser, 1955) ve *Steinernema feltiae* (Filipjev, 1934) (Rhabditida: Steinernematidae) türlerini içeren biyopreparatlar kullanılmıştır. Bu biyopreparatlar, 50, 100, 200 ve 400 IJs/5ml su dozlarında ayarlanarak, plastik kap içerisindeki 50 g sterilize edilmiş kumlu toprağa verilmiş, üzerine beşinci dönem *S. littoralis* larvalarından birer birey bırakılmıştır. Çalışma, 2022 yılında 27±2°C'de laboratuvar koşullarında 5 gün boyunca kontrolleri yapılarak gerçekleştirilmiştir. Çalışma sonucunda en yüksek ortalama ölüm oranları *S. carpocapsae* preparatı uygulamasında, 5. günde 400 ve 200 IJs dozlarında sırası ile %100 ve 95 olarak görülmüştür. *Steinernema feltiae* preparatı uygulamasında 5. günde, 200 ve 400 IJs dozunda %90 ortalama ölüm oranı görülmüştür. *Heterorhabditis bacteriophora* preparatı uygulaması ise en yüksek ortalama ölüm oranını, %75 ile 400 IJs dozunda ve 5. günde göstermiştir. Bu çalışma, *S. littoralis* ile mücadelede EPN'lerin kullanım olanaklarının belirlenmesinde kabul edilebilir bir adımdır.

**Anahtar sözcükler:** *Heterorhabditis bacteriophora*, *Spodoptera littoralis*, *Steinernema carpocapsae*, *Steinernema feltiae*

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## Introduction

*Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) adults are grey brown with yellow stripes on the forewings. They lay eggs on the back surface of cotton leaves in packets and covered with buff-colored hairs (TAGEM, 2008). Fourth and fifth instar larvae display cannibalism behavior and the last instar larvae descend to the soil and pupate. The pest can be found in cotton from July onwards. *Spodoptera littoralis* starts to cause damage right after hatching. The newly hatched larva first eats the eggshell, then gnaws the lower surface of the leaf and eats the epidermis and leaves it like a membrane. In this case, the leaf looks like a sieve. In later stages, the larva moves to other leaves and feeds and riddles the leaves with holes.

The search for alternative methods to chemical-based insect control began in the 1960s along with the determination of the negative effects of chemical pesticides on the environment, human, and animal health (Altıkat et al., 2009). Bans, strict rules, and sanctions in chemical control have accelerated the transition to biological control and it has become a necessity to conduct agricultural control by considering agro-ecosystem and sustainable agricultural production. This requires prioritizing alternative methods to chemical control, especially cultural measures, and, if necessary, integrating them with each other (TAGEM, 2011). Biological control is the use of parasitoids, predators, pathogens, antagonists, or competitive populations to suppress the pest population, reduce its density and damage level (Waterhouse & Norris, 1987). Therefore, biological control is the basis of integrated pest control (Gaugler et al., 1997).

Nematodes are called "Entomopathogenic Nematodes (EPN)", which are natural enemies of many insect species in the soil ecosystem and have a regulatory role in insect populations. The application of these nematodes as biological control agents in certain habitats provides effective and reliable control of harmful insect species (Adams & Nguyen, 2002). Current studies have focused on 8 families in terms of their potential to be used in biological control of insect pests with nematodes (Kaya & Stock, 1997; Stock, 2005). Nematodes belonging to the families Steinernematidae and Heterorhabditidae have a wide host distribution (Liu et al., 2000).

The third juvenile (J3) stage of EPNs, which is free-living in the soil and can search for and find their host, is called the 'infective juvenile' (IJs) stage, during which the larvae can remain alive in the soil for more than one year (Burnell & Stock, 2000; Koppenhöfer et al., 2000; Susurluk & Ehlers, 2008). Nematodes do not feed and develop during this period (Kaya & Gaugler, 1993). When infective juveniles find a suitable host in the soil, they enter the haemocoel of the insect using the insect's natural openings (stigma, mouth, anus) or in some cases directly through the cuticle (only in *Heterorhabditis*) (Bedding & Molyneux, 1982; Wang & Gaugler, 1998). When the infective juveniles reach the haemolymph of the host, they release the bacteria that they carry into the haemocoel of the host. The bacteria released into the haemocoel of the insect start to multiply rapidly and cause the host to die of septicemia within 48 hours with the extracellular enzymes and toxins they secrete (Hazır et al., 2003). Nematodes belonging to the genus *Heterorhabditis* spp. are mutualistically related to *Photorhabdus* spp. and nematodes belonging to the genus *Steinernema* spp. are mutualistically related to *Xenorhabdus* spp.

Considering that 90% of insect pests in agricultural areas complete at least one biological period in the soil, the host list of EPNs is very large (Klein, 1993). The virulence of nematodes on lepidopterans varies significantly (Mbata & Shapiro-Ilan, 2005).

Within the scope of biological control of *Spodoptera littoralis*, which causes extensive damage in agricultural areas, although there is no EPN-containing biopreparation licensed for this pest in Türkiye, preparations containing *Steinernema carpocapsae* (Weiser, 1955) and *Steinernema feltiae* (Filipjev, 1934) (Rhabditida: Steinernematidae) have been recommended by some companies and *Heterorhabditis bacteriophora* (Poinar, 1975) (Rhabditida: Heterorhabditidae) was found to be highly effective in the literature. In this study, it was aimed to determine the efficacy of preparations containing these species on *S. littoralis* at different doses (50, 100, 200 and 400 IJs) under laboratory conditions of 27±2°C and 50-65% relative humidity.

## Materials and Methods

### Preparations containing Entomopathogenic nematodes

The biopreparations of *Steinernema carpocapsae* (Ekobioset®), *Steinernema feltiae* (Nematac®), *Heterorhabditis bacteriophora* (Bioteam®) obtained from Bioglobal Company (Türkiye). Entomopathogenic nematode biopreparations used in the experiment were diluted with tap water and adjusted at dose levels of 50, 100, 200 and 400 IJ/5 ml tap water.

### Mass rearing of *Spodoptera littoralis* (Boisduval, 1833) individuals

For the artificial food used for feeding *Spodoptera littoralis* larvae, tap water (800 ml), ascorbic acid (4 g), sorbic acid (1.25 g), streptomycin sulfate (1 g), wheat germ (3 g), yeast (35 g), agar (14 g) and beans (266.5 g) were used by modifying the diet formulation of Doğan et al. (2021).

*Spodoptera littoralis* larvae were mass reared with artificial diet in plastic petri dishes with blotting paper placed inside. The blotting papers were changed, and the diet was refreshed every day until pupation. Pupated *S. littoralis* individuals were transferred to a separate petri dish and the adult individuals were expected to emerge. Cotton wool soaked with 14% honey water was used as adult diet. After the first adult emergence was observed, they were allowed to lay eggs in the area formed with 2 pieces of oviposition paper shaped as a cylinder. The egg clusters laid on the paper were cut and collected every other day. Mass rearing of *S. littoralis* individuals was performed under laboratory conditions at 27±2°C and 50-65% relative humidity.

### Mass rearing of *Galleria mellonella* (L., 1758)

In mass rearing of *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) larvae, artificial diet used to include honey (20%), glycerin (20%), wheat bran (20%), maize flour (15%), soya flour (10%), milk powder (10%), yeast (5%). Mass rearing of *G. mellonella* larvae was realized in jars containing food and in incubator at 60±5% relative humidity and 29±2°C. To prevent the larvae from escaping from the culture jars, the tops of the jars were covered with blotting paper, chrome screen wire and metal clamp. After the first adult individual was seen in the jars, filter paper was placed on the jars and the adults were allowed to lay their eggs there. The filter papers on which the eggs were laid were collected and placed in another jar and the eggs were removed.

### Experimental design

The biopreparations containing 3 different species of EPN were added separately to plastic containers of 60 mm diameter, 70 mm height and 100 ml volume. The biopreparations were added separately to each container containing 50 g of sterilized sandy soil at 4 different dose levels (50, 100, 200, and 400 IJs/5 ml of tap water). For the control character, only 5 ml of tap water without nematodes was added. One individual of 5<sup>th</sup> instar *S. littoralis* larvae was placed in each container and placed in the climate chamber (27±2°C) of Ege University, Faculty of Agriculture, Department of Plant Protection in 2022. During five days, the larvae were checked daily, and the dead individuals were removed.

The experiment was set up according to the random plots experimental design with 5 replications repeated 4 times and *S. littoralis* larvae were controlled at 24, 48, 72, 96, and 120 hours. At 24, 48 and, 72 hours, the larvae that were observed to die were taken into the White traps. The individuals whose death was observed at 96 and 120 hours were examined under stereomicroscope and light microscope by disrupting the body integrity with the help of forceps and scalpel, and the presence of EPNs inside were determined. The preparation containing the relevant entomopathogenic nematode species was recorded as the cause of death of individuals in whom the presence of entomopathogenic nematodes was detected.

Re-isolation was conducted to check the activity of the observed EPNs. The activity of the EPNs transferred to the tap water in the traps that is obtained from dead *S. littoralis* larvae was checked on the last stage *G. mellonella* larvae.

### Statistical analysis

The data obtained from the experiment were analyzed using IBM SPSS (Version 25.0) statistical software. Biological activity of EPNs was calculated using Abbott (1925)'s formula and square root transformation was applied. Mean mortality rates were compared by analysis of variance (ANOVA) and groups were evaluated according to Duncan's Multiple Range Test ( $p < 0.05$ ).

## Results

The effects of the biopreparations of three different EPNs (*S. carpocapsae*, *S. feltiae* and *H. bacteriophora*) at 4 different doses ranging from 50 to 400 IJs on *S. littoralis*, which progressed from 5<sup>th</sup> instar larvae to pupate under the soil were determined at 24, 48, 72, 96, and 120 hours.

### Evaluation of the efficacy of entomopathogenic nematode bio-preparation

In all EPN-containing preparations used in the application, mortality increased in direct proportion to the dose from the third day onwards. Only in the *S. feltiae* preparation, the mortality rate at the dose of 200 and 400 IJs on day 5 was the same. In the preparation containing *H. bacteriophora* species, the highest mortality rate of 75% was reached at the highest dose and on the last controlled day. In the preparation containing *S. carpocapsae* EPN, the highest mortality rate was observed on the 5<sup>th</sup> day (100%) at a dose of 400 IJs. In *S. feltiae* treatment, the highest mortality rate was 90% at 400 IJs dose on day 4 and 5 and 200 IJs dose on day 5 (Figure 1).

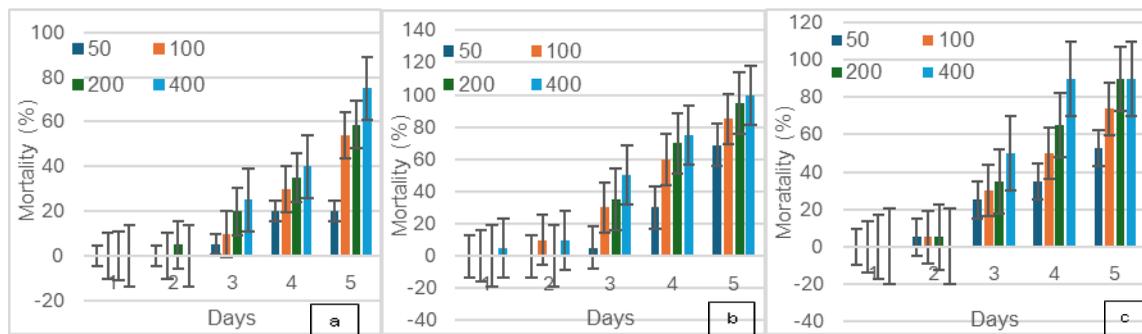


Figure 1. Mortality rates ( $X \pm SD$ ) of different species on *Spodoptera littoralis* (Boisduval, 1833) larvae on different days and doses: a) mortality rates of larvae treated with *Heterorhabditis bacteriophora* (Poinar, 1975) ( $F_{\text{dose}} = 0,610$ ;  $df_{\text{dose}} = 3$ ;  $16$ ;  $\text{sig}_{\text{dose}} = 0,618$ ) ( $F_{\text{day}} = 13,779$ ;  $df_{\text{day}} = 4$ ;  $15$ ;  $\text{sig}_{\text{day}} = 0,000$ ); b) mortality rates of larvae treated with *Steinernema carpocapsae* (Weiser, 1955) ( $F_{\text{dose}} = 1,056$ ;  $df_{\text{dose}} = 4$ ;  $15$ ;  $\text{sig}_{\text{dose}} = 0,412$ ) ( $F_{\text{day}} = 27,091$ ;  $df_{\text{day}} = 4$ ;  $15$ ;  $\text{sig}_{\text{day}} = 0,000$ ); c) mortality rates of larvae treated with *Steinernema feltiae* (Filipjev, 1934) ( $F_{\text{dose}} = 0,375$ ;  $df_{\text{dose}} = 3$ ;  $16$ ;  $\text{sig}_{\text{dose}} = 0,772$ ) ( $F_{\text{day}} = 23,144$ ;  $df_{\text{day}} = 4$ ;  $15$ ;  $\text{sig}_{\text{day}} = 0,000$ ).

### Evaluation of efficacy in terms of application doses

At the doses used in the application (50, 100, 200, and 400 IJs), the mortality rate increased in direct proportion to the dose, except for the 400 IJs dose of the biopreparation containing *S. feltiae* species from the 3<sup>rd</sup> day onwards (Figure 2).

At 50 IJs dose, the highest mortality rate was observed on the 5<sup>th</sup> day in the preparation containing *S. carpocapsae* species with 68.75%. From day 3 onwards, the mortality rates in *S. carpocapsae* and *S. feltiae* treatments increased in direct proportion and the difference between these averages was statistically significant. On day 5, the difference in mortality rates between *S. carpocapsae* and *S. feltiae* was not statistically significant (Figure 2).

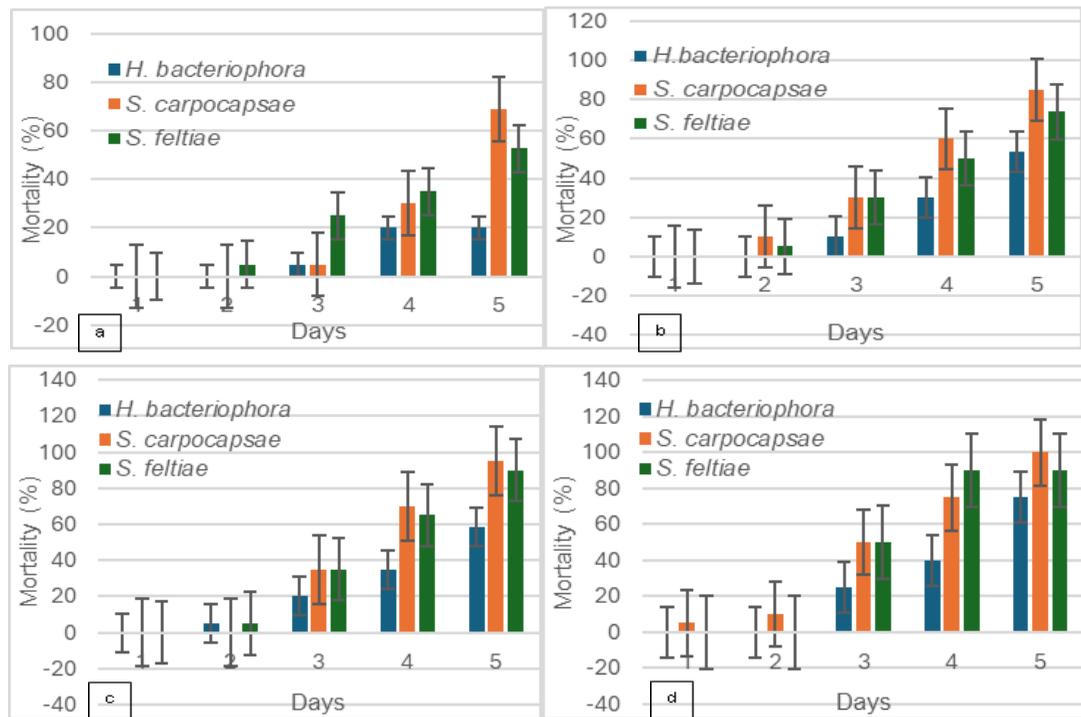


Figure 2. Mortality rates ( $X \pm SD$ ) of different doses on *Spodoptera littoralis* (Boisduval, 1833) larvae on different species and days: a) mortality rates of 50 IJs dose ( $F_{\text{species}} = 0,616$ ;  $df_{\text{species}} = 2$ ;  $12$ ;  $sig_{\text{species}} = 0,556$ ) ( $F_{\text{day}} = 7,285$ ;  $df_{\text{day}} = 4$ ;  $10$ ;  $sig_{\text{day}} = 0,005$ ); b) mortality rates of 100 IJs dose ( $F_{\text{species}} = 0,484$ ;  $df_{\text{species}} = 2$ ;  $12$ ;  $sig_{\text{species}} = 0,628$ ) ( $F_{\text{day}} = 20,503$ ;  $df_{\text{day}} = 4$ ;  $10$ ;  $sig_{\text{day}} = 0,000$ ); c) mortality rates of 200 IJs dose ( $F_{\text{species}} = 0,323$ ;  $df_{\text{species}} = 2$ ;  $12$ ;  $sig_{\text{species}} = 0,730$ ) ( $F_{\text{day}} = 22,014$ ;  $df_{\text{day}} = 4$ ;  $10$ ;  $sig_{\text{day}} = 0,000$ ); d) mortality rates of 400 IJs dose ( $F_{\text{species}} = 0,388$ ;  $df_{\text{species}} = 2$ ;  $12$ ;  $sig_{\text{species}} = 0,687$ ) ( $F_{\text{day}} = 20,930$ ;  $df_{\text{day}} = 4$ ;  $10$ ;  $sig_{\text{day}} = 0,000$ ).

At 100 IJs dose, the highest mortality rate was observed on the 5<sup>th</sup> day in the preparation containing *S. carpocapsae* species with 85%. At 100 IJs dose, the difference between the EPN treatments was not statistically significant in the first 4 days. On day 5, *S. carpocapsae* was the most effective treatment followed by *S. feltiae* (73.75%) and *H. bacteriophora* (53.75%), the difference between these species was statistically significant (Figure 2).

At 200 IJs dose, the highest mortality rate was observed on the 5<sup>th</sup> day in the preparation containing *S. carpocapsae* species with 95%. This mortality rate was followed by *S. feltiae* with 90% and statistically the same effect was observed. The difference between the average mortality rates observed from day 3 in *H. bacteriophora* and *S. carpocapsae* treatments was statistically significant, while the difference between the mortality rates between day 4 and day 5 in *S. feltiae* treatment was not significant (Figure 2).

At 400 IJs, the highest mortality rate of 100% was observed on day 5 in the preparation containing *S. carpocapsae* species. While the difference in mortality rates after the 2<sup>nd</sup> day was statistically significant in *H. bacteriophora* and *S. carpocapsae* treatments, there was no difference in the mortality rate of *S. feltiae* since no mortality was observed after the 4<sup>th</sup> day (Figure 2).

### Evaluation of activities in terms of days

In the first 2 days of the application, no mortality in any preparation and at any dose was statistically significant. On the 3<sup>rd</sup> day of EPN application, *S. carpocapsae* and *S. feltiae* treatments at a dose of 400 IJs killed half of the population of *S. littoralis* individuals with a mortality rate of 50%. At 50 IJs dose, except for the mortality rate in *S. feltiae*, the other doses (100, 200, and 400 IJs) were statistically the same for all species (Figure 3).

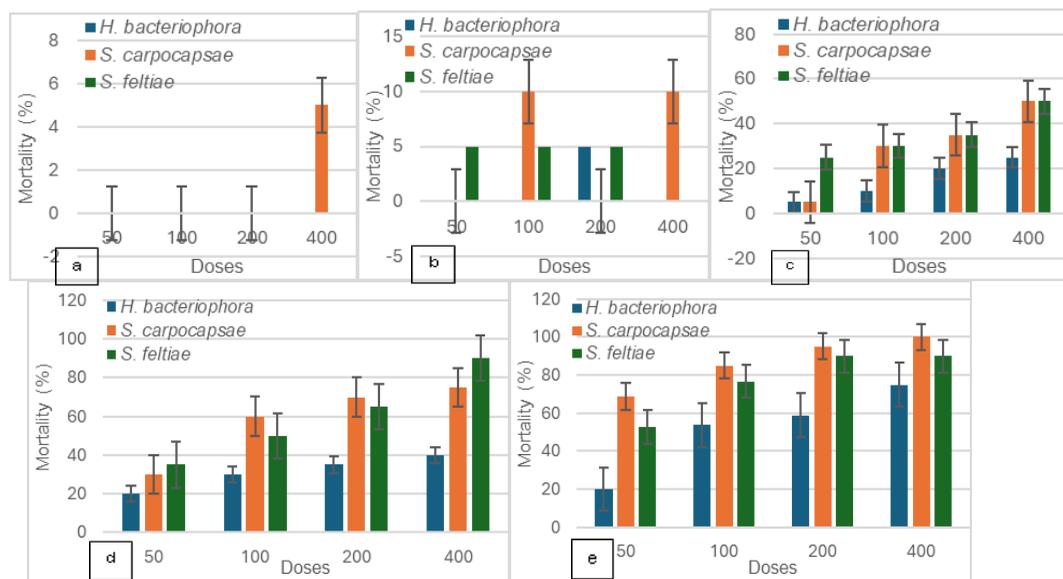


Figure 3. Mortality rates ( $X \pm SD$ ) of different days on *Spodoptera littoralis* (Boisduval, 1833) larvae on different species and doses: a) at the end of the first day ( $F_{\text{species}} = 1,000$ ;  $df_{\text{species}} = 2: 9$ ;  $sig_{\text{species}} = 0,405$ ) ( $F_{\text{dose}} = 1,000$ ;  $df_{\text{dose}} = 3: 8$ ;  $sig_{\text{dose}} = 0,441$ ); b) at the end of the second day ( $F_{\text{species}} = 0,955$ ;  $df_{\text{species}} = 2: 9$ ;  $sig_{\text{species}} = 0,421$ ) ( $F_{\text{dose}} = 0,296$ ;  $df_{\text{dose}} = 3: 8$ ;  $sig_{\text{dose}} = 0,827$ ); c) at the end of the third day ( $F_{\text{species}} = 2,364$ ;  $df_{\text{species}} = 2: 9$ ;  $sig_{\text{species}} = 0,150$ ) ( $F_{\text{dose}} = 3,434$ ;  $df_{\text{dose}} = 3: 8$ ;  $sig_{\text{dose}} = 0,072$ ); d) at the end of the fourth day ( $F_{\text{species}} = 3,079$ ;  $df_{\text{species}} = 2: 9$ ;  $sig_{\text{species}} = 0,096$ ) ( $F_{\text{dose}} = 2,633$ ;  $df_{\text{dose}} = 3: 8$ ;  $sig_{\text{dose}} = 0,122$ ); e) at the end of the fifth day ( $F_{\text{species}} = 3,862$ ;  $df_{\text{species}} = 2: 9$ ;  $sig_{\text{species}} = 0,062$ ) ( $F_{\text{dose}} = 2,722$ ;  $df_{\text{dose}} = 3: 8$ ;  $sig_{\text{dose}} = 0,114$ ).

On the 4<sup>th</sup> day of EPN application, the highest mortality rate belonged to *S. feltiae* treatment with 90% at the highest dose used (400 IJs). *S. carpocapsae* was the second most effective treatment with 75% mortality rate, but these two preparations had statistically the same average mortality rate (Figure 3).

On the last day of the experiment, *S. carpocapsae* had the two highest mortality rates with 100% at 400 IJs dose and 95% at 200 IJs dose. The mortality rates at 100 and 400 IJs are statistically different between the preparations (Figure 3).

## Discussion

Cotton leafworm, *S. littoralis*, which can produce 6-7 progeny per year in regions where climatic conditions are suitable for its biology, can cause up to 100% losses when this species is not controlled (Aydın, 2002; Ünlü & Kornoşor, 2003; Hadim & Gürkan, 2007; TAGEM, 2008; Yıldırım & Başpınar, 2008).

With the restriction of pesticides commonly used for chemical control, new control methods are being encouraged and emphasized (Lu et al., 2012; Barzman et al., 2015). Biological control in agriculture is one of the alternatives to chemical control (Stern et al., 1959; Waage & Greathead, 1988; Baker et al., 2020). Entomopathogenic nematodes are among the most important organisms successfully used in biological control (Ehlers, 1996).

The virulence of EPN on lepidopterans varies significantly (Mbata & Shapiro-Ilan, 2005). Studies with EPNs have concentrated on the families Steinernematidae and Heterorhabditidae. Kaya & Gaugler (1993) emphasized the need for more in-depth basic information on the biology of EPNs, including ecology, behavior, and genetics, to help understand the reasons underlying their success and failure as biological control agents. Selection of the most appropriate nematode species and/or strain is important in terms of efficacy and abiotic factors such as soil type, soil temperature and humidity. Appropriate matching of the nematode to the host requires virulence, host finding and ecological factors to be essential before application to the field.

Campos-Herrera et al. (2009) infected *S. littoralis* with *S. feltiae* and *S. carpocapsae* and observed that the mortality rate of *S. littoralis* infected with *S. feltiae* varied between 74-100%, while the mortality rate of individuals infected with *S. carpocapsae* was 71-82%. These studies were deemed worthy of further research on this subject.

Atwa & Hassan (2014) observed the activity of *H. bacteriophora* and *Steinernema glaseri* (Steiner, 1929) (Rhabditida: Steinernematidae) entomopathogenic nematodes on *S. littoralis* and *Temnorhynchus baal* (Reiche & Saulcy, 1856) (Coleoptera: Scarabaeidae) in 50, 100, 200 and 400 IJ doses on filter paper and 50 g soil. In 50 g of soil, *H. bacteriophora* nematode showed a mortality rate of 80-100% and *S. glaseri* nematode showed a mortality rate of 17-92% on *S. littoralis* and it was stated that the infection of EPNs may very highly depending on the experimental conditions.

Acharya et al. (2020a) applied entomopathogenic nematodes at a dose of 250 IJs to all larval stages of the species *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) and the 5th and 6th instar larvae of *S. frugiperda* were 100% killed by *Heterorhabditis indica* (Poinar, Karanukar and David, 1992) (Rhabditida: Heterorhabditidae), *Steinernema arenarium* (Artyukhovsky, 1967), *S. carpocapsae* and *Steinernema longicaudum* (Shen & Wang, 1992) (Rhabditida: Steinernematidae) killed 100% of the entomopathogenic nematodes. As a similar result to current study, in this study, *H. bacteriophora* species lagged behind the activities of other species with an average mortality rate of 53%.

Acharya et al. (2020b) conducted a similar study on *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae) with *H. indica*, *H. bacteriophora*, *S. carpocapsae* and *S. longicaudum* testing the activity of entomopathogenic nematodes and found a similar result to the results of current study, with *S. carpocapsae* having an average mortality rate of 100% and *H. bacteriophora* having the lowest mortality rate of 67%.

Yağcı et al. (2022) used *H. bacteriophora*, *S. carpocapsae* and *S. feltiae* species on the late larvae of *S. littoralis* at 4 different doses (0, 250, 500, and 1000 IJs/ml). The mortality rates of larvae were calculated at 48, 72, and 96 hours. The highest mortality rates of fifth instar larvae were found to be caused by *S. carpocapsae* (Tokat-Bakışlı 05) with 100% and *S. feltiae* (Tokat-Emir) with 92.12%, in parallel with our study, while *H. bacteriophora* (11KG) and *H. bacteriophora* (TOK-20) were found to have 87.62 and 49.28% mortality rates, respectively.

In another similar study, Nouh (2022) applied *H. bacteriophora*, *S. glaseri* and *S. carpocapsae* species at doses of 15, 30, 60, 120, and 240 IJs to the 3<sup>rd</sup> and 5<sup>th</sup> larval stages of *S. littoralis* and according to the results obtained at the end of the 6th day, the highest mortality rate was reached by *H. bacteriophora* species at 240 IJs dose with 100% in the 5<sup>th</sup> larval stage, followed by *S. carpocapsae* with 94% mortality rate. Unlike the findings of this study, the results of our study have indicated that at a dose of 200 IJs and at the end of 5 days, *H. bacteriophora* species displayed a mortality rate of 60% while *S. carpocapsae* species showed a mortality rate of 95%.

The results of this study clearly show that 5<sup>th</sup> instar larvae of *S. littoralis* are highly susceptible to the tested EPNs and that these beneficial organisms can be used as effective biological control agents against *S. littoralis*. All three EPN preparations tested showed different levels of efficacy against *S. littoralis*. In this study, the infectivity and mortality rate of *H. bacteriophora* species entomopathogen nematode was lower than the mortality rate of other EPNs used in this study, which may be due to the fact that symbiotic bacteria (*Xenorhabdus* spp.) may be due to the fact that symbiotic bacteria (*Photorhabdus* spp.) in Heterorhabditidae species EPNs settle in the host more successfully than symbiotic bacteria (*Photorhabdus* spp.) (Atwa & Hassan, 2014). Due to the difference in the host-finding behavior of *H. bacteriophora* in the application of EPNs in the present study, it is thought that the efficacy success may have been lower than other species.

In the current study, differences in mortality rates were observed between the species, and all of the EPNs which were used in the applications under laboratory conditions were highly effective on *S. littoralis*. Yet more studies need to be conducted in greenhouses and open areas by investigating the combined use of EPN species.

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