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Biological control potential of local entomopathogenic nematodes against the different stage larvae of cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)

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Abstract

Background: The cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is an economic pest on various crops worldwide. Farmers generally used to apply chemical pesticides to control the pest. The bio-control potential of the entomopathogenic nematodes (EPNs) as alternatives to harmful synthetic pesticides was examined in this study. The study aimed to determine the efficacy of EPNs isolates against the different stages of larvae of the cotton leafworm *S. littoralis* and the effect of time on mortality rate under laboratory conditions.

Results: EPNs isolates were tested at 4 different concentrations (0, 250, 500 and 1000 IJs/ml) in 150 ml plastic containers on last instar larvae (sixth instar) of *S. litorallis*. Experiments for third, fourth and fifth instar larvae of *S. litorallis* were carried out in petri dishes at different concentrations (0, 50, 100 and 200 IJs/ml) at 25 °C temperature. Mortality rates of larvae were calculated at 3 different times (48, 72, 96 h) after inoculation. The high mortality rate of last instar larvae (98.81%) of *S. littoralis* was caused by *Steinernema feltiae* (Tokat-Emir), followed by *S. carpocapsae* (Tokat-Bakışlı05) (95.24%) and then *H. bacteriophora* (11 KG) with (90.47%) at the highest concentration (1000 IJ/ml). The highest mortality rate of fifth instar larvae was caused by *S. feltiae* (Tokat-Bakışlı05) and *S. carpocapsae* (Tokat-Emir) with (100%) and (92.12%). In addition, the highest mortality rate of the 4th instar larvae was determined S. *feltiae* (Tokat-Bakışlı05) and S. *carpocapsae* (Tokat-Emir) isolates (98.87%) and (97.74%), respectively. Additionally, the highest larval mortality rate in the third stage by *S. feltiae* (Tokat-Bakışlı05) and *S. carpocapsae* (Tokat-Emir) was (100%) and (97.74%) at the highest concentration. Mortality rates of larvae were calculated at 3 different times after inoculation. The highest mortality rate counted in all isolates was determined 96 and 72 h after inoculation of EPNs.

Conclusions: All indigenous EPN isolates were found to be effective at different rates against *S. littoralis*. The results showed that these nematode species could be used against *S. littoralis* biological control programs.

Keywords: Efficacy, *Spodoptera littoralis*, Entomopathogenic nematodes, Biological control

Background

The cotton leafworm, *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae), is one of the most damaging lepidopterous pests in numerous commercial crops, inflicting significant economic losses on a wide range of ornamental, industrial, and crops in both

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greenhouses and open fields (Mohamed et al. 2019). *S. littoralis* is difficult to be controlled due to its resistance to synthetic pesticides (Ghulam et al. 2017). Biological agents such as parasitoids, predators, and entomopathogens (bacteria, fungi, nematodes, and viruses) have been used to control several pests (Atia et al. 2016). EPNs have high adaptability to different conditions, a wide variety of insect hosts, simple mass rearing, and the ability to resist some chemical pesticides. EPNs belong mainly to the 2 families: Heterorhabditidae and Steinernematidae, which are the most economically used against several pests (Koppenhöfer 2007). Around 100 valid species of Steinernema and 21 species of Heterorhabditis have been described from different countries of the World (Bhat et al. 2020). Many factors efficacious the distribution of EPNs to several regions (Laznik and Trdan 2012).

The present study aimed to evaluate the efficacy of local EPNs isolates [*Heterorhabditis bacteriophora* (Poinar, 1975) (Rhabditida: Heterorhabditidae), *Steinernema feltiae* (Filipjev, 1934) (Rhabditida: Steinernematidae) and *S. carpocapsae* (Weiser, 1955)] against different larval instars of the cotton leafworm and effect of the exposure time (48 h, 72 h, 96 h) on mortality rates under laboratory controlled conditions.

Methods

Mass rearing of *Spodoptera littoralis*

Spodoptera littoralis larvae were collected from cotton fields in the vicinity of Adana, Turkey, transferred to the laboratory, and reared at 25 ± 2 °C and 65–75 RH%. The larvae were fed on an artificial (Chen et al. 2000). The ingredients were mixed in a blender and stored at 4°C for not more than one week. Diet-fed larvae were reared in plastic boxes (10 cm height × 20 cm width × 30 cm length) containing the artificial diet. Adults were placed in the plastic cages (10 cm diameter by 20 cm high) closed on the upper extremity with tissue and were fed on 10% sugar solution (Santos et al. 2005). The culture was kept at 25 ± 2 °C, 65 ± 5% RH, and 16: 8 h (L: D) photoperiod.

Galleria mellonella larval growth

Last stage larvae of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) were mass-reared on a diet containing 445 g milk powder, 890 g flour, 222 g dry baker's yeast, 500 g honey, 500 g glycerin, 125 g beeswax. Diet was prepared according to Mohammed and Coppel, (1983). Glycerin, beeswax, and honey melted after adding other materials. Eggs were placed onto the food in one-liter jars and kept in an incubator at 23–24 °C.

Rearing of entomopathogenic nematodes

Entomopathogenic nematode juveniles of *Steinernema feltiae* (Tokat-Emir), *S. carpocapsae* (Tokat-Bakışlı05), *Heterorhabditis bacteriophora* (TOK-20), *H. bacteriophora* (11 KG) obtained from the Plant Protection Department of Gaziosmanpaşa University were used in this study. Infective juveniles of the EPNs isolates were reared on last instar larvae of greater wax moth *G. mellonella* according to Kaya and Stock (1997). Sixth (last) instar larvae of *Galleria* larvae were used for mass rearing of the EPNs juveniles at all experiments. Four different EPNs isolates (*S. carpocapsae* (Tokat-Bakışlı05), *H. bacteriophora* (11 KG), *H. bacteriophora* (TOK-20), *S. feltiae* (Tokat-Emir) were tested. Ten larvae were placed in Petri dishes (6 cm diameter) with Whatman No. 1 paper soaked with distilled water. Infective juveniles of nematode species were applied on *G. mellonella* larvae. The lid of the Petri dishes was wrapped by a parafilm and placed in an incubator at 23–25 °C. Larval mortality was counted and recorded daily. EPNs were collected from infected wax moth larvae using the "White trap" method (White 1927). The traps were placed for 3–7 days under the same conditions. EPN (IJs) were placed in flasks and then stored in a refrigerator at 10 °C. This process was repeated every 2 months to prevent the nematodes from losing their activity.

Bioassays

Studies were carried out in 150 ml plastic containers on last instar larvae (sixth instar) of *S. litorallis*, which were consisted of soil mixture (80% sand, 15% soil and 5% clay) sterilized at 121 °C (Chen et al. 1995). Last instar larvae were placed into soil mixture in plastic containers. Four different nematode concentrations (0, 250, 500 and 1000 IJs/ml) were tested. The soil moisture level was adjusted to 10% (w/w) by adding distilled water. Control plastic cups were added only distilled water. EPNs were inoculated onto the soil using a pipette in each cup. The treated plastic cups were kept at 25 °C, concentration was located onto soil and cups were covered by tulle. Laboratory trials were conducted with 10 individuals for each concentration of EPN isolate ($n = 10$ per EPN isolate per cont.) In each container, only one *S. littoralis* last stage larva was added. Trials were repeated 3 times under the same conditions on different dates. Other experiments were conducted for other stage larvae (third, fourth and fifth) *S. litorallis* in plastic Petri dishes (9 cm) that included artificial diet at the same conditions. Two laboratory-reared larvae of *S. litorallis,* taken from the stock culture, were placed in a new Petri dish. EPN isolates were prepared, using distilled water at 0, 50, 100, and 200 IJs/ml for third, fourth and fifth instar larvae and applied

directly into the Petri dish and then covered with a parafilm. All experiment was repeated 3 times at different dates. Experiments were conducted under the laboratory conditions (25 °C and 65% RH). Mortality rates of larvae were calculated at 3 different times (48, 72, 96 h) after inoculation. Dead *S. littoralis* larvae were placed using the White trap method. After one week, insect cadavers were examined in distilled water under a stereomicroscope. EPNs juveniles were obtained from each infected *S. littoralis* larvae.

Data analysis

Data obtained in the trials were converted to measurements' percentage, transformed using arc-sin transformation. Comparisons between concentrations were done using Duncan's range comparison test. And then analyzed with analysis of variance (One-way ANOVA). Comparisons between concentrations were done using Duncan's range comparison test. All statistical analyses were made SPSS 23.0 package program (IBM Corp 2013).

Results

Results were evaluated, 48, 72, 96 h after EPNs inoculation. The interaction among the parameters (isolates and doses)(times and isolates) investigated was statistically significant in the study. The results showed differences among the nematode concentrations and isolates.

According to the results, the percent mortality rate increased as the concentration of infective juveniles increased. EPN isolates caused different levels of mortality rates on the last larval instar larvae of *S. litorallis*. *Steinernema feltiae* (Tokat-Emir) caused 98,81% mortality. It was higher than the others (*S. carpocapsae* (Tokat-Bakışlı05) (95,24%), *H. bacteriophora* (11 KG) with 90,47%, *H. bacteriophora* (TOK-20) with 54,75%). The lowest mortality rate was recorded in all isolates at 250 IJs/ml concentrations. At 500 IJs/ml concentration, *S. feltiae* (Tokat-Emir) was found to have the highest mortality rate (95.24%) (Table 1).

According to the results, EPNs concentrations against larvae of *S. littoralis* were found more effective than controls (soils treated with distilled water). In addition, it was observed that the mortality rate increased proportionally as the exposure time increased. When the effect of time on larval death was examined, it was determined that the highest mortality rate was within 96 h post-infection.

The highest mortality rate (93.83%), counted 96 h post-exposure, was caused by *S. feltiae* (Tokat-Emir), followed by *S. carpocapsae* (Tokat-Bakışlı05) with (90.47%) *H. bacteriophora* (11 KG) isolate with (78.56%), and *H. bacteriophora* (TOK-20) with (54.75%) (Table 2). *H. bacteriophora* (TOK-20) caused the lowest mortality rate (36,88%) detected 48 h post-exposure. Morphological characteristics of both Heterorhabditidae and Steinernematidae infection are shown in Fig. 1.

Data presented in Table 3 showed the susceptibility of cotton leafworm fifth larval instar to infection with EPNs species. The highest mortality rates of fifth instar were determined at 200 IJs/ml concentration for all 4 EPNs species. Maximum mortality rate (100%) was reported at 200 IJs/ml concentration in the case of *S. carpocapsae* (Tokat-Bakışlı05) isolate, followed by *S. feltiae* (Tokat-Emir) with (92,12%), *H. bacteriophora* (11 KG) with (87,62%) and *H. bacteriophora* (TOK-20) with (49,28%) (Table 3). The efficacy of EPNs species on fourth stage larvae was examined, S. *carpocapsae* (Tokat-Bakışlı05) isolate was caused (98,87%) mortality rate, followed by S. *feltiae* (Tokat-Emir) (97,74%), H. *bacteriophora* (11 KG) (90,92%) and H. *bacteriophora* (TOK-20) (56.69%) at 200 IJs/ml cont. (Table 4). Likewise, results showed that all isolates highest mortality rate on third instar of cotton leafworm larvae for *S. carpocapsae* (Tokat-Bakışlı05)

Table 1 Efficacy of nematode isolates and concentrations mortality rate on 6th instar of cotton leafworm larvae

Nematode species	Concentrations												
	250 IJs/ml			500 IJs/ml			1000 IJs/ml			Control			
Steinernema carpocapsae (Tokat-Bakışlı05)	70.23 ± 14.94	a*	C**	85.71 ± 13.13	b	B	95.24 ± 10.86	ab	A	6.67 ± 1.67	$F = 11.22, p = 0.00$		
Steinernema feltiae (Tokat-Emir)	78.13 ± 7.45	a	B	95.24 ± 9.45	a	A	98.81 ± 3.57	a	A	8.89 ± 3.09	$F = 23.26, p = 0.00$		
Heterorhabditis bacteriophora (TOK-20)	38.07 ± 8.93	b	B	49.98 ± 16.08	c	AB	54.75 ± 10.42	c	A	6.67 ± 3.33	$F = 4.42, p = 0.02$		
Heterorhabditis bacteriophora (11 KG)	69.04 ± 9.94	a	A	78.56 ± 13.13	b	AB	90.47 ± 9.95	b	A	6.67 ± 3.33	$F = 6.60, p = 0.01$		
	$F = 22.65, p = 0.00$			$F = 15.44, p = 0.00$			$F = 29.24, p = 0.00$						

* Means within column bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

** Means within lines bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

Table 2 Mortality rates of cotton leafworm larvae at different exposure times

	Time									
	48 h			**72 h**			**96 h**			
Steinernema carpocapsae (Tokat-Bakışlı05)	70.23 ± 17.59	a*	B**	90.47 ± 11.30	ab	A	90.47 ± 11.30	a	A	$F = 4.87, p = 0.02$
Steinernema feltiae (Tokat-Emir)	84.52 ± 13.25	a	A	93.83 ± 9.80	a	A	93.83 ± 9.80	a	A	$F = 1.82, p = 0.18$
Heterorhabditis bacteriophora (TOK-20)	36.88 ± 11.30	b	B	51.17 ± 12.12	c	A	54.75 ± 11.72	b	A	$F = 5.95, p = 0.01$
Heterorhabditis bacteriophora (11 KG)	76.18 ± 12.88	a	A	78.56 ± 15.16	b	A	83.33 ± 14.29	a	A	$F = 0.63, p = 0.54$
Control	6.67 ± 2.56			7.50 ± 2.50			7.50 ± 2.50			
	$F = 11.96, p = 0.00$			$F = 14.61, p = 0.00$			$F = 12.64, p = 0.00$			

* Means within column bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

** Means within lines bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

Table 3 Efficacy of nematode isolates and concentrations mortality rate on fifth instar of cotton leafworm larvae

Nematode	**Concentrations**									
	50 IJs/ml			**100 IJs/ml**			**200 IJs/ml**			**Control**
Steinernema carpocapsae (Tokat-Bakışlı05)	88.75 ± 3.55	a*	B**	93.27 ± 2.91	a	B	100.00 ± 0.00	a	A	3.33 ± 0.00	$F = 5.51, p = 0.01$
Steinernema feltiae (Tokat-Emir)	87.63 ± 3.68	a	A	89.87 ± 3.37	a	A	92.12 ± 2.25	b	A	1.11 ± 1.11	$F = 0.19, p = 0.83$
Heterorhabditis bacteriophora (TOK-20)	17.56 ± 2.46	c	B	28.87 ± 4.01	b	B	49.28 ± 7.87	c	A	2.22 ± 1.11	$F = 9.04, p = 0.00$
Heterorhabditis bacteriophora (11 KG)	72.96 ± 4.72	b	A	79.76 ± 6.29	a	A	87.62 ± 4.03	b	A	0.00 ± 0.00	$F = 2.17, p = 0.14$
	$F = 42.83, p = 0.00$			$F = 24.56, p = 0.00$			$F = 27.37, p = 0.00$			

* Means within column bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

** Means within lines bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

Table 4 Efficacy of nematode isolates and concentrations mortality rate on fourth instar of cotton leafworm larvae

Nematode	**Concentrations**									
	50 IJs/ml			**100 IJs/ml**			**200 IJs/ml**			**Control**
Steinernema carpocapsae (Tokat-Bakışlı05)	82.93 ± 4.49	a*	B**	90.92 ± 4.31	a	AB	98.87 ± 1.13	a	A	3.33 ± 0.00	$F = 6.03, p = 0.01$
Steinernema feltiae (Tokat-Emir)	92.05 ± 2.83	a	A	95.48 ± 3.44	a	A	97.74 ± 1.50	ab	A	3.33 ± 0.00	$F = 1.37, p = 0.27$
Heterorhabditis bacteriophora (TOK-20)	17.76 ± 2.27	c	C	30.34 ± 2.70	c	B	56.69 ± 6.24	c	A	2.22 ± 1.11	$F = 22.22, p = 0.00$
Heterorhabditis bacteriophora (11 KG)	37.21 ± 3.86	b	C	62.36 ± 4.10	b	B	90.92 ± 3.15	b	A	2.22 ± 1.11	$F = 40.21, p = 0.00$
	$F = 56.22, p = 0.00$			$F = 47.57, p = 0.00$			$F = 28.15, p = 0.00$			

* Means within column bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

** Means within lines bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

with (100%), followed by *S. feltiae* (Tokat-Emir) isolates with (97,74%), *H. bacteriophora* (11 KG) with (69.28%) and *H. bacteriophora* (TOK-20) with (62.47%) (Table 5).

The interaction between EPNs species and post-treatment time for nematode infection was significant ($F = p < 0.05$). The mortality rate was observed after 48 h, and the rate was directly proportional to the EPNs exposure time. In experiments, the highest mortality rates of fifth instar *S. litorallis* larvae were counted 72 and 96 h after treatment with all 4 EPNs species at 200 IJs/ml. The highest mortality rate of fifth larvae was counted, 96 h for *S. carpocapsae* (Tokat-Bakışlı05) with (98.86%), followed by *S. feltiae* (Tokat-Emir) with (96,58%), *H. bacteriophora* (11 KG) isolate with (89.74%), and *H. bacteriophora* (TOK-20) with (44.16%) (Table 6). Higher mortality rate of fourth larvae occurred at 96 h post-treatment, with (98.85%) mortality caused by *S. feltiae* (Tokat-Emir) likewise, *S. carpocapsae* (Tokat-Bakışlı05); *H. bacteriophora* (11 KG), *H. bacteriophora* (TOK-20) achieved 96.55%, 72.42% and 43.70% mortality, respectively (Table 7). When the mortality rate of third stage cotton leafworm larvae at different exposure times was examined, mortality rate was determined (97.70%) at *S. feltiae* (Tokat-Emir), followed by *S. carpocapsae* (Tokat-Bakışlı05) (95.40%), *H. bacteriophora* (11 KG) (65.53%) and *H. bacteriophora* (TOK-20) (54.04%) (Table 8).

Discussion

Obtained results revealed that EPNs virulence differed significantly among EPN species. According to results, the highest mortality rates were found in *S. carpocapsae* (Bakışlı05) and *S. feltiae* (Tokat-Emir) isolates, followed by *H. bacteriophora* (11 KG), and *H. bacteriophora* (TOK-20), respectively. In addition, the highest mortality rate was observed on *S. littoralis* larvae 72 and 96 h after inoculation and mortality started from the first count in the trials. This study may provide the basis for

Table 5 Efficacy of nematode isolates and concentrations mortality rate on third instar of cotton leafworm larvae

Nematode	Concentrations											
	50 IJs/ml			100 IJs/ml			200 IJs/ml			Control	
Steinernema carpocapsae (Tokat-Bakışlı05)	81.82 ± 3.69	b*	C**	89.78 ± 2.95	a	B	100.00 ± 0.00	a	A	6.67 ± 0.00	$F = 20.33, p = 0.00$
Steinernema feltiae (Tokat-Emir)	89.80 ± 3.80	a	B	89.76 ± 2.41	a	B	97.74 ± 2.26	a	A	0.00 ± 0.00	$F = 3.68, p = 0.04$
Heterorhabditis bacteriophora (TOK-20)	16.82 ± 2.65	d	B	54.51 ± 6.78	b	A	62.47 ± 4.77	b	A	1.11 ± 1.11	$F = 22.99, p = 0.00$
Heterorhabditis bacteriophora (11 KG)	45.37 ± 4.71	c	B	56.73 ± 3.25	b	B	69.28 ± 4.48	b	A	2.22 ± 1.11	$F = 8.31, p = 0.00$
	$F = 54.66, p = 0.00$			$F = 18.35, p = 0.00$			$F = 60.06, p = 0.00$				

* Means within column bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

** Means within lines bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

Table 6 The mortality rate of fifth stage cotton leafworm larvae at different exposure times

Nematode	Time									
	48 h			72 h			96 h			
Steinernema carpocapsae (Tokat-Bakışlı05)	87.68 ± 3.67	a*	B**	95.48 ± 2.46	a	AB	98.86 ± 1.14	a	A	$F = 4.45, p = 0.02$
Steinernema feltiae (Tokat-Emir)	82.08 ± 2.80	a	B	90.96 ± 2.65	ab	A	96.58 ± 1.71	ab	A	$F = 9.04, p = 0.00$
Heterorhabditis bacteriophora (TOK-20)	18.23 ± 3.92	c	A	33.31 ± 4.83	c	AB	44.16 ± 8.22	c	A	$F = 5.33, p = 0.01$
Heterorhabditis bacteriophora (11 KG)	64.16 ± 4.78	b	B	86.44 ± 3.39	b	A	89.74 ± 2.96	b	A	$F = 9.30, p = 0.00$
Control	0.83 ± 0.83			1.67 ± 0.96			2.50 ± 0.83			
	$F = 44.19, p = 0.00$			$F = 35.68, p = 0.00$			$F = 30.90, p = 0.00$			

* Means within column bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

** Means within lines bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

Table 7 The mortality rate of fourth stage cotton leafworm larvae at different exposure times

Nematode	Time									
	48 h			72 h			96 h			
Steinernema carpocapsae (Tokat-Bakışlı05)	81.92 ± 5.30	a*	B**	94.26 ± 2.50	a	A	96.55 ± 2.44	a	A	$F = 4.43$, $p = 0.02$
Steinernema feltiae (Tokat-Emir)	87.57 ± 3.30	a	B	98.85 ± 1.15	a	A	98.85 ± 1.15	a	A	$F = 10.84$, $p = 0.28$
Heterorhabditis bacteriophora (TOK-20)	23.14 ± 3.83	c	B	37.95 ± 6.45	c	AB	43.70 ± 8.10	c	A	$F = 2.90$, $p = 0.08$
Heterorhabditis bacteriophora (11 KG)	51.40 ± 8.09	b	A	66.68 ± 7.67	b	A	72.42 ± 8.27	b	A	$F = 2.16$, $p = 0.14$
Control	1.67 ± 0.96			3.33 ± 0.00			3.33 ± 0.00			
	$F = 23.83$, $p = 0.00$			$F = 31.27$, $p = 0.00$			$F = 20.02$, $p = 0.00$			

* Means within column bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

** Means within lines bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

Table 8 The mortality rate of third stage cotton leafworm larvae at different exposure times

Nematode	Time									
	48 h			72 h			96 h			
Steinernema carpocapsae (Tokat-Bakışlı05)	87.17 ± 4.83	a*	A**	92.02 ± 2.85	a	A	95.40 ± 1.82	a	A	$F = 1.68$, $p = 0.21$
Steinernema feltiae (Tokat-Emir)	86.44 ± 3.39	a	B	93.16 ± 2.96	a	AB	97.70 ± 1.52	a	A	$F = 4.09$, $p = 0.03$
Heterorhabditis bacteriophora (TOK-20)	33.31 ± 5.39	b	A	46.44 ± 8.16	b	A	54.04 ± 10.07	b	A	$F = 1.53$, $p = 0.24$
Heterorhabditis bacteriophora (11 KG)	46.88 ± 6.06	b	B	58.97 ± 3.82	b	AB	65.53 ± 3.86	b	A	$F = 3.94$, $p = 0.03$
Control	1.67 ± 1.67			2.50 ± 1.60			3.33 ± 1.36			
	$F = 21.10$, $p = 0.00$			$F = 22.27$, $p = 0.00$			$F = 22.12$, $p = 0.00$			

* Means within column bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

** Means within lines bearing the same letter are not significantly different (Duncan's test, $p > 0.05$)

Fig. 1 *Spodoptera littoralis* larvae infected with Steinernematidae (**a**) and Heterorhabditidae (**b**)

the application of these local EPNs species against *S. littoralis*.

Many studies have been conducted on the biological control of pests, using different bio-control agents such as (EPNs) (Orozco et al. 2014). They are eco-friendly tools that have no harmful effects on animals, humans and the environment. Sobhy et al. (2020) reported that the concentration of 400 IJs/dish for *S. monticolum* (Rhabditida: Steinernematidae) achieved up to (97.77%) of the mortality rate of the fifth instar larva of *S. littoralis*. Yan et al. (2019) found 2 isolates of *Steinernema* sp. 64–2, *S. longicaudum* (Shen and Wang 1991) (Rhabditida: Steinernematidae), 4 isolates of *S. carpocapsae* and 2 isolates of *H. indica*, caused higher mortality rates in the second, third and fourth instars of *S. litura* Fab. (Lepidoptera, Noctuidae) than the other isolates, with mortality > 90% after 48 h exposure. Abdel-Razek and Abd-Elgaward (2007) reported 7 EPNs strains tested against the last instar larvae of *S. littoralis* and *G. mellonella* in Petri dishes and sand column assay. In sand column trials strains, *Heterorhabditis* sp. ELB., *Steinernema riobrave* Cabanillas, Poinar & Raulston, 1994 (Rhabditida: Steinernematidae) and *S. carpocapsae* showed the highest activity against *S. littoralis* with (100%) mortality within 24 h post-exposure. Shairra and Noah (2014), *H. bacteriophora* Poinar (HP88 strain), *S. riobrave* and *Metarhizium anisopliae* (Metschn.) Sorokin, 1883 (Hypocreales: Clavicipitaceae) and *Beauveria bassiana* (Bals.-Criv.) Vuill, 1912 (Hypocreales: Cordycipitaceae) as well the combined effect of both against third instar larvae of the cotton leafworm. In another study, Abdel-Razek (2006) studied the susceptibility of the *S. littoralis* to the nematode *S. carpocapsae* (All) and *H. bacteriophora. H. bacteriophora* and recorded 100% mortality rate 96, 90 and 48 h post-exposure of *S. littoralis* larvae. In addition, *S. carpocapsae* (All) reported (100%) mortality 120, 90, and 56 h post-exposure, respectively. Atwa and Shalaby (2014) evaluated the efficacy of the *H. bacteriophora* (HP88 strain) and *Steinernema glaseri* Steiner (NJ strain) Glaser, 1932 (Rhabditida: Steinernematidae) against the fifth and third instar larvae of *S. littoralis* under laboratory conditions. *H. bacteriophora* was the most effective one on the fifth instar larvae of the pest. Shaurub et al. (2016) reported that *S. riobrave* and *H. bacteriophora* or between either nematode species or *B. bassiana* would result in the efficient control of *S. littoralis* when they are simultaneously or sequentially applied. Ahmed et al. (2014) reported that the activity of some larval enzymes was also affected due to infection. Invertase and Trehalase activity increased with the infection on *S. littoralis* by *S. feltiae, S. riobrave,* and *H. bacteriophora.* Nouh (2021) showed laboratory applications of the 2 EPNs isolates against the *S. littoralis*, *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae) were determined. The third instar larvae of *A. ipsilon* and *S. littoralis* were more sensitive than the pupae. *Heterorhabditis* sp. strain TAN5 was the highest reproduction and the most effective on both *A. ipsilon* and *S. littoralis* larvae and pupae were the highest. Abd ElAzim (2022) examined *H. taysearae* (Rhabditida: Heterorhabditidae) against *S. littoralis* using different concentrations. Data showed that 150 IJs/larvae *H. taysearae* caused a 100% mortality rate. In addition Caoili et al. (2018) *H. indica* PBCB was the most virulent to *S. litura* (8.89 IJ per larva). *Steinernema abbasi* MBLB was the most virulent to *O. furnacalis* (10.98 IJ per larva), but non-significantly different to *S. litura* (17.08 IJ per larva.

Conclusions

Overall, concentrations of EPN isolates were more effective than the negative control (distilled water). This study showed highest mortality rates of all EPNs isolates against the cotton leafworm recorded at high concentrations at 25 °C temperature. The results indicated that the nematode species; *S. carpocapsae* (Tokat-Bakışlı05), *H. bacteriophora* (11 KG), *Heterorhabditis bacteriophora* (TOK-20*), S. feltiae* (Tokat-Emir) were efficacious on different instar larvae of cotton leafworm. Generally, *S. carpocapsae* (Bakışlı05) and *S. feltiae* (Tokat-Emir) isolates were the most effective against larvae than the other isolates. Therefore, these EPN species could be potentially used as biological control agents to *S. littoralis.* Additionally, they can use them in field applications for the safe control of pests that maybe serve as an alternative to synthetic chemicals.

Abbreviations

S littoralis: Spodoptera littoralis; *EPNs*: Entomopathogenic nematodes; *IJs*: Infective juveniles; *H bacteriophora*: Heterorhabditis bacteriophora; *S carpocapsae*: Steinernema carpocapsae; *S feltiae*: Steinernema feltiae; *G mellonella*: Galleria mellonella.

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Author contributions

M.Y and F.D.E participated in setting the work planning and executing the experimental work. C.Y analyzed the all data (statistical analyses) in study. C.Y and G.B rearing *S. littoralis* larvae for study. F.D.E and M.Y mass rearing entomopathogenic nematodes and participated in experimental studies. İ.K providing entomopathogen nematode isolates for work. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interest.

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