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Influence of Entomopathogenic Nematodes (EPN) on *Spodoptera frugiperda* (J. E. Smith) and related alterations in certain enzymatic processes

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Abstract
Background: The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is a major threat to many crops, with larvae attacking the plants at all growth stages.
Materials: A study examined if nematodes (*Steinernema carpocapsae* (Sc) and *Heterorhabditis bacteriophora* (HP88)) could be effective against fall armyworm (*S. frugiperda*) infestations on okra plants. The alternations in enzymatic activities of the insect pest larvae were determined 48 hours after exposure to the nematodes.
Results: Under laboratory conditions, entomopathogenic nematodes, *S. carpocapsae*, exhibited greater virulence compared to *H. bacteriophora* against the second larval stage of *Spodoptera frugiperda*. The highest concentrations of nematodes recorded the highest mortality in larvae of *Spodoptera frugiperda*. *S. carpocapsae* killed all larvae in their second instar, 100% of them, four days after treatment with the highest concentrations (300 and 200 IJs/ml). Also, when using *H. bacteriophora*, complete mortality was obtained 5- days post-treatment. In contrast, entomopathogenic nematodes *S. carpocapsae* and *H. bacteriophora* have the same virulence against the fourth larval stage of *S. frugiperda*.
Also, when using *S. carpocapsae* at a concentration (number) of 100 IJs/ml, four days after treatment, the greatest death rate (86.15%) was recorded.
The study examined the protein content and lipid peroxide activity of *S. frugiperda* larvae infected with entomopathogenic nematodes. The total protein content was highest in the untreated larvae, while those infected with *H. bacteriophora* had the lowest content. The fall armyworms (*S. frugiperda*) in their sixth larval stage had the most lipid peroxide activity when treated with both nematodes (*S. carpocapsae* and *H. bacteriophora*). However, the highest levels of chitinase activity were seen in worms treated with *S. carpocapsae* specifically during their fourth larval stage.

Keywords: Entomopathogens, nematodes, fall armyworm, enzymatic activities

Introduction
A prominent invasive polyphagous pest, *Spodoptera frugiperda* (J. E. Smith) fall armyworms (Lepidoptera: Noctuidae) harm over 353 plant species, with a focus on maize, sorghum, sugarcane, turfgrass, cotton, and vegetable crops (Gamil 2020; Timilsena *et al*. 2022) [8, 24]. The *S. frugiperda* was initially discovered in Komombo (Aswan Governorate) maize fields in 2019 (Dahi *et al*. 2020, Gamil 2020) [4, 8].
There are a number of control measures that can be used to lessen the effects of FAW, such as synthetic insecticides, botanicals like neem extracts, and biopesticides like viruses (such multiple nucleopolyhedrovirus) or the bacterium *Bacillus thuringiensis* (Bt), crops that are genetically engineered and contain Bt toxins, but also mechanical control practices such as handpicking of caterpillars, or cultural control such as push and pull cropping (Guo *et al*., 2020; Wan *et al*., 2021) [9, 25]. Fortunately, the lack of effective alternatives has led to chemical pesticides becoming the mainstay of FAW management quite quickly, in previously untreated maize-growing areas, this situation has resulted in a huge flow of insecticides (Tambo *et al*., 2020) [23]. The increased use of synthetic insecticides on a large scale has the potential to cause negative impacts on ecosystems and public health (Rani *et al*., 2021) [17]. Unfortunately, their use also significantly lowers the numbers of important natural enemies and can cause FAW populations become more resistant. Thus, there is an urgent need for easily accessible, secure, efficient, and long-lasting substitutes.

Entomopathogenic nematodes (EPN) are microscopic round worms that are soil-dwelling and are naturally occurring in soils all over the world. EPN are frequently employed mostly as biological control in agriculture against soil pests because of their capacity to infect and destroy a variety of insects (Koppenhofer *et al*., 2020) [12]. Lepidopteran larvae, such as FAW, are highly pathogenic to numerous EPN species or strains (Fallet *et al*., 2022) [7].

Entomopathogenic nematodes (EPNs), parasitic nematodes from the Steinernematidae and Heterorhabditidae families, are potential biological control tools for managing insect pests. The insect's natural openings, including the mouth, anus, and spiracles, enable EPNs to enter and regurgitate symbiotic bacteria they carry in their gut (*Xenorhabdus* spp. and *Photorhabdus* spp. In the Steinernematidae and Heterorhabditidae, respectively). The host insect dies from septicemia or toxemia as a result of bacterial proliferation producing a number of metabolites and poisons. Each EPN has a different level of virulence based on the kind of EPN, the species of host insect, and the stage of the host (Yan *et al*. 2020) [26]. Numerous investigators from various nations investigated the vulnerability of various EPNs strains in *S. frugiperda* (Lalramnghaki *et al.* 2021) [13].

Thus, the purpose of the current study was to evaluate the susceptibility of *S. frugiperda* second and fourth larval instars to EPNs "*S. carpocapsae* and *H. bacteriophora*", as well as the accompanying alteration in some enzymatic activities.

Materials and Methods

1. Rearing *Spodoptera frugiperda*

Larvae of *S. frugiperda* were taken from okra plants that were infected. in glass jars and transferred to be reared under laboratory conditions (26±2 °C and 65±5% RH). The collected larvae were fed on fresh okra leaves until they pupated, and they were each placed in a cup with a diameter of (5 cm and a height of 10 cm). The pupae were sexed, one male and one female were introduced into cages for mating and oviposition. The cages were provided with sugar solution to activate female egg-laying.

2. Tested entomopathogenic nematodes, *Steinernema carpocapsae* (Sc) and *Heterorhabditis bacteriophora* (HP88)

Nematodes, *S. carpocapsae* (Sc) and *H. bacteriophora* (HP88) were acquired from DR/ Ahmed Azazy, Pest Physiology Department of Plant Protection Research Institute, Agricultural Research Centre (ARC), Giza, Egypt. The larger wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), larvae were used to cultivate nematodes at a temperature of (25±1 °C). Using white traps, infectious juveniles (IJs) that emerged from wax moth larvae were collected twice a week. Nematode viability was assessed using a stereomicroscope; a specimen was deemed alive if it moved or reacted to a needle touch. When over 98% of IJs were viable, EPN suspensions were employed for the studies.

3. Experimental design and application of the EPNs

For the experiment, a completely randomized design (CRD) was employed, with three treatments consisting three different IJ nematode suspension concentration (numbers) (100, 200, and 300 IJs ml−1) and a control group that got the same amount of sterilized distilled water. Every EPN isolate and nematode suspension was examined independently for the second and fourth *S. frugiperda* larval instars. With ten larvae per replication, the experiments were conducted four times. The larvae were each given a detached okra leaf as food and placed individually in a Petri dish with a diameter of 5.5 cm. The larvae and okra leaf in each treatment got a topically applied one milliliter of nematode suspension containing varying densities of IJs; the control treatment received the same application. Every day, the food in the insect rearing room was changed, and the larvae were kept at 25±1 °C with a 14:10 (light: dark) photoperiod and 60±10% relative humidity.

4. Assessment of mortality

The larval mortality was measured 48 hours after the inoculation, and observations were kept records for ten days. When a larva did not react to the forceps' contact, it was determined that it was dead. Dead larvae were kept apart so that the white trap technique could be used to track the emergence of nematodes from the cadaver. The larvae were recorded as nematode killed only if they displayed signs of nematode emergence. The 10-day accumulated mortality percentage of the tested samples was computed with the following formula.

$$\text{Mortality rates reported} = \frac{\text{Total sum of larvae that have died}}{\text{Total sum of nematode treated larvae}} \times 100$$

5. Enzymatic activity

Crude extract was prepared from the fourth and sixth instars *S. frugiperda* larvae treated with EPNs after 48 hours of exposure were compared to the control group. For every treatment, three replicates were assigned. Ten pre-starved larvae were given enough treated okra leaves in each replicate, and the larvae were kept there for 24 h. The surviving larvae of each treatment were then ready for biochemical analysis in the Insect Physiology Laboratory, Plant Protection Research Institute, Dokki, Giza, Egypt.

5.1. Preparation of insect homogenates

Batches of *Spodoptera frugiperda* 4th and 6th instar larvae of different EPNs treatments as well as the control were weighed. A teflon homogenizer with a crushed ice jacket was used to mechanically homogenize each batch in 10 volumes (W/V) of 0.1 M phosphate buffer, pH 7, for a duration of two minutes. The homogenates were then centrifuged using a cooling centrifuge for 30 minutes at 4°C and 4000 rpm. The activities of chitinase, lipid peroxide (malondialdehyde), acetylcholinesterase (A.Ch.E.), and soluble protein concentration were assessed using the resulting supernatant.

5.2. Estimating the total protein content

Using bovine serum albumin as a standard, the Bradford (1976) [3] method was used to estimate the total proteins.

5.3. Estimating the lipid peroxide (malondialdehyde)

Malondialdehyde (lipid peroxide) activity was determined using the Satoh *et al.* (1978) [18] and Satoh (1979) [19] technique.

5.4. Estimating the acetylcholinesterase (A. Ch. E.) activity

Using acetylcholine bromide (A. Ch. Br) as the substrate, acetylcholinesterase (A. Ch. E.) activity was determined in accordance with the methodology outlined by Simpson *et al.* (1964) [21].

5.5. Estimating the chitinase activity

According to Ishaaya and Casida (1974) [10], chitinase was measured using 3,5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexosamine released on chitin digestion.

Statistical Analysis

Using the SAS program, the data were subjected to analysis of variance (ANOVA) and the means were compared using the LSD test at 0.05 levels (SAS Institute, 1988).

Results and discussion

The efficiency of two entomopathogenic nematodes in laboratory settings *Steinernema carpocapsae* (Sc) and *Heterorhabditis bacteriophora* (HP88) against *Spodoptera frugiperda*

Data in Table (1) show the influence of tested entomopathogenic nematodes on the mortality percentage of second instar larvae of *Spodoptera frugiperda* under laboratory conditions. On the second day, there were no deaths at any concentration. After three days of treatments with an infection caused by *Steinernema carpocapsae*, the maximum mortality rate was 96.67% at a concentration of 300 IJs. However, after four days of treatment with the same concentration of *Steinernema carpocapsae*, the mortality rate increased to 100%. *Heterorhabditis bacteriophora* killed all larvae at the 5th day after treatment at the concentration of 300 IJs. So, entomopathogenic nematodes, *Steinernema carpocapsae* was more virulence than *Heterorhabditis bacteriophora* against the 2nd instar of *Spodoptera frugiperda* larvae. In general, the maximum mortality was observed at the highest used concentrations. After 4 days of treatment, *S. carpocapsae* caused 100% mortality of the second larval instar at a high concentration (300 and 200 IJs/ml). Additionally, the greatest mortality rate (100%) was recorded five days after treatment with 300 and 200 IJs/ml when *Heterorhabditis bacteriophora* was used.

Fig 1: *Spodoptera frugiperda* larval mortality following EPN application, *Heterorhabditis bacteriophora*

Fig 2: *Spodoptera frugiperda* larval instar mortality following application of EPNs, *Steinernema carpocapsae*

Table 1: Percentage of mortality of *Spodoptera frugiperda* larvae in their second instar treated with entomopathogenic nematodes in a laboratory setting (28±5 °C, 65±10 RH).

Days after treatment	Control	*Steinernema carpocapsae* (Sc) con. / *S. frugiperda* larvae			*Heterorhabditis bacteriophora* (HP88) con. / *S. frugiperda* larvae		
		100 IJs	200 IJs	300 IJs	100 IJs	200 IJs	300 IJs
2nd	0	0.00	0.00	0.00	0.00	0.00	0.00
3rd	0	69.45	93.33	96.67	38.30	83.33	90.00
4th	0	11.05	6.67	3.33	9.26	13.33	6.67
5th	0	0.00	0.00	0.00	4.74	3.33	3.33
6th	0	0.00	0.00	0.00	8.85	0.00	0.00
Total	0	80.5	100	100	61.15	99.99	100

The data provided in Table (2) illustrates how tested entomopathogenic nematodes affect the mortality rate of *Spodoptera frugiperda* larvae in the fourth instar in a lab setting. With all concentrations, there was no mortality on the second day. The infection with *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* reached 100% mortality after four days from treatment with the same concentration from these two pathogens. The highest mortality occurred at the concentration of 300 IJs (93.33%) after three days from treatment. Because of this, entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* are equally virulent against *Spodoptera frugiperda* larvae in their fourth instar.

S. carpocapsae and *H. bacteriophora* caused 100% mortality to the 4th larval instar after 5 days of treatment with a concentration used (200 IJs/ml). Additionally, when using *S. carpocapsae*, the highest mortality rate (86.15%) was obtained 4-day post-treatment with a concentration used (100 IJs/ml).

In general, the highest used concentrations recorded the highest mortality. Reducing the use of chemicals in pest control management is critically needed., biological control presents a viable approach that is less harmful to the environment than dangerous chemicals for the FAW's long-term management. Many researchers from around the world have looked into how well different nematode strains work against *S. frugiperda*. (Mohamed and Shairra 2023) [15], and they showed that, while at varying rates and concentrations, distinct strains killed FAW larvae. The results of this study demonstrated that the larval mortality rates for FAW varied depending on the nematode species, post-exposure times, and developmental instar larvae.

Table 2: Percentage of mortality in fourth-instar *Spodoptera frugiperda* larvae treated with entomopathogenic nematodes in a lab setting (28±5 °C, 65±10 RH).

Days after treatment	Control	*Steinernema carpocapsae* (Sc) con/ *S. frugiperda* larvae			*Heterorhabditis bacteriophora* (HP88) con/ *S. frugiperda* larvae		
		100 IJs	200 IJs	300 IJs	100 IJs	200 IJs	300 IJs
2nd	0	0.00	0.00	0.00	0.00	0.00	0.00
3rd	0	61.20	80.33	93.33	21.80	86.67	93.33
4th	0	24.95	10.00	6.67	5.40	10.00	6.67
5th	0	0.00	10.00	0.00	4.80	3.33	0.00
6th	0	0.00	0.00	0.00	15.70	0.00	0.00
Total	0	86.15	100	100	47.7	100	100

Additionally, the treatment of *S. carpocapsae* nematodes was more potent and pathogenic against all FAW instars, resulting in 100% death within 4-5 days of exposure. The Rwandan strain of *S. carpocapsae* (RW14-G-R3a-2) caused 100% mortality in the second and third instars fairly quickly, but the rate reduced to 75% in the sixth instar, according to Fallet *et al.* (2022) [7] findings, which were partially supported by our data. Additionally, after three to five days of infection with *S. carpocapsae*, (Sayed *et al*., 2022) [20] discovered that the third and fifth instar larvae had the highest 100% mortality rates, with rates of 72.2 and 77.8%, respectively. Furthermore, only the (1st–3rd) larvae were demonstrated to be very susceptible to *S. carpocapsae*, whereas the (4th-6th) larvae were more susceptible to other strains, including *S. arenarium* and *S. longicaudum*. Nonetheless, *H. indica* infection of FAW early instars was highly likely, and this finding was in line with earlier research by (Acharya *et al.* 2020) [1]. The dominance of *S. carpocapsae* nematode over *H. indica* in every FAW instar studied, irrespective of IJs concentrations, was further explained by the data. Numerous more research (Fallet *et al*. 2022; Sayed *et al*. 2022 and Mohamed and Shairra 2023) [7, 20, 15] corroborated the effectiveness of the Steinernematidae genera against other Spodoptera spp., as a biocontrol agent, including *S. frugiperda*. These investigations also confirmed the findings of this study. The morphological features of nematode strains, their resistance to host-immune reactions, and the specialization of the host insect could all contribute to the variations in virulence and infectivity between the Heterorhabditidae and Steinernematidae families. Previous studies have suggested that the physical characteristics, sizes, behaviors, and immune defense systems of larvae in developmental instars may eventually account for the variations in their susceptibility and mortality rates (Elbrense *et al.* 2021) [6].

Total amount of protein

The findings are outlined in Table (3) and shown in Fig. (3). show the total amount of protein in whole homogenates of *Spodoptera frugiperda* 4th and 6th larval instar due to infection with entomopathogenic nematodes under laboratory conditions. In comparison to the other entomopathogens, the untreated *Spodoptera frugiperda* (4th larval instar) exhibited the greatest amount of total protein content (19.48 mg/g. b. wt. /min.). The lowest protein contents (0.59 mg/g. b. wt. /min.) were assessed in *S. frugiperda* larvae infected with entomopathogenic nematodes (*Heterorhabditis bacteriophora*). While the corresponding amount of protein contents in *Steinernema carpocapsae* treatments was 5.03 mg/g. b. wt./min.

For *Spodoptera frugiperda* 6th larval instar, the corresponding total protein ratios owing to *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* treatments were 1.90 and 1.34, respectively, in comparison with the baseline untreated (control).

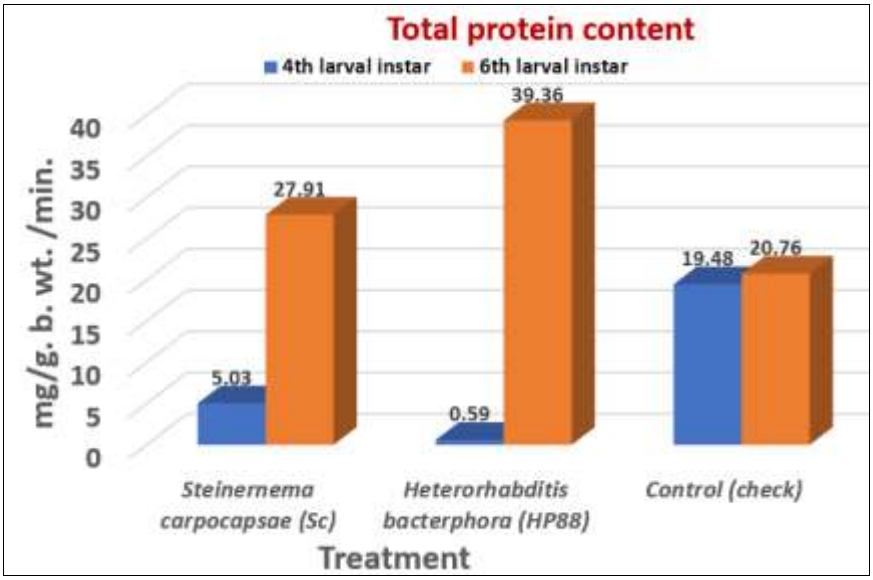

Fig 3: Total protein content in whole homogenates of the 4th and 6th larval instars of *Spodoptera frugiperda* treated with entomopathogenic nematodes under laboratory conditions (28±5 °C, 65±10 RH)

Table 3: Total protein content in whole homogenates 4th and 6th larval instar of *Spodoptera frugiperda* treated with entomopathogenic nematodes under laboratory conditions (28±5 °C, 65±10 RH)

Treatment	*Spodopta frugipera* larval instar	Total protein Content (mg/gm body weight)	Total protein content ratio
Steinernema carpocapsae (Sc)	4th	5.03±0.02	0.26
	6th	27.91±0.28	1.34
Heterorhabditis bacteriophora (HP88)	4th	0.59±0.00	0.03
	6th	39.36±0.23	1.90
Control (check)	4th	19.48±0.28	1.00
	6th	20.76±0.09	1.00
L.S.D.		58.51	

Total protein content is expressed as: mg/g. b. wt. /min.

$$\text{Total protein content ratio} = \frac{\text{Amount of total protein of different treatments}}{\text{Amount of total protein of (untreated check)}}$$

Lipid peroxide (Malondialdehyde) activity

The findings are outlined in Table (4) and shown in Fig. (4). reveal that lipid peroxide activity ratios for *Spodoptera frugiperda* 4th larval instar were lower in *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* compared to the untreated ones. However, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* treatment exhibited the highest activity ratio (2.53 and 2.37, respectively) in case of treated *Spodoptera frugiperda* 6th larval instar. In the same direction, the highest lipid peroxide activity was highest in *S. frugiperda* 6th larval instar treated with *Steinernema carpocapsae* (625.72) and *Heterorhabditis bacteriophora* (587.93), but lowest in case of treating the 4th larval instar by *Steinernema* carpocapsae (218.88) and *Heterorhabditis bacteriophora* (185.41).

Table 4: Lipid peroxide (Malondialdehyde) activity in whole homogenates 4th and 6th larval instars of *Spodoptera frugiperda* treated with entomopathogenic nematodes under laboratory conditions (28±5 °C, 65±10 RH)

Treatment	*Spodopta frugipera* larval instar	Lipid Peroxide (Malondialdehyde) activity (nmol/g. tissue)	Activity ratio
Steinernema carpocapsae (Sc)	4th	218.88±2.73	0.68
	6th	625.72±2.77	2.53
Heterorhabditis bacteriophora (HP88)	4th	185.41±1.87	0.58
	6th	587.93±2.77	2.37
Control (check)	4th	320.23±10.02	1.00
	6th	247.77±2.78	1.00
L.S.D.		749.50	

Activity is expressed as: Malondialdehyde nmol / g. tissue in sample: Tissue = A Sample/ A Standard X 10/ g. tissue used

$$\text{Activity ratio} = \frac{\text{Enzymatic activity in larvae in different treatments}}{\text{Enzymatic activity in larvae of control (untreated)}}$$

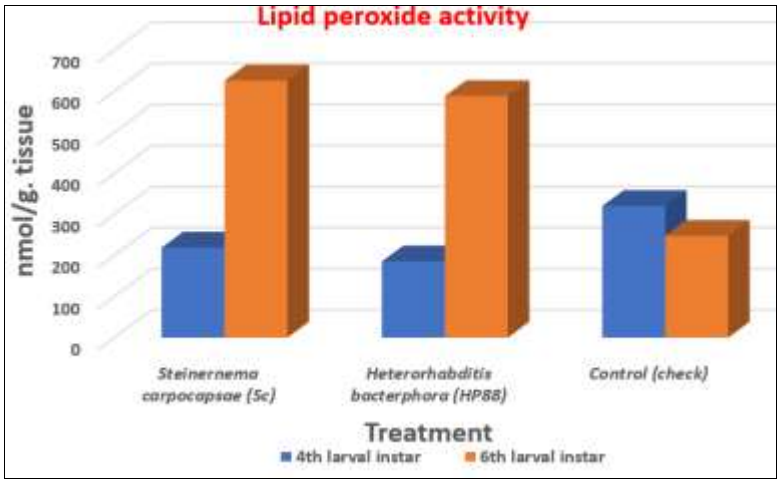

Fig 4: Lipid peroxide (Malondialdehyde) activity in whole homogenates of *Spodoptera frugiperda* 4th and 6th larval instars as influenced by entomopathogenic nematodes under laboratory conditions (28±5 °C, 65±10 RH)

Acetylcholinesterase (A.Ch.E.) activity

By eliminating acetylcholine released at impulse synapses and potentially along axons, acetylcholine esterase (A.Ch.E.) is essential to the maintenance of the activity of neurons. Information provided in Table (5) and (Fig. 5) reveals the inhibitory effect of entomopathogenic nematodes against acetylcholinesterase activity. *Heterorhabditis bacteriophora* (260.13 µg /min/gm body weight) applications induced higher acetylcholinesterase activity in the 4th instar larvae compared with larvae infected with *Steinernema carpocapsae* (162.62 µg /min/gm body weight). In the same direction, *Heterorhabditis bacteriophora* (265.58 µg /min/gm body weight) and *Steinernema carpocapsae* (256.28 µg /min/gm body weight) applications induced higher acetylcholinesterase activity in the 6th larval instar of *Spodoptera frugiperda*.

Table 5: Acetylcholinesterase activity in whole homogenates of 4th and 6th larval instar of *Spodoptera frugiperda* treated with entomopathogenic nematodes under laboratory conditions (28±5 °C, 65±10 RH)

Treatment	*Spodopta frugipera* larval instar	Acetylcholinesterase Activity (µg AchBr/min/gm body weight)	Activity ratio
Steinernema carpocapsae (Sc)	4th	162.62±0.55	1.09
	6th	256.28±1.16	1.42
Heterorhabditis bacteriophora (HP88)	4th	260.13±19.42	1.74
	6th	265.58±0.55	1.47
Control (check)	4th	149.47±0.85	1.00
	6th	180.26±0.85	1.00
L.S.D.		128.29	

Activity is expressed as: µg AchBr release / gm body weight /min.

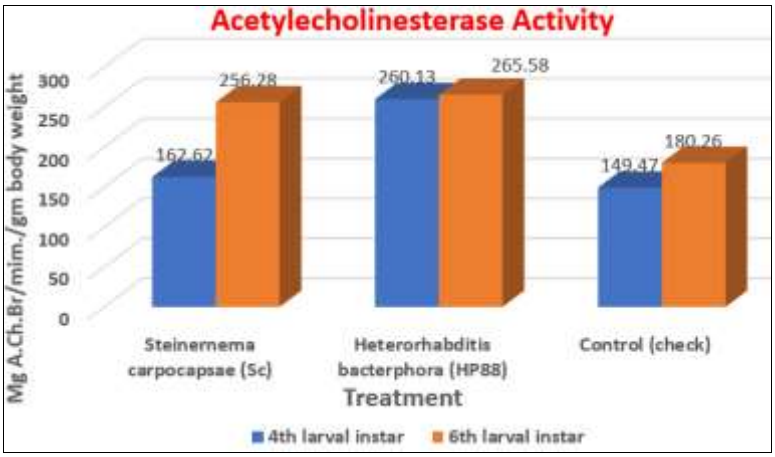

Fig 5: Acetyl cholinesterase activity in whole homogenates of *Spodoptera frugiperda* 4th and 6th larval instars infected with entomopathogenic nematodes under laboratory conditions (28±5 °C, 65±10 RH)

Activity of chitinase

Results in Fig. (6) and Table (6) makes clear that chitinase activity was highest (121.22) in the homogenate of *S. frugiperda* larvae (4th instar) treated with *Steinernema carpocapsae*, followed by the untreated (check) larvae with the activity value of 63.62. Although, the chitinase activity was lower in *S. frugiperda* 6th larval instar treated with *Steinernema carpocapsae* (53.54) followed by *Heterorhabditis bacteriophora* (42.39) compared to the untreated ones.

Insect chitinases and chitinase-like proteins can be classified into multiple families using phylogenetic analysis, as demonstrated by Arakane and Muthukrishnan (2010) [2]. Blocking the expression of the *S. frugiperda* chitinase gene had an effect on the old epidermis's chitin breakdown and growth of new epidermis (Liu *et al*., 2022) [13]. Furthermore, the increase in chitin content, inhibited the larvae ability to moult normally. Merzendorfer and Zimoch (2003) [14] have shown that structural remodeling involving chitin is essential for insect growth and morphogenesis. Because of this, chitin synthases and chitinolytic enzymes are found in a variety of tissues within insects' bodies.

The study's conclusions are in line with findings of Hamama *et al.* (2015) [9], who observed that enzymatic activity variations following bioinsecticide treatment suggest that these enzymes are affected by alterations in the midgut's physiological balance. El-Sheikh (2012) [5] investigated how *Bacillus thuringiensis* affect *Spodoptera. littoralis* and discovered that the enzymes that hydrolyze carbohydrates, such as trehalase, considerably decreased, and amylase insignificantly decreased when compared to the untreated sample.

Table 6: Chitinase activity in whole homogenates of fourth and sixth larval instar of *Spodoptera frugiperda* treated with entomopathogenic nematodes under laboratory conditions (28±5 °C, 65±10 RH)

Treatment	*Spodopta frugipera* larval instar	Chitinase Activity (µg N-acetylglucosamine released / g. b. wt. / min.)	Activity ratio
Steinernema carpocapsae (Sc)	4th	121.22±0.26	1.91
	6th	53.54±1.66	0.41
Heterorhabditis bacteriophora (HP88)	4th	15.47±0.26	0.24
	6th	42.39±0.35	0.32
Control (check)	4th	63.62±0.65	1.00
	6th	131.51±13.83	1.00
L.S.D.		129.37	

Each value represents the average of three replicates± S.E Activity is expressed as: µg N-acetylglucosamine released / g. b. wt. / min.

$$\text{Activity ratio} = \frac{\text{Enzymatic activity in larvae in different treatments}}{\text{Enzymatic activity in larvae of control (non-treated)}}$$

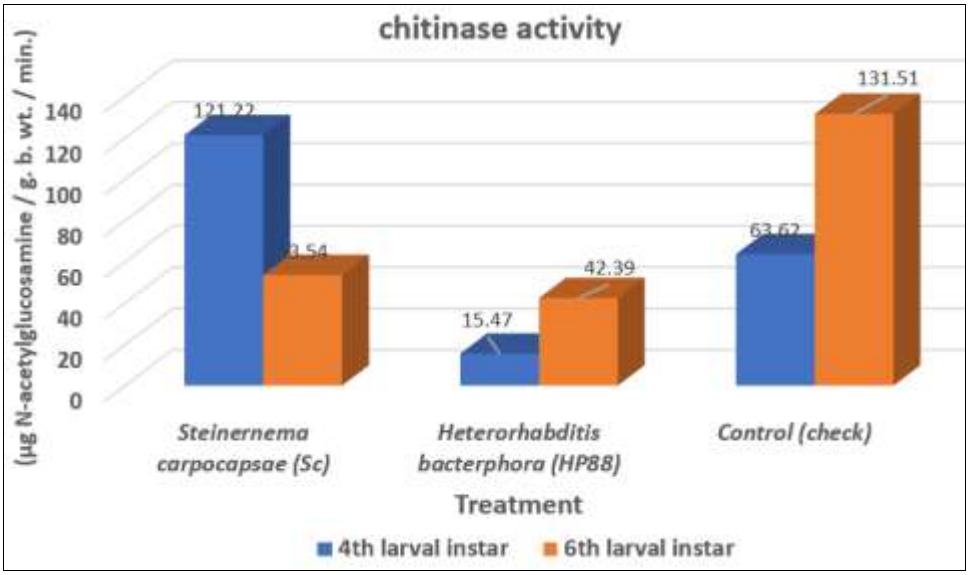

Fig 6: Chitinase activity in whole homogenate of *Spodoptera frugiperda* 4th and 6th larval instars treated with entomopathogenic nematodes under laboratory conditions (28±5 °C, 65±10 RH)

Conclusion

The total protein content in *Spodoptera frugiperda* larvae significantly varied due to infection by entomopathogenic nematodes. The untreated 4th larval instar exhibited the highest protein content (19.48 mg/g. b. wt./min.), while larvae infected with *Heterorhabditis bacteriophora* showed the lowest protein levels (0.59 mg/g. b. wt./min.). For the 6th instar, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* treatments resulted in protein contents of 39.36 mg/g and 27.91 mg/g, respectively, compared to the control (20.76 mg/g). These findings underscore the impact of entomopathogenic nematodes on the biochemical profiles of *S. frugiperda* larvae under laboratory conditions.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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