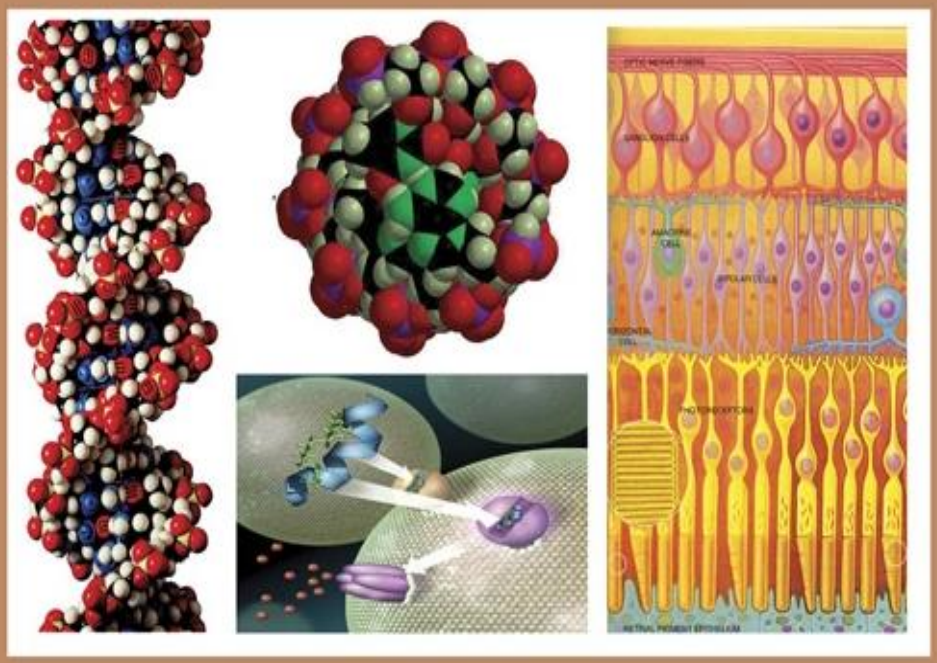
EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

C

PHYSIOLOGY & MOLECULARBIOLOGY



ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 12 No. 2 (2020)

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 12(2) pp.97-109(2020)*

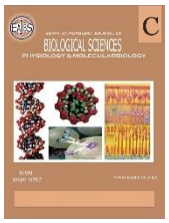
Egypt. Acad. J. Biolog. Sci., 12(2):97-109(2020)

Egyptian Academic Journal of Biological Sciences

C. Physiology & Molecular Biology

ISSN 2090-0767

www.eajbsc.journals.ekb.eg



Biochemical Virulence of Some Entomopathogenic Nematodes on *Galleria mellonella* Larvae (Lepidoptera: *Galleridae*)

Khater, K. S.[1], El-lakwah, S.F.[2], Abd-Elmonem, H. M.[2], Ahmed, F.A.[1], and Shoukry, I. F.[1],

1-Zoology Department, Faculty of Science - Zagazig University

2-Plant Protection Research Institute, Dokki, Giza, Egypt

E.Mail: k.shoukry@zu.edu.eg - k_s_kamel@hotmail.com

ARTICLE INFO

Article History

Received:27/8/2020

Accepted:19/11/2020

Keywords:

Entomopathogenic nematodes, *Heterorhabditis bacteriophora*(HP88), *Steinernema carpocapsae* (S.C), *Steinernema scapterisci* (S.S), *Steinernema glaseri* (S. g).

ABSTRACT

The present study aimed to verifies the effect of four strains of entomopathogenic nematodes, *Heterorhabditis bacteriophora* (HP88), *Steinernema carpocapsae* (S.C), *Steinernema scapterisci* (S.S), *Steinernema glaseri* (S. g) against the last larval instar of the greater wax moth *G. mellonella*. The biochemical alterations due to nematodes infection were conducted after four exposure times 6, 12, 24 and 48hrs and by 20, 50 and 100 infective juveniles (IJs) concentrations. Results demonstrate that all treatments provoke dysfunction in carbohydrate hydrolyzing enzymes (amylase, Trehalase and Invertase) and α- and β- esterase enzymes. At 20 (IJs) all treatments cause a significant reduction in the activity of amylase, in contrary both *S. scapterisi* (S.S) and *S. glaseri* (S. g) caused a non-significant increase at 6h after treatments. Significant elevation in the activity of amylase enzyme after 6h recorded by 50 IJs. The maximum level was reported after treatment with *H. bacteriophora* (HP88) (510.50±3.33 µg glucose/g. b. wt.) with a change percentage of 47.24% compared to control. Meanwhile, *H. bacteriophora* (HP88) strain caused a significant reduction in the activity of the enzyme (235.70±2.58 and 175.60±6.53 µg glucose/g. b. wt.) with a percentage of change, -59.86% and -68.67% after 12 and 48h, respectively compared with control. Also, HP88 treatment exhibited inhibition in trehalase activity at 100 (IJ) as well as, the measured values of the enzyme are (1431.08±3.33, 743.14±4.89 and 467.17±3.08 µg glucose/g. b. wt.) with inhibition percentage of -42.08, -41.00 and -52.50% after 6, 24 and 48hrs, respectively after treatments. The invertase enzyme activity showed a significant decrease at 20 (IJ) after 6 and 12 hrs and reversed action recoded, a significant increase after 24hrs with all strains as compared to control. At 50(IJ) The treatment with (S. g) caused an initial increase in α-esterase activity then, a significant decrease in the enzyme activity recorded and gave (270.10±1.85 and 234.00±2.21 µg β-naphthol /g. b. wt.) with percentages of -30.00 and -44.00 % after 24 and 48hrs, respectively of treatment compared with control. The treatment with (S.C) had a significant decrease in β esterase activity and reached (189.90±2.93µg β-naphthol /g. b. wt.) with percentage of -46.60% after 12hrs from treatment. On the contrary there was increase in β esterase enzyme recorded the highest values (442.20±2.25 and 523.40±2.61µg β-naphthol /g. b. wt.)), respectively after 24 and 48hrs of treatments.

INTRODUCTION

Galleria mellonella, honeycomb moth belongs to the family Galleridae, this insect is also known as the greater wax moth and distributed throughout the world. It has been reported in twenty-seven African countries. *G. mellonella* larvae are known to parasitized on combs of honeybee hives. The larvae tunnel through the honeycombs that contain honeybee larvae and their honey stores. The tunnels they create are lined with silk, which entangles and starves emerging bees, in a phenomenon known as galleriasis. Tunnels also result in huge destruction of the combs. As a result of larval activities, honey is wasted as it seeps outside when cell caps are consumed. Because of the serious economic loss caused by this species, numerous control methods including chemical fumigants and heat treatment have been used (Kwadha *et al.*, 2017). These methods can induce multiple side effects on man and non-target organisms. The search for new, safe biopesticides is being conducted all over the globe. The biological control agents especially nematodes consider important alternatives for chemical insecticides. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) are good biocontrol agents beside they cause rapid death of the insect host, they did not cause any side effects on non-target organisms. Entomopathogenic nematodes (EPNs) proven to have many advantages such as wide host range, rapid host death, actively seek and invade their hosts (Shapiro-Ilan *et al.*, 2012). The infective juveniles (IJs) of nematode penetrate through natural openings as mouth, anus and spiracles, or in some species of *Heterorhabditis spp*. through the cuticle and enter the hemocoel of the host releasing their symbiotic bacteria into the haemolymph of their host (Dowds and Peters, 2002). The lethal procedures caused by insecticidal active substances are often related to the decline in activity of some enzymes in the host insects (Grewal et al., 2005). Insects activate some defensive mechanisms, as Phenoloxidase, haemocytes, antioxidant and detoxification enzymes (Kunc *et al.*, 2017) and (Lalitha *et al.*, 2018). little information based on the enzymatic aspects of this pest after exposure to biopesticides, entomopathogenic nematodes (EPNs).

The present work focuses on determination of biochemical effects of the four EPNs, *H. bacteriophora*, *S. carpocapsae*, *S. scapterisci*, *S. glaseri* on some carbohydrate hydrolyzing enzymes (amylase, trehalase, invertase) and esterases (α-estrases and β-estrases) enzymes activities of last instar larvae of *G. mellonella*.

MATERAILS AND METHODS

Rearing Technique:

The greater wax moths were obtained from Plant Protection Research Institute and reared on a semi-synthetic artificial diet as described by Ibrahim *et al.* (1984). The insect cultured were reared in the laboratory at 28±2^{o}C and 60-70% R.H. for several generations far from any insecticidal contamination. The larvae could grow on a semi-synthetic diet layer of 5-7 cm then placed in a glass jar (9.40 cm diameters x 1.50 cm high) capacity and covered with plain paper fitted in place with two rubber bands. The larvae were supplied with an artificial diet to the developing till pupation. The larval faces and debris were cleaned out daily. After pupation, pupae were collected and transferred to wide glass jars until adult emergence. The emerged adults were collected and placed in pairs, males, and females per each glass jars as well as untreated adults. The deposited eggs were collected daily and transferred to clean glass jars then incubated at the previously mentioned condition to carry out the different experiments.

Entomopathogenic Nematodes:

The entomopathogenic nematode (EPNs) were obtained from a stock culture maintained for several generations in the laboratory of the Department of insect physiology, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. The four species of EPNs used for this investigation are *Heterorhabditis bacteriophora poiner* (HP88), *Steinernema*

carpocapsae (S.C), *Steinernema scapterisi* (S.S) and *Steinernema glaseri* (S.g).

Biochemical Studies:

Larval samples of *G. mellonella* conducted for biochemical assays were collected at 6, 12, 24 and 48hrs post-treatment with three concentrations, 20,50 and100 infective juvenile of the four nematode species, the treated larvae were weighed and homogenized in distilled water using a Teflon homogenizer. The homogenates were centrifuged at 5000 r. p. m for 30 minutes at 10ºC. The supernatants were kept in a deep freezer until use for estimation of carbohydrate hydrolyzing enzymes (amylase, trehalase, and invertase) and esterases (α- and β- esterase) enzymes.

1-Carbohydrate Hydrolyzing Enzymes:

The method used to estimate the digestion of starch, trehalose, and sucrose by amylase, trehalase, and invertase enzymes, respectively were conducted according to those described by **Ishaaya and Swiriski (1976)**.

2-Determination of Estrases:

α-estrases and β-estrases, as non-specific estrases, were determined colorimetrically according to the method described by (Van Asperen, 1962).

Statistical Analysis:

The significance of the main differences in biological and biochemical trials was determined by analysis of variance (ANOVA). Means were compared using L.S.D. (5% significance level). Data were subjected to statistical analyses via a software package Costat® Statistical Software (1990) a product of Cohort Software, Monterey, California.

RESULTS

The biochemical response on carbohydrate digestive enzymes (amylase, trehalase, and invertase) and esterases (α and βesterase) of the greater wax moth larvae of *G. mellonella* was assessed after treatments with both three different concentrations of the infective juveniles (20, 50 and 100 IJs / larva) of the four entomopathogenic nematode, *H. bacteriophora*, *S. carpocapsae*, *S. scapterisci*, *S. glaseri.*

Amylase Enzyme:

At 20 (IJ):

The results in table (1) signified that at 20 (IJ) all treatments cause a significant reduction in the activity of amylase, in contrary both *S. scapterisi* (S.S) and *S. glaseri* (S. g) caused a non-significant increase at 6h after treatments. The treatment with *H. bacteriophora* (HP88) had a high significant and significant decrease in the enzyme activity (204.00±3.42 and 261.90±2.16 µg glucose /g. b. wt.) with reduction percentage of -56.79 and -53.28% after 24 and 48h, respectively. Treatment with (S.S) leads to a significant reduction of the activity of the enzyme (287.10±2.58, 174.90±3.11 and 180.50±7.41 µg glucose/g. b. wt.) with reduction percentage of -51.11 %, -62.95% and -67.81% after 12, 24 and 48h, respectively.

At 50 (IJ):

High significant elevation in the activity of the amylase enzyme after 6h was recorded. The maximum level was reported after treatment with *H. bacteriophora* (HP88) (510.50±3.33 µg glucose/g. b. wt.) with a change percentage of 47.24% compared to control (346.7±3.02 µg glucose/g. b. wt.). Meanwhile, *H. bacteriophora* (HP88) strain caused a highly significant reduction in the activity of the enzyme (235.70±2.58 and 175.60±6.53 µg glucose/g. b. wt.) with a percentage of change, -59.86 and -68.67% after 12 and 48h, respectively compared with control. The data elucidated that, the treatment of *S. carpocapsae* (S.C) had a highly significant reduction in the amylase activity and recorded (206.40±2.18 and 197.30±1.56 µg glucose/g. b. wt.) with a percentage of -56.27 and -64.80% after 24 and 48hrs, respectively (Table 1).

At 100 (IJ):

Clear initial elevation in amylase activity levels at 6h recorded then decrease at the end of all treatments (Table 1). The treatment with (S. g) caused significant elevation to reach its maximum level to (520.00±3.04 µg glucose/g. b. wt.) and change percent 49.99% after 6h of treatment. In case of (S.S) treatment, there was a highly

significant reduction in the activity of the enzyme which attained (335.10±1.80 µg glucose/g. b. wt.) with reduction percent equal - 29.02% and (253.30±3.25 mg glucose /g. b. wt.) with reduction percent -54.81% after 24 and 48hrs, respectively. Also, the treatment of (S. g) strain caused a highly significant inhibition in the enzyme activity and reached its maximum level to (243.60±2.09 µg glucose/g. b. wt.) with a percentage of -58.52% after 12 h of treatment as compared to control.

Table 1: Activity of amylase enzyme in last instar larvae of *G. mellonella* treated with entomopathogenic nematodes, HP88, S.C, S.S and S. g after 6h., 12h., 24h., and 48h. exposure times**.**

Treatments	Amylase (µg glucose /g. b. wt.) At 20 IJ /hours							
	6h		12h		24h		48h	
	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)
HP88	202.50±2.44 b	-41.59	442.60±5.64 b	-28.04	204.00±3.42c	-56.79	261.90±2.16b	-53.28
S.C	316.10±8.83 a	-8.82	478.20±5.23ab	-18.58	387.60±3.24a	-17.90	249.90b±1.41c	-55.41
S. S	361.20±1.67 a	4.18	287.10±2.58c	-51.11	174.90±3.11c	-62.95	180.50±7.41d	-67.81
S. g	353.90±1.71a	2.07	387.10±3.59bc	-34.08	296.70±3.51b	-37.15	194.40±2.23cd	-65.33
Control	346.70±3.02a	-	587.30±4.73a	-	472.10±1.38a	-	560.50±3.67a	-
P	**		**		**		*	
At 50 IJ /hours								
HP88	510.50±3.33a	47.24	235.70±2.71c	-59.86	250.30±2.58bc	-46.98	175.60±6.53b	-68.67
S.C	428.20±2.50ab	23.52	464.20±3.78b	-20.97	206.40±2.18c	-56.27	197.30±1.56b	-64.80
S. S	488.30±2.66a	40.84	297.00±1.75c	-49.42	281.90±2.15b	-40.29	217.30±2.06b	-61.24
S. g	388.60±3.38b	12.09	323.00±2.53c	-45.01	255.30±1.73bc	-45.93	217.00±1.64b	-61.28
Control	346.70±3.02b	-	587.30±4.73a	-	472.10±1.38a	-	560.50±3.67a	-
P	**		**		**		**	
At 100IJ /hours								
HP88	404.80±3.20ab	16.76	325.20±1.51b	-44.62	401.00±3.18bc	-15.05	293.30±3.74c	-47.68
S.C	498.40±3.92a	43.75	327.40±3.90b	-44.25	593.60±4.85a	25.73	291.60±1.50c	-47.97
S. S	420.00±6.55ab	21.15	502.60±3.09a	-14.43	335.10±1.80c	-29.02	253.30±3.25c	-54.81
S. g	520.00±3.04a	49.99	243.60±2.09b	-58.52	375.10±2.61c	-20.55	402.10±4.37b	-28.26
Control	346.70±30.21b	-	587.30±47.38a	-	472.10±13.84b	-	560.50±36.73a	-
P value	*		**		**		**	

Each datum represents the mean of five replicates.
Means within a column followed by the same letter are not significantly different (LSD test, P<0.05).
Significant difference, p*<0.05, high significant difference, p** < 0.01.
(HP88**,** *Heterorhabditis bacteriophora*; SA, specific activity; S.C, *Steinernema carpocapsae*; S.g, *Steinernema glaseri*; S.S, *Steinernema scapterisci*; RA, relative activity).

Trehalase Enzyme:

At 20 (IJ):

The results presented in table (2) showed that the treatment with HP88 provoke a highly significant decrease in enzyme activity (708.45±2.79 µg glucose /g. b. wt.) with a reduction percentage of -43.80% after 24h of treatment. For (S.S) treatment, there was a highly significant reduction of the enzyme activity (1207.77±3.14 µg glucose/g. b. wt.), with reduction percentage -51.70%; (1367.22±2.22 µg glucose /g. b. wt.), with reduction percentage -17.60% and (876.45±2.79 µg glucose/g. b. wt.), with reduction percentage -10.90% after 6, 12 and 48hrs, respectively compared with control(2503±3.54).

At 50 (IJ):

The results in table (2) indicated that the (S.S) treatment had a highly significant decrease in the activity of the enzyme (1310.39±3.45 µg glucose/g. b. wt.), with -

21.00% reduction percentage and (736.45±5.27 µg glucose/g. b. wt.), with -25.20% reduction percentage after 12 and 48h, respectively. For (S. g) treatment, there was a highly significant reduction of enzyme activity (1457.24±4.22 µg glucose/g. b. wt.), with -41.80% reduction percentage; (878.71±4.72 µg glucose/g. b. wt.) with a percentage of -30.30 % after 6 and 24h, respectively.

At 100 (IJ):

Data represented in the same table showed that, the treatment with (S.C) elicited a highly significant decrease in the activity of enzyme reached to (1264.95±1.73 µg glucose/g. b. wt.) with reduction percentage of -49.50% after 6hrs of treatment. Also, HP88 treatment exhibited high significant inhibition in trehalase activity as well as, the measured values of the enzyme are (1431.08±3.33, 743.14±4.89 and 467.17±3.08 µg glucose/g. b. wt.) with inhibition percentage of -42.08, -41.00 and -52.50% after 6, 24 and 48hrs, respectively after treatments. For (S. g.) treatment there was a highly significant reduction in the activity of the enzyme attained (1398.36±5.42 µg glucose /g. b. wt.), with a -44.10 % reduction percentage after 6 hrs from treatment compared with control. Furthermore, (S.S) caused a highly significant reduction reached (1424 ±4.42 µg glucose/g. b. wt.) with -43%.

Table 2: Activity of trehalase enzyme in last instar larvae of *G. mellonella* treated with the entomopathogenic nematode, HP88, S.C, S.S and S. g after 6h., 12h., 24h., and 48h. exposure times.

Treatments	Trehalase (µg glucose /g. b. wt.) At 20IJ /hours							
	6h		12h		24h		48h	
	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)
HP88	2024.91±7.77c	-19.1	1579.70±2.81a	-4.79	708.45±2.79b	-43.8	1149.52±3.68b	16.81
S.C	2287.57±4.66b	-8.61	1564.02±3.60a	-5.74	1376.04±4.52a	9.2	1272.71±2.48a	29.33
S. S	1207.77±3.14c	-51.7	1367.22±2.22b	-17.6	1336.04±4.41a	6.03	876.45±2.79d	-10.9
S. g	1703.75±1.01d	-31.9	1567.22±3.79a	-5.54	1347.34±4.46a	6.92	906.11±1.12cd	-7.93
Control	2503.06±3.54a	-	1659.18±3.49a	-	1260.09±3.46a	-	984.11±5.45c	-
P	**		**		**		**	
At 50IJ /hours								
HP88	1683.61±3.25b	-32.7	1518.71±5.81b	-8.47	1450.40±4.08a	15.10	879.03±2.25a	-10.7
S.C	1507.34±4.32cd	-39.8	1437.24±4.24bc	-13.4	1378.19±3.31a	9.37	870.11±3.16a	-11.6
S. S	1608.19±2.76c	-35.8	1310.39±3.45c	-21.0	1196.04±2.95b	-5.08	736.45±5.27b	-25.2
S. g	1457.24±4.22d	-41.8	1358.19±5.28c	-18.1	878.71±4.72c	-30.3	896.45±2.57a	-8.91
Control	2503.06±3.54a	-	1659.18±3.49a	-	1260.09±3.46b	-	984.11±5.45a	-
P	**		**		**		**	
At 100IJ /hours								
HP88	1431.08±3.33b	-42.80	1350.4±2.25b	-18.6	743.14±4.89c	-41.00	467.17±3.08c	-52.5
S.C	1264.95±1.73c	-49.50	1367.64b±2.16	-17.6	973.48b±2.95	-22.70	746.11b±5.41	-24.2
S. S	1424.95±4.42b	-43.1	1353.64±2.15b	-18.4	953.48±1.54b	-24.3	716.11±9.02b	-27.2
S. g	1398.36±5.42b	-44.1	1213.48±2.30c	-26.9	973.48±1.14b	-22.7	796.45±4.62b	-19.1
Control	2503.06±3.54a	-	1659.18±3.49a	-	1260.09±3.46a	-	984.11±5.45a	-
P	**		**		**		**	

Each datum represents the mean of five replicates.

Means within a column followed by the same letter are not significantly different (LSD test, P<0.05).

High Significant difference p** < 0.01. (HP88**,** *Heterorhabditis bacteriophora*; SA, specific activity; S.C, *Steinernema carpocapsae*; S.g, *Steinernema glaseri*; S.S, *Steinernema scapterisci*; RA, relative activity).

Invertase Enzymes:

At 20 (IJ):

Remarkably, all treatments always elicited a highly significant reduction in invertase activity at all intervals apart from the treatment after 24hrs which resulted in a highly significant elevation in the activity of invertase (Table 3). The maximum elevation recorded (1632.53±1.85 µg glucose /g. b. wt.) with a percentage of 33.12% after 24hrs of treatment with (S.C) strain. In case of (HP88) treatment, there was a highly significant reduction of the enzyme activity attained (1323.22±2.69 µg glucose/g. b. wt.) and -47.60% reduction percentage after 6hrs from treatment. Significant decrease in the activity of the enzyme reached to (1430.40±3.99 and 516.17±4.29 µg glucose/g. b. wt.) with reduction percentage of -20.60 and -41.00% reduction percentage after 12 and 48hrs of treatment with (S.S), respectively. In case of (S. g) treatment, a significant reduction in enzyme activity was found after 48hrs of reached to (493.60±2.29 µg glucose /g. b. wt.) with a reduction percentage of -43.60% as compared to control.

At 50 (IJ):

In case of (HP88) treatment, there was a highly significant reduction in the enzyme activity attained (1588.59±3.47 ,1251.69±7.63, 959.49±3.13 and 412.72±5.62 µg glucose/g. b. wt.) with reduction percentage of -37.0%, -30.50, -21.80 and -52.80 %after 6, 12, 24 and 48hrs of treatment, respectively compared to control. The results in the same table showed that the (S.C) treatment caused a significant decrease in the enzyme activity and reached to (1516.42±3.20 µg glucose /g. b. wt.) and percentage of -40.00 % after 6hrs of treatment.

At 100 (IJ):

Generally, all EPNs species produced a significant reduction in enzyme activity after 12 and 24hrs of treatment. At (S.C) strain a significant decrease in the enzyme activity reached to (886.84±7.21 µg glucose/g. b. wt.) with a percentage of change equal -27.70 % after 24 hrs of treatment, respectively compared to control. In addition (S.S) treatment exhibited significant inhibition in invertase enzyme activity recorded (414.59±3.58 µg glucose /g. b. wt.) with change percent -52.60 % after 48hrs of treatment compared with control. A high significant reduction was recorded after 6 and 48hrs of treatment. (HP88) the treatment causes a decrease in the enzyme activity reached to (1488.26±6.33, and 559.98±3.01µg glucose/g. b. wt.) with change percent of -41.10, and -36.00% after 6, and 48hrs from treatment, respectively.

Table 3: Activity of invertase enzyme in last instar larvae of *G. mellonella* treated with entomopathogenic nematodes (HP88, S.C, S.S, and S. g) after 6h., 12h., 24h., and 48h. exposure times.

Treatments	Invertase (µg glucose/ g. b. wt.) At 20IJ /hours							
	6h.		12h.		24h.		48h.	
	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)
HP88	1323.22±2.69d	-47.60	1460.10±4.33ab	-18.90	1431.69±4.03ab	16.74	536.17±6.42b	-38.70
S.C	1503.11±3.39cd	-40.50	1718.49±1.59ab	-4.60	1632.53±1.85a	33.12	771.31±5.06a	-11.90
S. S	1731.61±8.30b	-31.50	1430.40±3.99b	-20.60	1614.40±1.74a	31.64	516.17±4.29b	-41.00
S. g	1604.42±3.59bc	-36.50	1643.85±3.86ab	-8.74	1420.10±2.97ab	15.80	493.60±2.29b	-43.60
Control	2527.48±1.23a	-	1801.38±1.29a	-	1226.38±8.29b	-	875.18±5.53a	-
P	**		**		**		**	
At 50IJ /hours								
HP88	1588.59 [b] ±3.47	-37.10	1251.69±7.63b	-30.50	959.49±3.13b	-21.80	412.72±5.62c	-52.80
S.C	1516.42±3.20b	-40.00	1582.85±2.32ab	-12.10	1377.29±5.018a	12.31	698.84±1.52b	-20.10
S. S	1676.42±8.79b	-33.70	1303.85±2.46b	-27.60	1317.29±1.95a	7.41	715.18±1.99b	-18.30
S. g	1596.09±3.09b	-36.90	1363.85±3.30b	-24.60	1317.29±2.81a	7.41	839.18±4.29a	-4.11
Control	2527.48±1.23a	-	1801.38±2.072a	-	1226.38±1.29a	-	875.18±5.53a	-
P	**		*		*		**	
At 100IJ /hours								
HP88	1488.26±6.33b	-41.10	1341.19±4.59b	-25.50	960.10±4.85b	-21.70	559.98±3.01b	-36.00
S.C	1653.76±1.88b	-34.60	1562.76±7.14ab	-13.30	886.84±7.21b	-27.70	494.59±0.15bc	-43.50
S. S	1513.76±4.59b	-40.10	1402.22±2.45b	-22.20	914.84±3.99b	-25.40	414.59±3.58c	-52.60
S. g	1493.76±5.40b	-40.90	1542.22±4.63ab	-14.40	986.84±3.66b	-19.50	474.59±4.88bc	-45.80
Control	2527.48±1.23a	-	1801.38±2.07a	-	1226.38±1.29a	-	875.18±5.50a	-
P	**		*		*		**	

Each datum represents the mean of five replicates.
Means within a column followed by the same letter are not significantly different (LSD test, P<0.05).
A significant difference, p*<0.05, highly significant difference, p** < 0.01. (HP88**,** *Heterorhabditis bacteriophora*; SA, specific activity; S.C, *Steinernema carpocapsae*; S.g, *Steinernema glaseri*; S.S, *Steinernema scapterisci*; RA, relative activity).

Alpha Esterase Enzymes (α-esterase):

Table (4) illustrated the variations in values of α-esterase activity in treated greater wax moth with the four nematode strains and significant differences after the four exposure times of treatment.

At 20 IJs:

The results in table (4) showed that HP88 treatment also caused a reduction in α-esterase reaching (42.38±2.46 and 58.04±1.20 µg α-naphthol/g. b. wt.) with a percentage of -62.20% and -38.90 after 12 and 24 hrs of treatment, respectively. In addition, treatment with (S.C) causes a highly significant decrease in the enzyme activity and reached to maximum values (55.86±2.02 and 57.68±4.22 µg α-naphthol /g. b. wt.) with percentages of change -34.00 and -62.50% after 6 and 12hrs of treatment, respectively. In case of (S.S) treatment, there was a highly significant reduction in the activity of α - esterase enzyme after 6 and 12hrs of treatment and gave (51.26±1.62 and 56.97±4.12 µg α-naphthol /g. b. wt.) with a percentage of -39.40and -62.90 % compared to control.

At 50 IJs:

The treatment of *G. mellonella* larvae with all EPNs caused a highly significant inhibition in the enzyme activity. (HP88) treatment caused a significant reduction in the activity and reached to (51.99±3.10, 44.03±1.19 and 38.28±2.47 µg α-naphthol/g. b. wt.) with a percentage of -38.60, -71.30 and -44.80 % after 6, 12 and 24hrs of treatment. The treatment with (S.C) strain caused the initial decrease in α-esterase enzyme activities

reached to (36.47±3.56 and 30.09±3.77 µg α-naphthol /g. b. wt.) with a percentage of -76.30and -56.60 % after 12 and 24hrs, respectively. Also, in case of (S. g.) treatment, there was a high significant reduction of enzyme activity reaching (41.18±4.19 and 47.36±2.19 µg α-naphthol /g. b. wt.) with -73.20% and -48.30% change after 24 and 48hrs of treatment compared to control.

At 100 (IJ):

The results showed that the treatment of last instar larvae with (HP88) provoke a significant reduction of the enzyme activity gave (48.01±2.64 and 31.00±2.79 µg α-naphthol /g. b. wt.) with a percentage of – 68.80 and – 66.20% after 12 and 48hrs of treatment. In case of (S.C), high significant decreased in the activity of the enzyme after 6 hrs and 12hrs reaching (48.15±2.42 and 67.49±3.05 µg α-naphthol /g. b. wt.) with a percentage of -43.10 and – 56.10%, respectively. Additionally (S.S) treatment, there was a significant inhibition of α – esterase activity and reaching (69.49±3.18 and 45.89±2. 4 µg α-naphthol /g. b. wt.) with a percentage of -54.80 and -49.90% after 12 and 48hrs of treatment, respectively as compared to control. Also, after 12 and 48hrs of treatment with (S. g) significant inhibition of α – esterase activity recorded (69.29±3.97 and 47.49±2. 47 µg α-naphthol /g. b. wt.) with percentage of -54.80 and -48.20%. In contrary a high significant increase in the enzyme activity reported after 24 hrs of treatment reached 75.06±6.00 with change percent 8.27%.

Table 4: Activity of (α-esterase) enzyme in last larval instar of *G. mellonella* treated with entomopathogenic nematodes, HP88, S.C, S.S and S. g after 6h., 12h., 24h., and 48h. exposure times

Treatments	α-esterase (µg β-naphthol /g. b. wt.) At 20IJ /hours							
	6hrs		12hrs		24hrs		48hrs	
	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)
HP88	66.71±3.73b	-21.20	58.04±1.20c	-62.20	42.38±2.46d	-38.90	58.12±2.88c	-36.60
S.C	55.86±2.02c	-34.00	57.68±4.22c	-62.50	89.06±6.03a	28.46	85.35±3.04a	-6.87
S. S	51.26±1.62c	-39.40	56.97±4.12c	-62.90	62.38±2.23c	-10.00	64.52±2.54c	-29.60
S. g	53.06±3.8c	-37.30	83.15±2.28b	-45.90	77.99±5.59ab	12.50	77.82±2.03b	-15.10
Control	84.64±3.67a	-	153.60±3.87a	-	69.32±b2.98c	-	91.65±2.09a	-
P value	**		**		**		**	
At 50IJ /hours								
HP88	51.99±3.10c	-38.60	44.03±1.19b	-71.30	38.28±2.47bc	-44.80	49.38±3.67bc	-46.10
S.C	62.17b±3.84	-26.50	36.47b±3.56	-76.30	30.09c±3.77	-56.60	55.56b±1.96	-39.40
S. S	61.64bc±4.06	-27.20	42.50±3.05b	-72.30	33.99±1.01bc	-51.00	53.16±1.99bc	-42.00
S. g	56.01bc±1.86	-33.80	41.18±4.19b	-73.20	40.53±3.79b	-41.50	47.3±2.196c	-48.30
Control	84.64±3.67a	-	153.60±3.87a	-	69.32±2.98a	-	91.65±2.09a	-
P value	**		**		**		**	
At 100IJ /hours								
HP88	57.21±0.87b	-32.40	48.01±2.64c	-68.80	65.52±1.75b	-5.48	31.00±2.79c	-66.20
S.C	48.15±2.42b	-43.10	67.49±3.05b	-56.10	81.84±5.22a	18.06	50.41±4.11b	-45.00
S. S	54.23±4.19b	-35.90	69.49±3.18b	-54.80	65.34±1.31b	-5.75	45.89±2. 4b	-49.90
S. g	81.83±4.33b	-3.31	69.29±3.97b	-54.90	75.06±6.00ab	8.27	47.49±2.74b	-48.20
Control	84.64±3.67a		153.60±3.87a		69.32±2.98b		91.65±2.09a	
P value	**		**		**		**	

Each datum represents the mean of five replicates.
Means within a column followed by the same letter are not significantly different (LSD test, P<0.05).
High significant difference p** < 0.01. (HP88**,** *Heterorhabditis bacteriophora*; SA, specific activity; S.C, *Steinernema carpocapsae*; S.g, *Steinernema glaseri*; S.S, *Steinernema scapterisci*; RA, relative activity).

Beta esterase Enzyme (β -esterase):

Data obtained from table (5) clarify that, all EPNs caused a fluctuation by increasing and decreasing in values of β-esterase enzyme activity not only at different nematode strains but also at different concentrations after four exposure times of experiment.

At 20 (IJ):

EPNs show a significant difference between each other. The results showed that, in case of (HP88) treatment, there was a significant reduction of the enzyme activity attained (188.60±7.62 and 190.79±3.80 µg β-naphthol /g. b. wt.) with percentages of -45.80 and -54.50% after 6 and 48hrs of treatment. The treatment with (S.C) had a significant decrease and variable effect in the activity of the enzyme and reached (189.90±2.93µg β-naphthol /g. b. wt.) with a percentage of -46.60% after 12hrs from treatment. On the contrary, in the same species, there was a highly significant increase in β esterase enzyme recorded the highest values (442.20±2.25 and 523.40±2.61µg β-naphthol /g. b. wt.)), respectively after 24 and 48hrs of treatments. For the treatment with (S.S), there was a highly significant reduction of enzyme activity recorded (256.50±3.12 µg β-naphthol/g. b. wt.) with percentage -33.50% and (175.50±2.75 µg β-naphthol /g. b. wt.) and -58.10 % after 24 and 48hrs of treatment compared with control. Also, S. g treatment cause high significant inhibition in the activity of β-esterase enzyme reached to (177.30±2.03 µg β-naphthol/g. b. wt.) with -57.70% after 48hrs of treatments.

At 50 (IJ):

The results in the same table cleared that, in (HP88) treatment at 6hrs initial significant increase in the enzyme activity(384.80±2.29 µg β-naphthol/g. b. wt.), then enzyme activity reversed and highly significant reduction reached (246.50±3.67µg β-naphthol/g. b. wt.) with -30.70% after 12hrs of treatment. In case of (S.C) treatment, a significant increase in values of β-esterase enzyme activity after the first three times of experiment was recorded. The highest increase recorded 533.20±2.26 with a percentage of 50.01% at 12hrs of the treatment, with the exception at the end of the experiment high significant reduction in the enzyme activity reached (185.70±3.61 µg β-naphthol /g. b. wt.) and -55.70 % after 48hrs. For (S.S) treatment, there was a significant reduction of enzyme activity achieved (285.20±2.90 with -18%) after 6hrs of treatment and a highly significant reduction (265.80±3.62 and 248.10±1.47 µg β-naphthol /g. b. wt.) with treatment percentages of -25.20 and -40.80 % after 12 and 48hrs of treatments, respectively. The treatment with (S. g) caused an initial increase then causes a disturbance in its values to the end of the experiment. A significant decrease in the enzyme activity estimated and gave (270.10±1.85 and 234.00±2.21 µg β-naphthol /g. b. wt.) with percentages of -30.00 and -44.00 % after 24 and 48hrs, respectively of treatment compared with control.

At 100 IJ:

The results showed that all the treatments with EPNs caused a disturbance in the enzyme activity. The highest increase was recorded after 24hrs of treatment with (S.C) and gave (574.80±3.54 µg β-naphthol /g. b. wt.) with 49.03% compared to control. In case of HP88 treatment caused a significant increase in the activity of β-esterase enzyme after four times of experiment. While the lowest value recorded after treatment with (S.S) which had a highly significant reduction of the enzyme activity after 6hrs recorded (164.50±1.33 µg β-naphthol /g. b. wt.) with a percentage of -52.70% comparing with control.

Table 5: Activity of β esterase enzyme in last instar larvae of *G. mellonella* treated with entomopathogenic nematode, HP88, S.C, S.S, and S. g after 6h., 12h., 24h., and 48h. exposure times.

Treatments	(β-esterase) (µg β- naphthol/ g. b. wt.) /hours At 20IJ /hours							
	6hrs		12hrs		24hrs		48hrs	
	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)	SA (Mean ±SE)	RA (%)
HP88	188.60±7.62b	-45.80	264.90±3.38cd	-25.50	494.50±6.47a	28.21	190.79±3.80c	-54.50
S.C	298.80±49.22a	-14.10	189.90±2.93d	-46.60	442.20±2.25a	14.63	523.40±2.61a	24.93
S. S	280.50±1.19ab	-19.30	441.10±1.48a	24.11	256.50±3.12b	-33.50	175.50±2.75c	-58.10
S. g	338.00±4.52a	-2.80	306.80±1.39c	-13.70	404.80±2.99a	4.94	177.30±2.03c	-57.70
Control	347.70±3.38a	-	355.40±3.02b	-	385.70±1.52a	-	419.00±3.58b	-
P value	*		**		**		**	
At 50IJ /hours								
HP88	384.80±2.29ab	10.67	246.50±3.67c	-30.70	300.70±3.85c	-22.10	343.62±2.80a	-18.00
S.C	449.80±3.80a	29.34	533.20±2.26a	50.01	403.20±1.53ab	4.52	185.70±3.61b	-55.7
S. S	285.20±2.90c	-18.00	265.80±3.62c	-25.20	475.60±3.07a	23.31	248.10±1.47b	-40.80
S. g	355.00±2.42bc	2.10	378.90±5.23b	6.61	270.10±1.85c	-30.00	234. 0±2.21b	-44.00
Control	347.70±3.38bc	-	355.40±3.02bc	-	385.70±1.52a	-	419.00±3.58a	-
P value	*		**		**		**	
At 100IJ /hours								
HP88	394.00±2.90b	13.32	369.50±3.26ab	3.95	499.60±3.24a	29.53	518.70±3.49a	23.80
S.C	477.40±3.17a	37.29	449.60±5.87a	26.49	574.80±3.54a	49.03	374.40±6.15b	-10.60
S. S	164.5.00±1.33c	-52.70	254.20±4.42b	-28.50	233.40±2.68c	-39.50	314.40±2.92b	-25.00
S. g	242.5.00±2.41c	-30.30	298.40±2.74b	-16.10	560.10±1.12a	45.22	354.40±2.37b	-15.40
Control	347.70±3.38b	-	355.40±3.02ab	-	385.70±1.52b	-	419.00±3.58ab	-
Pvalue	**		*		**		*	

Each datum represents the mean of five replicates.
Means within a column followed by the same letter are not significantly different (LSD test, P<0.05).
A significant difference, p*<0.05, highly significant difference, p** < 0.01. (HP88**,** *Heterorhabditis bacteriophora*; SA, specific activity; S.C, *Steinernema carpocapsae*; S.g, *Steinernema glaseri*; S.S, *Steinernema scapterisci*; RA, relative activity).

DISCUSSION

Little studies describe the effects of sublethal doses of entomopathogenic nematodes infection on carbohydrate hydrolyzing enzymes and estreases in *G. mellonella*. The trend of almost all treatments in the present investigation provokes a significant reduction in the activities of amylase, trehalase and invertase as compared to control. Reduction of amylase, trehalase and invertase activities lead to the disturbance in carbohydrates metabolism and consequently affect the chitin synthesis. Carbohydrates are generally used as energy supply while sugars serve as an energy reserve for the metabolic processes and in response to stress conditions imposed on an organism. Amylase enzyme is necessary to hydrolyze carbohydrates present in larvae. Most treatments showed an inhibitory action on amylase activity that, in harmony with the results obtained by (Zółtowska, 2006) who reported a significant reduction in the activity of α amylase enzyme after treatment of last larval instar of *G. mellonella* with *S. affinis* and *S. feltiae* at all exposure periods except a significant increase recorded after 12, 18 and 24 of *S. feltiae* infection. (Ahmed *et al.*, 2014). Declared that, amylase activity decreased in *Spodoptera littoralis* larvae with the infection by *H. bacteriophora*, *S. riobrave* and *S. feltiae.*

Trehalase enzyme hydrolyses the disaccharide trehalose, the main sugar in

insect haemolymph to glucose. The results of this investigation prove that trehalase activity significantly decreased after treatment with most nematode concentrations. These results are in accordance with the findings of many authors, Candy and Kilby (1962) announced the disturbance in trehalase activity in desert locust might inhibit the supply of glucose necessary for chitin reinforcement. Dmitryjuk *et al.* (2001) recorded a slight decrease in the activity of trehalase in the third larval instar of *G. mellonella* infected with the infective juvenile of *S. affinis* compared to the control. (Zółtowska, 2006) prove a significant decrease in the activity of trehalase of seventh larval instar of *G. mellonella* larvae following 6 and 12hrs of infection with *S. affinis* and *S. feltiae* compared with control experiment. On contrary, (Zółtowska, 2004) declared that the activity of hydrolases amylase, maltase, and trehalase following 48hrs infection of (20 IJ/insect) with *Heterorhabditis zealandica* was higher than that of control. The value of trehalase enzyme activity recorded a high significant increase at 18 and 24 hrs compared with control. Ahmed *et al.* (2014) pronounced an increase in invertase and trehalase activity with the infection of *S. littoralis* by *S. riobrave* and *H. bacteriophora* species. Ibrahim *et al.* (2015) stated that the activity of carbohydrate digestive enzymes (amylase, trehalase, and inveratse) reduces following nematode infection. A fluctuation in invertase activity was recorded in *H. zealandica* infected 4th larval instar of *Agrotis ipsilon,* while an increase in invertase activity in larvae infected with *S. abbasi* was detected till 24hrs of infection then the invertase activity was decreased compared with untreated larvae.

Esterases are large classes of enzymes, they categorized as can break ester bond through hydrolysis. Most enzymes of this group are important in metabolism (Sivakumarm and Maya, 1991), but also play a critical role in detoxification (Shen and Dowd, 1991). Enzymatic changes in the concentrations of alpha and beta esterase in nymphs of *Schistocerca gregaria* were evaluated by Shairra and Awad (2011) in response to *H. bacteriophora* infection. The level of alpha esterase has significantly increased by increasing time, while levels of beta esterase have dropped significantly by increasing post-inoculation time. Gaber *et al.* (2018) prove that the activity of β esterase significantly decreased in the 5th nymphal instar of desert locust with increasing nematodes concentration compared with control.

REFERENCES

Ahmed, F.N., Maklad, A.M.H., Yassin, S. A., Abolmaaty, S.M. (2014): Biochemical effects of *Steinernema feltiae, Steinernema riobrave and Heterorhabditis bacteriophora* on *Spodoptera littoralis* larvae. *Egyptian Academic Journal of Biological Sciences,* 6(1): 23-34. DOI: 10.21608/ eajbsc.2014.16044.

Candy, D.J., Kilby, B.A. (1962): Studies on chitin synthesis in desert locust. *Journal of Expermintal Biology,* 39: 129-140.

Costat Statistical Software (1990): Microcomputer program analysis. Version 4.2, Cohort Software, Berkeley, CA.

Dmitryjuk, M., Lopieńska, E., Zołtowska, K. (2001): The concentration of trehalose and activity of trehalase from *Galleria mellonella* larvae infected by *Steinernema affinis,* Bovien 1937 (Nematoda: Rhabditida: Steinernematidae). *Wiadomości parazytologiczne,* 47(3):305-309.

Dowds, B. C. A., Peters, A. (2002): Virulence mechanisms. Pp. 79–98 *in* R. Gaugler, ed. Entomopathogenic Nematology. New York: CABI.

Gaber, M. A. M., Shamseldean, M. S. M., Nabil, M. I., Rabia, M. M. (2018): Impact and biochemical changes of Egyptian and imported entomopathogenic nematodes on the desert locust *Schistocerca gregaria* (Forskal, 1775) (Orthoptera: Acrididae). *Middle East Journal of Agriculture Research,*7 (4): 1528-1544.

Grewal, P., Ehlers, R. U., Shapiro, I. D. I.

(Eds.), (2005): Nematodes as biological control agents. Wallingford, UK.

Ibrahim, S. H., Ibrahim, A. A., Fayad, Y. H. (1984): Studies on mass rearing of the wax moth, *Galleria mellonella* L. and its parasite *Apanteles galleriae* Wilk. with some biological notes on the parasite. *Agricultural Research Review*, 62 (1): 349-353.

Ibrahim, S.A.M., Taha, M.A., Salem, H.H.A., Farghaly, D.S. (2015): Changes in enzyme activities in *Agrotis ipsilon* (Lepidoptera, noctuidae) as a response to entomopathogenic nematode infection. *International Journal of Advanced Research,* 3: 111-118.

Ishaaya, I., Swirski, E. (1976): Trehalase, invertase and amylase activities in black scale *Saissetia oleae* and their relation to host adaptaility. *Journal of Insect Physiology,* 16: 1025-1029.https://doi.org/10.1016/0022-1910(76)90087-1.

Kunc, M., Arefin, B., Hyrsl, P., Theopold, U. (2017): Monitoring the effect of pathogenic nematodes on locomotion of Drosophila larvae.*Fly,*11(3):208-217. https://doi.org/ 10.1080/ 19336934.2017.1297350.

Kwadha, C. A., Ong'amo, G. O., Ndegwa, P. N., Raina, S. K., Fombong, A. T. (2017): The biology and control of the greater wax moth, *Galleria mellonella*. *Insects,* 9; 8(2): 61. DOI: 10.3390/ insects8020061.

Lalitha, K., Karthi, S., Vengateswari, G., Karthikraja, R. Perumal, P., Shivakumar, M. S. (2018): Effect of entomopathogenic nematode of *Heterorhabditis indica* infection on immune and antioxidant system in Lepidopteran pest *Spodoptera litura* (Lepidoptera: Noctuidae). *Journal of Parasitic Diseases*, 42 (2): 204-211. DOI: 10.1007/s12639-018-0983-1.

Shairra, S.A., Awad, H.H. (2011): Effect of the Entomopathogenic Nematode *Heterorhabditis bacteriophora* (Hp 88) and the Garlic Extract *Allium sativum* on the Immune Challenge of the Desert Locust, *Schistocerca gregaria* (Forskal). *Egyptian Journal of Biological Pest Control,* 21(1), p.11.

Shapiro-Ilan, D.I., Han, R., Dolinksi, C., (2012): Entomopathogenic nematode production and application technology. *Journal of Nematology,* 44(2), p.206.

Shen, S.K., Dowd, P.F. (1991): 1-Naphthyle acetate esterase activity from cultures of the symbiont yeast of the cigarette beetle (Coleoptera: Anobiidae). *Journal of Economic Entomology*, 84(2): 402-407. https://doi.org/ 10.1093/jee/84.2.402.

Sivakumaran, S., Maya, Z. B, (1991): Electrophoretic characterization of esterase in the greenbug, *Schizaphis germanium*. *Journal of the Kansas Entomological Society,* 64: 357-362.

Van Asperen, K. (1962): A study of housefly esterase's by means of a sensitive colorimetric method. *Journal of Insect Physiology,* 8(4): 401-416. https:// doi. org/10.1016/00221910(62)90074-4.

Zółtowska, K. (2004): Content of saccharides and activity of alpha-glycosidases in *Galleria mellonella* larvae infected with entomopathogenic nematodes *Heterorhabditis zealandica*. *Wiadomości parazytologiczne*, 50 (3):495-501.

Żółtowska, K., Łopieńska E. (2006): Content of glycogen and trehalose and activity of alpha-amylase and trehalase in *Galleria mellonella* larvae infected with entomopathogenic nematodes *Steinernema affinis* and *S. feltiae. Wiadomości parazytologiczne,* 52(2): 103-7.

ARABIC SUMMARY

القدرة البيوكيميائية لبعض النيماتودا الممرضة للحشرات علي يرقات فراشة الشمع الكبري جاليريا ميلونيلا (حرشفية الاجنحة: جاليريدي)

كريمه شكرى خاطر[1] و سهير فيصل اللقوة[2] وهند محمد عبد المنعم[2] و فرج عبد الحليم أحمد[1] و ابراهيم فتحى شكري[1]

1-قسم علم الحيوان- كلية العلوم- جامعة الزقازيق
2- معهد بحوث وقاية النباتات-الدقي- الجيزة

استهدفت الدراسة توضيح تأثير اربعة اجناس من النيماتودا الممرضة للحشرات هيتيروبيتيديس بكتيروفورا *Heterorhabditis bacteriophora* (HP88) و شتينيرنيما كاربوكابسي *Steinernema carpocapsae* (S.C) و شتينيرنيما سكابترسي *Steinernema scapterisci* (S.S) و شتينيرنيما جلاسري *Steinernema glaseri* (S. g) ضد العمر اليرقي الاخيرلفراشة الشمع الكبري وتمت دراسة بعض التغيرات البيوكيميائية بعد فترات تعرض6و12و24و48 ساعة بتركيزات 20و50و100 من الاطوار المعدية. وأثبتت الدراسة وجود خلل في وظائف الانزيمات المحللة للكربوهيدرات (الاميليزو التريهاليزو الانفرتيز) وكل من انزيمي الالفا والبيتا استريرز.حيث أظهرت النتائج انخفاض معنوي في نشاط انزيم الاميليز بعد المعاملة ب20طور معدي ولكن علي النقيض التعرض ل شتينيرنيما سكابترسي (S.S) و شتينيرنيما جلاسري (S. g) تسبب في زيادة غير معنوية بعد 6ساعات من المعاملة.وسجلت زيادة معنوية في نشاط الاميليز بعد 6ساعات من المعاملة ب50طور معدي وكانت النسبة الملحوظة بهيتيروبيتيديس بكتيروفورا (HP88) (510.50±3.33 ميكرو جرام جلوكوز/جم) بنسبة 47.24% مقارنة بالمجموعة الضابطة بينما سجل نفس النوع انخفاض معنوي لنشاط الانزيم(235.70±2.58 و175.60± 6.53) بنسبة 59.86% و 68.67% بعد 12و 48ساعة.

سجلت جميع المعاملات خفضا معنويافى نشاط إنزيم التريهاليز مع زيادة فترات التعرض. أيضا العاملة ب 100طور معدي هيتيروبيتيديس بكتيروفورا (HP88) أدي الي انخفاض معنوي في نشاط انزيم التريهاليز(1431.08 3.33± و 743.14±4.89و 467.17±3.08 ميكرو جرام/جم) بنسب 42.08 ٪ و41.00 ٪ و 52.50 ٪ بعد 6 و24 و 48ساعة.

شهد نشاط انزيم الانفرتيزخفض معنوي عند المعاملة 20طور معدي بعد 6و12ساعة وايضا سجل زيادة معنوية بعد 24ساعة في كل المعاملات ثم سجا خفض معنوي مرة أخري بعد 48ساعة بكل الانواع .

تسببت جميع الانواع من النيماتودا الممرضة للحشرات في تذبذب في نشاط الفاوبيتا استيريزليس فقط مع اختلاف فترات التعرض بل ولكن ايضا مع اختلاف التركيزات. وسجلت فروق معنوية في نشاط كل من انزيم الفا استيريزمقارنة بالمجموعة الضابطة حيث كان هناك خفض في مستوى نشاط انزيم الفا استيريز وأدت المعاملة ب50طور معدي من شتينرنيما جلاسري (S. g) الي زيادة اولية في نشاط ألفا استيريز وبعد ذلك سجل خفض معنوي (270.10 1.85±و234.00 2.21±ميكروجرام ألفانفثول /جم) بعد 24و 48ساعة بنسبة انخفاض تساوي 30.00% و 44.00% علي التوالي مقارنة بالتجربة الحاكمة. بينما سجلت المعاملة بشتينرنيما كاربوكابسي (S. C) خفضا معنويا في نشاط انزيم بيتا استيريز(189.90 ± 2.93ميكروجرام بيتانفثول / جم) بنسبة 46.60%بعد 12ساعة ، علي النقيض سجلت زيادة معنوية في نشاط انزيم بيتا استيريز(242.2 ± 2.25و523.4 ± 2.61ميكروجرام بيتانفثول / جم) أيضا بعد 24و48ساعة من المعاملة ب50 طور معدي ب شتينرنيما كاربوكابسي (S. C).