Biochemical Effects of *Heterorhabditis bacteriophora, Steinernema carpocapsae, Steinernema scapterisi* and *Steinernema glaseri* on *Galleria mellonella* Larvae (Lepidoptera: Pyrallidae)

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ABSTRACT

The present study aimed to investigate the biochemical effect of four entomopathogenic nematode strains, *Heterorhabditis bacteriophora poiner* (HP88)*, Steinernema carpocapsae* (S.c), *Steinernema scapterisi* (S.s) and *Steinernema glaseri* (S. g) against the last larval instar of the greater wax moth *G. mellonella*. The biochemical variations due to nematodes infection were estimated after 6, 12, 24 and 48hrs exposure times and by 20, 50 and 100 infective juveniles (IJs) concentrations. The content of total protein showed significant decreases at all treatments compared with control, meanwhile, a significant increase was determined after 24hrs of treatment with 100 IJs of both (S. c) and (S.s) strains. The most pronouncing reduction estimated 48 hrs post-treatment with (S. c) strain and scored 15.58±1.5 mg/g. b. wt. with a reduction percent -74.60% post-inoculation with 50 IJ. Clear dysfunction in protease, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities demonstrated after all treatments. At 100 (IJs), all treatments provoked a significant reduction in protease activity except after 48hrs of treatment with HP88, a non-significant increase (82.98±2.43 μg tyrosine/min/ g. b. wt.) was reported. All tested entomopathogenic nematodes induced a high significant increase in AST activity compared to control. At 100 (IJ) the increasing percentage reached its maximum level (195.60%) after 24hrs of the treatment with (S. g) as relative activity of control. Fluctuation in ALT activity by elevation and inhibition was recorded in comparison with the control experiment. High significant increase of the enzyme activity from 6 to 48hrs (604.64±4.46 and 518.96±4.43 μg pyruvate/g. b. wt.) with percentages of 88.97% and 114.10% clearly determined at 100(IJ) with (S. c) treatment compared to control. On the other hand, a highly significant decrease in ALT activity was recorded (167.82±1.84 μg pyruvate /g. b. wt.) with a reduction percentage of -64.20 % of treatment with (S. g) after 24hrs at 50 IJs as compared with control.

INTRODUCTION

Galleria mellonella (Lepidoptera: Pyrallidae), the greater wax moth is well known for its parasitization on honeybees and their hives. It is considered as one of the most common enemies of bees through feeding on pollen, wax, and honey besides destroying the weak colonies and old combs (Rahman *et al.,* 2017).

The larvae of his pest make tunnels in the wax and produced a mass of webs and leave debris behind it (Ellis *et al.,* 2013). This species is one of the most economically important pests of bee wax in the globe leads to serious economic loss to beekeeping (Oh *et al.,* 1995; Wojda, 2017). The larvae caused severe damage in tropical and sub-tropical regions and are believed to be one of the factors causing the large decline in both feral and wild honeybee populations (Kwadha *et al.,* 2017). Besides injuring wax combs and destroying frames and soft wooden parts at pupation in the hive, larvae can transfer bacterial disease (Charrière and Imdorf, 1997). Management of the greater wax moth relies mainly on multiple uses of chemical pesticides; these control methods can induce multiple side effects on human health and non-target organisms. The search for safe biopesticides is being conducted all over the world to develop new bioactive agents.

Biological control agents especially nematodes upgraded and are realized as important alternatives for chemical insecticides. Among the most suitable biological control agents for controlling the greater wax moth are the entomopathogenic nematodes of the families Heterorhabditidae and Steinernematidae (Koppenhofer *et al.,* 2006). Which are considered excellent biocontrol agents because they cause rapid death of the insect host without side effects on mammals. Entomopathogenic nematodes (EPNs) are effective in controlling a variety of economically important pests including the larvae *G. mellonella.* Dowds and Peters (2002) reported that the infective juveniles of nematode penetrate through natural openings, mouth, anus and spiracles, or in some species (*Heterorhabditis* spp.) through the cuticle and enter the hemocoel of the host releasing their symbiotic bacteria into the haemolymph of their host. These bacteria propagate and produce toxins. The insecticidal effect of both *Steinernematidae* and *Heterorhabditidae* may be due to the discharge of active substances, comprising toxins and proteases (Ffrench-Constant *et al.,* 2007; Toubarro *et al.,* 2009). Stefanovska *et al.* (2008) indicated that *H. bacteriophora* application induced moderately mortality against *G. mellonella* larvae. Nematodes of genus *Heterorhabditis* have associated symbiotic bacteria belonging to genus *Photorhabdus*. Toxins produced by *Photorhabdus* have several biochemical properties that affect physiology in several insect species. The toxins produced by symbiotic bacteria cause immune suppression and fatal septicemia in the insect host (Ullah *et al.,* 2014; El-Roby and Hussein 2019). The lethal effects are often correlated with changes in some enzyme's activities in the host insects (Grewal *et al.,* 2005). Insects activate multiple defense mechanisms in response to nematode infection (Kunc *et al.,* 2017; Lalitha *et al.,*2018).

MATERIALS 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 $28\pm2^{o}C$ and 60-70% R.H. for several generations far away from any insecticidal contamination. The larvae could grow on a semi-synthetic diet layer of 5-7 cm then transferred to a glass jar capacity (9.40 cm diameters x 1.50 cm high) and covered with plain paper fitted in place with two rubber bands. The larval faces and debris were cleaned out daily. After pupation, pupae were collected and transferred to clean wide glass jars until adult emergence. The emerged adults were collected and placed in pairs, males, and females per each glass jars. 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. Four species of EPNs were used for this study, *Heterorhabditis bacteriophora poiner* (HP88), *Steinernema carpocapsae* (S. c), *Steinernema scapterisi* (S.s) and *Steinernema glaseri* (S. g).

Biochemical Studies:

Larval samples of *G. mellonella* used for conducted biochemical assays were collected at 6, 12, 24 and 48hrs post-treatment with three concentrations of 20, 50 and 100 infective juveniles (IJ) 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 determination of total protein content, the activities of protease, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes.

Determination of Total Protein Content:

Colorimetric determination of total soluble protein in the total homogenate was carried out as described by (Gornall *et al.* 1949). The principle of this method is based on that protein in the presence of an alkaline cupric sulfate, produces a violet purple color, the intensity of which is proportional to their concentration. Briefly, a volume of 0.2 ml of larval homogenate was added to 5ml of Biuret reagent and incubated for 30 min at 20-25 ºC. The absorbance of the sample against a blank Biuret reagent was measured at a wavelength of 546 nm.

Determination of Protease Activity:

The activity of protease was estimated according to the method of Gessesse and Gashe (1997). The reaction mixture in a total volume of 2 ml was composed of 1% gelatin, 0.05 M glycine-NaOH buffer, pH 10 and appropriately diluted enzyme. The reaction was terminated by adding an equal volume of 10% trichloroacetic acid (TCA) after 20 minutes of incubation at 55 ºC, after this,

0.5 ml of clear supernatant was mixed with 2.5 ml of 0.5 M Na_2CO_3 and 0.5 ml of 1:1 Folin- Ciocalteau's phenol reagent. After 30 minutes at 25 ºC, the absorbance of the solution was measured at 660 nm in a spectrophotometer against a reagent blank.

Determination of Transaminases Activity:

Both Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) enzyme activities were determined calorimetrically according to the method described by Reitman and Frankle (1957). The reacting mixture consisted of 1ml of a mixture of phosphate buffer (pH =7.4) 0.2 (mM) α-ketoglutaric and 200 (mM) L-alanine or L- aspartate, 0.2(ml) of larval homogenate was then added to the reaction mixture. The mixture was incubated for 30min. Then NaOH was added after, 10 (ml) of 0.4 N. Optical density of the produced brown color is measured after 5min. using a spectrophotometer at 520(nm). The enzyme activity is expressed as μ gm. pyruvate/gm. larval weight /min.

Statistical Analysis:

The results were analyzed by one–way analysis of variance (ANOVA) using costat statistical software (Costat® Statistical Software, 1990) a product of Cohort Software, Monterey, California). Means were compared using L.S.D. (5% significance level).

RESULTS

Total Protein:

Generally, all treatments caused a highly significant decrease in the content of total protein as compared with untreated control except for control with 50 IJ after 24hrs a significant reduction has been estimated (Fig.1).

At 20 IJ:

Data in figure (1a) demonstrates that treatment with (HP88) induced a significant decrease in the total protein content and scored 25.92±2.72 and 37.39±1.95 mg/g. b. wt. with reduction percent (-37.80 %) and (-39.00%) after 24 and 48hrs of treatment, respectively compared to control (41.69±3.83 and

61.31±2.68 mg/g. b. wt.). In the case of (S.C) strain treatment caused a highly significant reduction in total protein content and reached its maximum level of 21.06±1.97 mg/g. b. wt. (-54.80%) after 12hrs of treatment. While in (S.S) treatment, a highly significant reduction was found 29.31±0.68 mg/g. b. wt. with a reduction percent -47.90 % after 6hrs of treatment compared to control (46.60±2.46 mg/g. b. wt.). Also, (S. g) treatment showed a highly significant reduction of total protein content and gave 28.83±1.88 mg/g. b. wt. with a reduction percent -53.00 % after 48 hrs from treatment.

At 50 IJ:

A high significant decreased in total protein content was conducted after 48 hrs of (HP88) treatment (34.88±1.72 mg/g. b. wt.) with a reduction percent, -43.10% compared to control (61.31±2.68 mg/g. b. wt.). A high significant reduction in total protein content was recorded following treatments with (S.C) as 20.67±1.20 mg/g. b. wt. with -55.60 % after 12 hrs and 15.58±1.5 mg/g. b. wt. with -74.60 % after 48hrs compared with control (46.60±2.46 and 61.31±2.68 mg/g. b. wt.). The (S. g) treatment induced the reduction in total protein reaching 41.37±3.32 and 26.05 ±2.44 mg/g. b. wt. by reduction percent -26.40 % and -57.50, respectively after 6 and 48hrs of treatment (Fig. 1b) compared to control (56.20±2.19 and 61.31±2.68 mg/g. b. wt.).

At 100 IJ:

Notably, the total content of protein was highly reduced after 48hrs of treatment with all EPNs comparing with control. The results indicated that the treatment of (HP88) strain showed a significant reduction in the total protein content after 6, 12, 24 and 48hrs reaching 26.84± 2.39 mg/g. b. wt. (-52.20%), 21.47± 2.11 mg/g. b. wt. (-53.90), 25.18±1.76 mg/g. b. wt. (-39.60%) and 17.05±1.67 mg/g. b. wt. (-72.20%), respectively. After 48 hrs of treatment with (S. g) high significant reduction recorded in total protein content and estimated as 18.32±2.68 with reduction

percent -70.1%. On the contrary treatments with (S. c) and (S.s) strains to cause a highly significant increase after 24 hrs determined as 54.49±3.08 and 47.24±2.70 with increasing percent 30.71% and 13.32%, respectively (Fig. 1c).

Protease Activity:

There was a significant difference in protease activity between the four nematode strains and control after the four exposure times (Fig.2)

At 20 IJ:

The results cleared that, the treatment with (HP88) and (S. c) strains show a highly significant reduction in the activity of the enzyme and reached (33.02± 4.37and 57.67±2.91μ g tyrosine /min/g. b. wt. (-56.60% and -24.20%) and 40.93± 4.39 and 61.73±2.53μg tyrosine/min/g. b. wt. (-62.40 % and -43.30%) after 6 and 12hrs of treatment, respectively. Then the activity is reversed, and a significant increase determined (68.30±2.39 and 72.06±3.69 μ g tyrosine /min/g. b. wt.) and finally returned to decrease after 48hrs of treatments. In the case of (S.S) treatment, there was a highly significant reduction of the enzyme activity and attained (42.04±2.37d μ g tyrosine /min/g. b. wt.) with -61.40 % after12hrs from treatment. The results also indicated that the treatment with (S. g) caused a significant decrease in the enzyme activity gave (36.28±2.21 μ g tyrosine /min/g. b. wt.) with -54.90% after 48hrs from treatment compared to control (80.50±2.18 μ g tyrosine /min/g. b. wt.) (Fig2a).

At 50 IJ:

The present data cleared that, the treatment with (HP88) show different effects at different exposure times. A high significant reduction in enzyme activity recorded after 6hrs, 12hrs and 24hrs of treatments 52.02±3.53, 55.59±4.09 and 53.28±2.59 μ g tyrosine /min/g. b. wt., respectively. The activity of the enzyme reversed after 48hrs and significantly increased by 6.76%. A fluctuated effect is recorded after S.C treatments. Initial high significant elevation was recorded after 6h

and attained 90.56±5.10 μ g tyrosine /min/g. b. wt. and the activity reversed as highly significant reduction after 12hrs attained 72.58±5.28 μ g tyrosine /min/g. b. wt. and then high significant increase recorded after both 24 and 48hrs post-application. The significant reduction in activity reached 46.20±2.70 μ g tyrosine /min/g. b. wt. with reduction percent -57.60% and 45.18±2.19 μ g tyrosine /min/g. b. wt. with reduction percent -43.90 % after 12 and 48hrs from (S.S) treatment. In addition (S. g) treatment, there was a significant reduction in the enzyme activity gave (41.74±3.39 and 47.94±1.97 μ g tyrosine /min/ g. b. wt.), with reduction percent -45.10 and -56.00 % after 6 and 12hrs from treatment (Fig. 2b).

At 100 IJ:

All treatments provoke a highly significant reduction in protease activity except after 48hrs of treatment with HP88, the non-significant increase reached 82.98±2.43 μ g tyrosine /min/ g. b. wt. Also, treatment with (S.C) caused a highly significant increase with its maximum values (154.30 ±3.38 and 151.80 ±3.09 μ g tyrosine /min/ g. b. wt.) and 41.62% and 143.80% after 12 and 24hrs of the post-infection period, respectively compared with control (108.90±1.99 and 62.26±1.74 μ g tyrosine /min/ g. b. wt.). On the other hand, the enzyme activity showed a highly significant reduction in the treatment with (S.S) and (S. g) reached its maximum values after 12and 48hrs (Fig.2c).
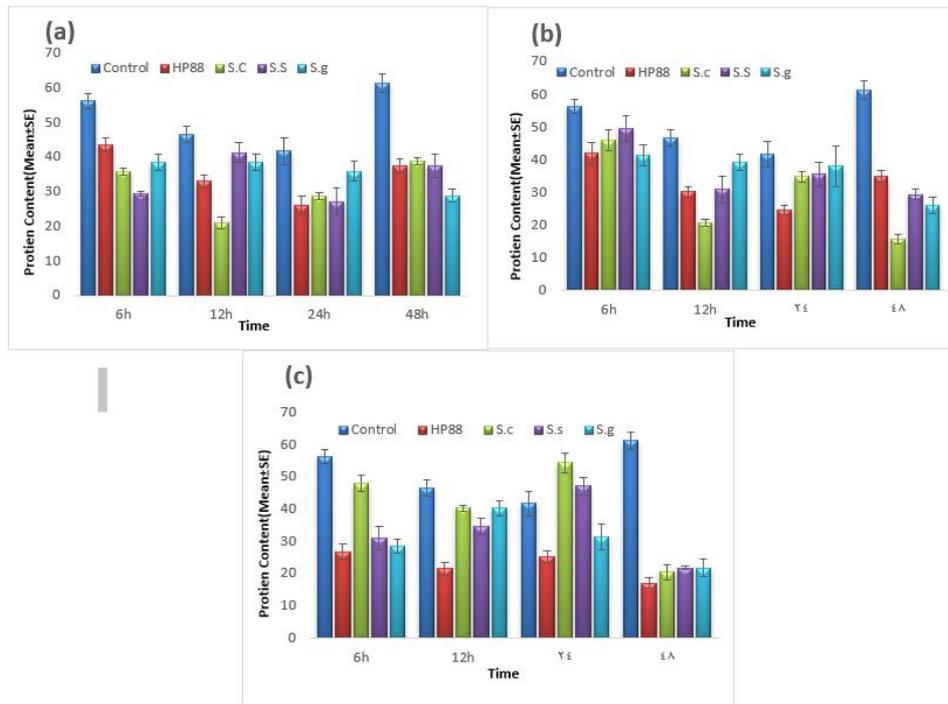
Fig. 1: Changes in total protein content of *G. mellonella* larvae treated with HP88, S.c, S.s and S. g after 6h., 12h., 24h., and 48h. exposure times. a) at 20IJ, b) at 50IJ, c) at 100IJ. Each bar represents the (mean ±SE). (HP88**,** *Heterorhabditis bacteriophora*; S.C, *Steinernema carpocapsae*; S.g, *Steinernema glaseri*; S.S, *Steinernema scapterisci*).
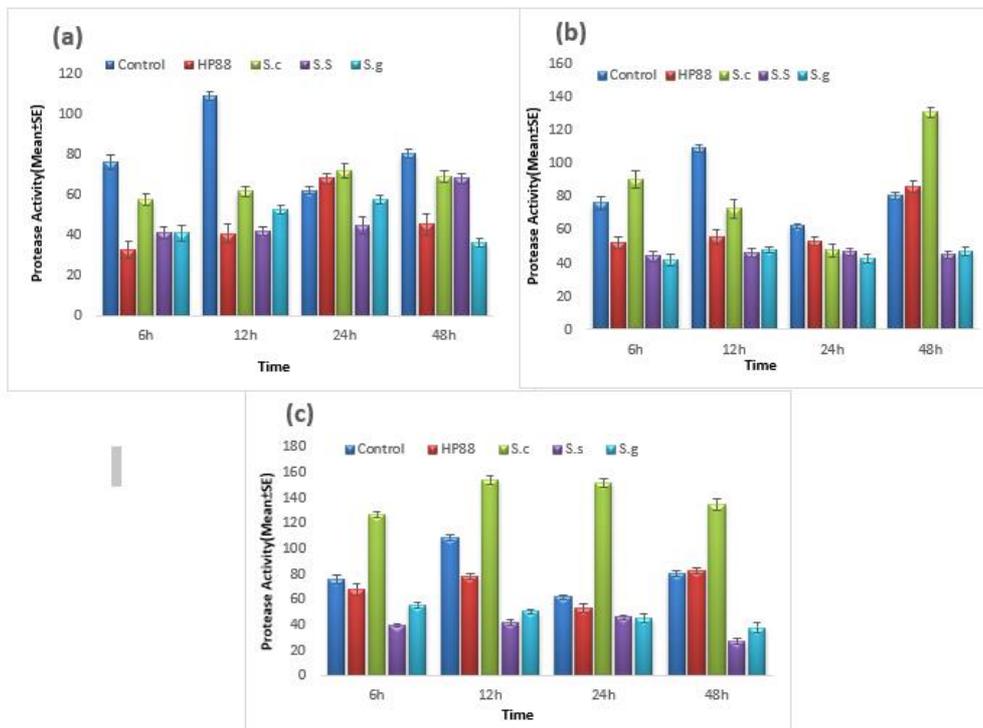

Fig. 2: Activity of Protease enzyme of *G. mellonella* larvae treated with HP88, S.c, S.s and S. g. after 6h., 12h., 24h., and 48h. exposure times. a) at 20IJ, b) at 50IJ, c) at 100IJ. Each bar represents the (mean ±SE). (HP88**,** *Heterorhabditis bacteriophora*; S.C, *Steinernema carpocapsae*; S.g, *Steinernema glaseri*; S.S, *Steinernema scapterisci*).

Aspartate Aminotransferase Enzyme (AST) Activity:

Data in figure (3) showed that all tested entomopathogenic nematodes recorded fluctuated increases in AST activity compared to control.

At 20 IJs:

The results indicated that all treatments cause a highly significant increase in the enzyme activity, the most promoting effect, highly significant increase recorded following treatment with 48hrs of both (S.C), (S. g) and 6 hrs of HP88 by 68.94±3.18, 61.34±0.65 and 49.24±2.05 µg pyruvate /g. b. wt., with elevation percent 135.70%, 109.70 and 103.30, respectively (Fig. 3a).

At 50 IJ:

The results showed that in case HP88, shows a highly significant increase in AST activity and their values are (52.89±0.56 and 49.5±0.10 µg pyruvate/g. b. wt.) with percentages of 118.40 and 102.2%, respectively. The treatment with (S.C) caused an increase in the activity of AST and reached its highest value (56.49 ±5.46 µg pyruvate /g. b. wt.) with 93.10% after 48hrs of treatment. Meanwhile, after 12hrs of treatment with S.C treatment which caused a significant reduction in AST activity attained 29.96±1.79 µg pyruvate/g. b. wt. and -9.26%. Application of the (S.S) treatment caused a highly significant increase in the enzyme activity reached (45.31±2.45 and 53.44±5.45 µg pyruvate /g. b. wt.) with 87.11 and 82.68% after 6 and 48hrs of treatment, respectively compared with control. Exposure of the larvae of greater wax moth to the tested (S. g) causes a highly significant increase of the enzyme activity and gave (52.8±1.27 and 60.49±2.30 µg pyruvate g. b. wt.) after 24 and 48hrs from treatment compared to control (Fig. 3b).

At 100 IJ:

The increasing percentage reached its maximum level after 24hrs of the treatment with S. g treatment and recorded (72.36±4.15 µg pyruvate /g. b. wt.) with 195.60% as relative activity of control. While the lowest activity occurs after treatment with HP88 and reached to (35.19±0.13 µg pyruvate /g. b. wt.) with 6.58 % and (36.91±1.37 µg pyruvate /g. b. wt.) with 26.15% after 12and 48hrs of treatment, respectively comparing with control (Fig. 3c).

Alanine Aminotransferase (ALT) Activity:

Results in figure (4) cleared that, the treatment of greater wax moth larvae using the four strains of nematodes showed significant differences between these strains and control after the exposure times. Generally, fluctuation by increasing and decreasing in ALT activities of treated larvae compared to control was noticed depending on strain and concentrations.

At 20 IJ:

Results indicated a significant increase in ALT activities with all treatments after 6 and 12hrs reached to (453.02±3.98 and 567.33±3.36 µg pyruvate /g. b. wt.) with increasing percentages of 41.59 and 97.53% for HP88 treatment, respectively compared with control. On the other hand, all the treatments caused a highly significant decrease in the enzyme activity and reached (148.53±4.46 µg pyruvate /g. b. wt.) with -38.70% after 48hrs of treatment by (S.C) strain. In case of (S.S) treatment, there was a highly significant reduction of the enzyme activity after all exposure times and scored 245.69±1.62 µg pyruvate /g. b. wt. with -23.20%; 227.87±27.26 µg pyruvate /g. b. wt. with -20.70% and 231.01±4.76 µg pyruvate /g. b. wt. with -50.70% after 6, 12 and 24hrs of

treatment, respectively compared to control (Fig. 4a).

At 50 IJ:

All treatments caused an initial increase in ALT activities recorded the highest increase of 595.20±3.02 μ g pyruvate /g. b. wt. with an increasing percentage of 86.02% and the lowest value was 299.48±3.53 μ g pyruvate /g. b. wt. with a percentage of 4.27% for S.C after 6 and 12hrs of treatment. Moreover, results showed a significant decrease in ALT activities ranged between 167.82±1.84 and 447.82±3.64 μ g pyruvate /g. b. wt. with reduction percentages of -64.20% and -4.27% of treatment with S. g and S.C after 24hrs as compared with control. The treatment with (S.S) significantly reduces the enzyme activity and attained (165.01±1.67 μ g pyruvate/g. b. wt.) and -31.90 % after 48hrs of treatment (Fig. 4b).

At 100 IJ:

The results indicated that there were significant differences between the tested nematode strains and control after 6, 24, and 48hrs from treatment. The non -significant increase was recorded after 12h with all strains.

There was a fluctuation in enzyme activity clearly reported. An initial high significant increase was also found in the activity of the enzyme in treatment with (HP88) strain which was 486.17±2.35 and 262.72±2.21 μ g pyruvate/g. b. wt. with 51.95 and the activity dropped 24hrs post-infection with -12.60% and returned to increase after 48hrs by 8.37 % after treatment. Treatment with (S.c) caused an increase of the enzyme from 6 to 48 hrs recorded (604.64±4.46 and 518.96±4.43 μ g pyruvate/g. b. wt.) with percentages of 88.97 and 114.10% compared to control. Treatment with (S.s) cause elevation in enzyme activity at 6 and 12hrs post-infection and the activity decreased after 24hrs (292.99±3.09 μ g pyruvate/g. b. wt. with percent - 37.40%) and returned to increase after 48hrs (494.30±3.31 with 103.90%). While in (S. g) treatment there was a significant decrease and reaching 210.56±2.82 μ g pyruvate /g. b. wt. with -34.20 percentage and (251.31±2.53 μ g pyruvate /g. b. wt. with -46.30% after 6 and 24hrs respectively compared to control (Fig. 4c).
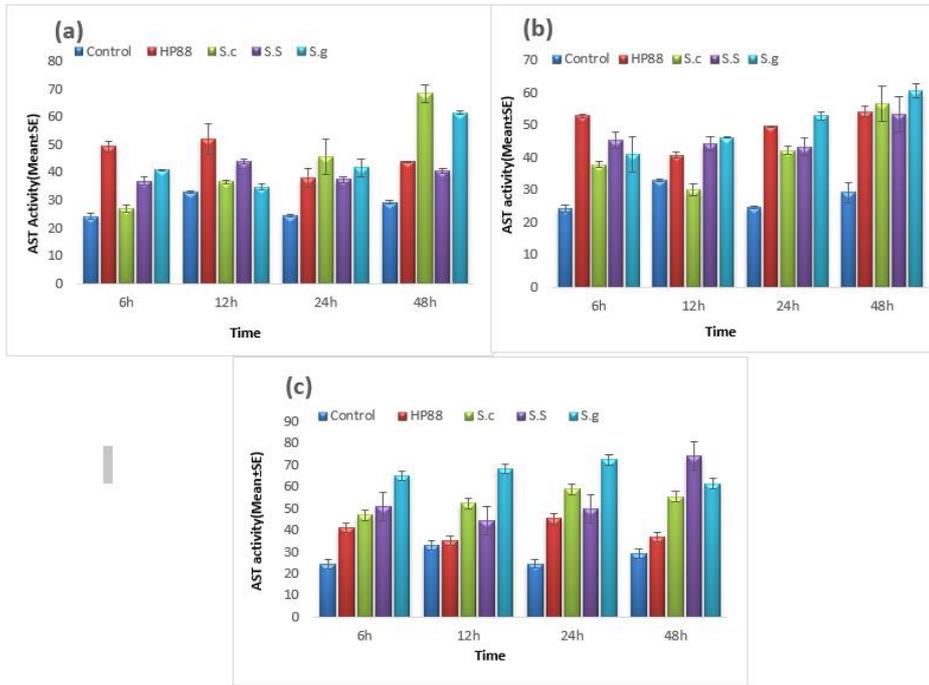
Fig. 3: Activity of Aspartate aminotransferase enzyme (AST) enzyme on larvae of *G. mellonella* treated with HP88, S.c, S.s and S. g. after 6h., 12h., 24h., and 48h. exposure times. a) at 20IJ, b) at 50IJ, c) at 100IJ. Each bar represents the (mean ±SE). (HP88, *Heterorhabditis bacteriophora*; S.C, *Steinernema carpocapsae*; S.g, *Steinernema glaseri*; S.S, *Steinernema scapterisci*).

Fig. 4: Activity of Alanine aminotransferase enzyme (ALT) enzyme in larvae of *G. mellonella* treated with HP88, S.c, S.s and S. g. after 6h., 12h., 24h., and 48h. exposure times. a) at 20IJ, b) at 50IJ, c) at 100IJ. Each bar represents the (mean ±SE). (HP88, *Heterorhabditis bacteriophora*; S.C, *Steinernema carpocapsae*; S.g, *Steinernema glaseri*; S.S, *Steinernema scapterisci*)
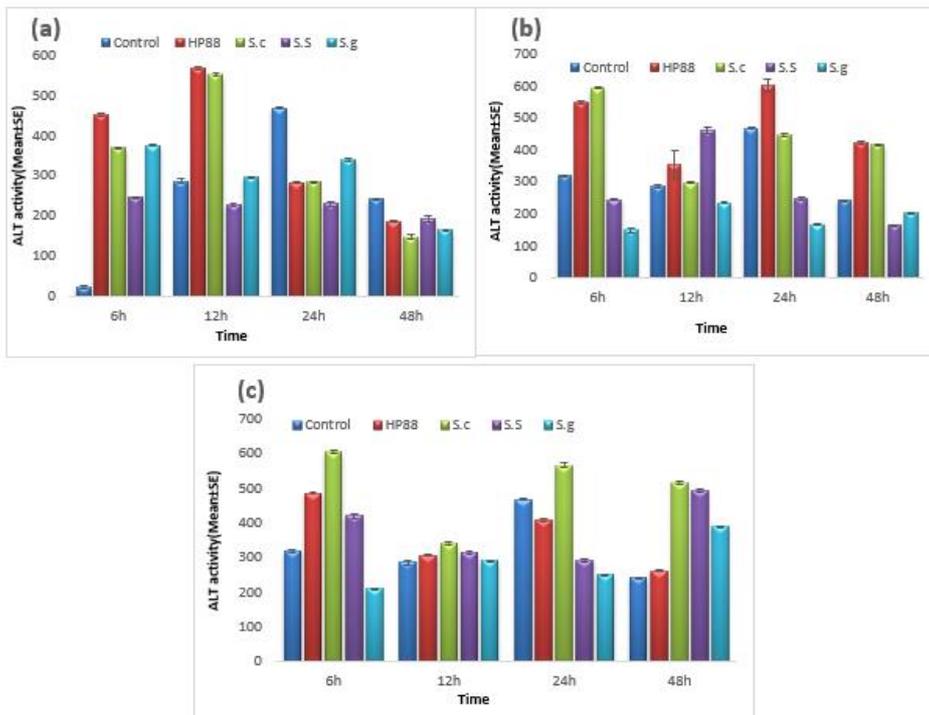
DISCUSSION

Little studies describe the effects of sublethal doses of entomopathogenic nematodes infection on the physiology of *G. mellonella* larvae. Almost all treatments in the present study caused a highly significant reduction in total protein content as compared with control; the highest reduction was recorded after 48 hrs of infection with the four tested nematodes. The most pronounced reduction is recorded with 50IJ of S.C treatment. These results are in harmony with Hassan *et al.* (2016) who found a decrease in total protein content of *Agrotis ipsilon* at 48 hrs post-treatment with *H. bacteriophora* and *S. glaseri.* El-Sadawy *et al.* (2009) reported a reduction in protein content in *Parasarcophaga aegyptiaca* and *Argas persicus* when infected with *H. bacteriophora* and *S. riobrave*. El-Bishry (1989) reported a reduction in haemolymph protein of *S. littoralis* at 30 hrs post-nematodes infection. Reduction in total protein content may be due to the proteolytic activity, this activity leads to host death. On contrary, treatments with (S. c) and (S.s) strains to cause a highly significant increase after 24 hrs with 100 IJs. These findings are in accordance with the findings of Gaber *et al.* (2018) who recorded elevation in total proteins in the 5th nymphs of the *S. gregaria* after 48 hrs post-treatment with infective juveniles (IJs) of *H. bacteriophora* nematode strain as compared to the control.

Enzymes are biological catalysts that accelerate cellular, chemical, and metabolic processes (Bar-Even *et al.,* 2011). The activity of some larval enzymes was affected due to infection by the four tested nematodes. Proteases cleave the peptide bonds in the insect food to release amino acids, which the body can then use for energy. Clear dysfunction in protease activity demonstrated. At 100 (IJs), all treatments provoked a significant reduction in protease activity except after 48hrs of treatment with HP88, a non-significant increase (82.98±2.43 µg tyrosine/min/ g. b. wt.) was reported. The decrease in protease activity in the current study may be due to the digestion of insect protein by the symbiotic bacteria post-infection. These findings are in accordance with the result of (Ibrahim *et al.,* 2015). Whereas a significant increase in protease activity was recorded in 48hrs post-treatments with 20IJ of HP88 recorded by the same author.

The transaminase enzymes, both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have been correlated with protein synthesis in many insect tissues. They play a major role in the formation of non-essential amino acids and the maintenance of the amino acid balance in insects (Meister, 1957). Also, transaminases are the key enzymes in gluconeogenesis and the metabolism of nitrogenous products (Pant and Kumar, 1980). The changes in their levels were used as an indicator of protein and amino acid metabolism (Mordue and Galdsworthy, 1973; Surhott, 1976). All tested entomopathogenic nematodes induced a highly significant increase in AST activity, the highest activity of AST occurs after exposure of the larvae to the highest lethal concentrations of infective juveniles (IJs) of the four EPNs compared with control. At 100 (IJ), the elevation percentage reached its maximum level (195.60%) after 24hrs of the treatment with (S. g) as relative activity of control. Fluctuation in ALT activity by elevation and inhibition was recorded in comparison with the control experiment. High significant increase of the enzyme activity from 6 to 48hrs (604.64±4.46 and 518.96±4.43 µg pyruvate/g. b. wt.) with percentages of 88.97% and 114.10% clearly determined at 100(IJ) with (S. c) treatment compared to control. On the other hand, a highly significant decrease in ALT activity was recorded (167.82±1.84 µg pyruvate /g. b. wt.) with a reduction percentage of -64.20 % of treatment with (S. g) after 24hrs at 50 IJs as compared with control.

It is remarkable that, the Steinernematodae species have the strongest effect on the activities of AST enzyme. The

obtained results in our work recorded a highly significant increase in AST activity after infection with all four EPN by 100IJs and ALT enzymes provoke fluctuations in activity in *G. mellonella* at different exposure time with EPN compared with the control. Few previous works studied the biochemical effects of EPNs on AST and ALT activities in insects. (Ahmed *et al.,* 2014) stated that activities of transaminases, (ALT) and (AST) showed a highly significant decrease in *S. littoralis* with the infection by *H. bacteriophora* and *S. riobrave*. Transaminases (ALT and AST) enzymes help in the production of energy and serve as a link between protein and carbohydrates metabolism and are known to be affected during pathological conditions (Eltebari *et al.,* 2005). The elevation in GOT activity after treatment suggests the mobility of amino acids during the stress exerted by certain toxic substances to meet the energy needs (Zeba and Khan, 1995). Increasing or decreasing in (GPT) activity may be due to the effect on the synthesis of this enzyme which alters the cell biology (Nath, 2000). The decrease in AST and ALT may be attributed to the binding of the tested compounds with enzyme active site that leads to inhibition in AST and ALT activity (El- Sheikh *et al.,* 2005).

In conclusion changes in total protein content and enzyme activities under nematode infection indicate the biochemical response to the nematode infection. This overreaction often leads to the death of the host insects. Finally, the entomopathogenic nematodes can be produced commercially and applied as a powder or aqueous suspensions of infective juveniles (IJs) to protect beehives through infecting harmful *G. mellonella* larvae.

REFERENCES

Ahmed, F.N., Maklad, A. H. M., 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, *C.Physiology and molecular biology,* Vol.6(1): 23-34. http:// doi.org/ 10.21608/ eajbsc.2014.16044.

Bar-Even, A., Noor, E., Savir, Y., Liebermeister, W., Davidi, D., Tawfik, D. S., Milo, R. (2011): The moderately efficient enzyme: evolutionary and physicochemical trends shaping enzyme parameters. *Biochemistry*, 50(21): 4402–4410. http://doi.org/10. 1021bi2002289.

Charrière, J.D., Imdorf, A. (1997): Protection of honeycombs from moth damage. Swiss Bee Research Center Federal Dairy Research Station, CH-3003, Communication, Nr. 24, Liebefeld, Bern, Switzerland, 3-4. 15pp.

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

Dowds, B.C.A., Peters, A. (2002): Virulence mechanisms Entomopathogenic nematology. New York, NY: CABI pp79-98. http://doi.org/10.1079/ 9780851995670.0000.

El- Bishry, M. H. (1989): Studies on the utilization of the entomopathogenous nematodes in controlling some pests in Egypt. Ph D Thesis Fac Agric Cairo Univ 81pp.

El- Roby, A. S. M. H., Hussein, S. M. (2019): Behavior of bio-and chemical insecticides in tomato ecosystem in Minia Governorate. *Acta Ecologica Sinica*, 39(2):152-156. https://doi.org/10. 1016/j.chnaes.2018.08.004.

Ellis, J.D., Graham, J.R., Mortensen, A. (2013): Standard methods for wax moth research. *Journal of Apicultural Research,* 52(1): 1-17 https://doi.org/10.3896/IBRA.1.52.1 .10

El-Sadawy, H.A., Abou-Nour, A. A., Sobh, H. A., Ghally, S. E. (2009): Biochemical changes in

Parasarcophaga egyptiaca and *Argas persicus (persicargas)* haemolymph infected with entomopathogenic nematode. *Nature Science*, 7 (6): 70-81.

El-Sheikh, T.A., Abdel-Khalik. A. A., Farghali, A.A., Abdel-Aal, A. E. (2005): Biochemical and histological effects of Flufenoxuron and two biocides on the cotton leafworm, *Spodoptera littoralis* (Boisd.). *Egyptian Journal of Agricultural Research*, 2 (2): 799- 809.

Etebari, K., Mirhodeini, S. Z., Matindoost, L. (2005): A study on intraspecific biodiversity of eight groups of silkworm (*Bombyx mori*) by biochemical markers. *Insect Science*, 12: 87-94.

Ffrench, C. R. H., Dowling, A., Waterfield, N. R. (2007): Insecticidal toxins from Photorhabdus bacteria and their potential use in Agriculture. *Toxicon,* 49 (4): 436-51. https:// doi. org/10.1016/j.toxicon.2006. 11.019

Gaber, 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.

Gessesse, A., Gashe, B. A. (1997): Production of alkaline protease by alkaliphilic bacteria isolated from an alkaline Soda lake. *Biotechnology Letters*, 19(5)**:** 479-481.

Gornall, J. G., Bardwill, G. J., David, M. M. (1949): Determination of serum protein by mean of Biuret reaction. *Journal of Biological Chemistry*, 117:751-766.

Grewal, P., Ehlers, R. U., Shapiro, I. D. I. (2005): Nematodes as biological control agents. Wallingford, UK.

Hassan, H. A., Shairra, S. A., Ibrahim, S. S. (2016): Virulence of entomopathogenic nematodes *Steinernema glaseri* and *Heterorhabditis bacteriophora poinar* (HP88 strain) against the black cutworm, *Agrotis ipsilon*. *Egyptian Academic Journal of Biological Sciences,A.Entomology,* 9 (1): 33 – 48. https:// doi.org/10. 21608/EAJBSA.2016.12853.

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.

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

Koppenhofer, A. M., Grewal, P. S., Fuzy, E. M. (2006): Virulence of the entomopathogenic nematodes *Heterorhabditis bacteriophora*, *Heterorhabditis zealandica*, and *Steinernema scarabaei* against five white grub species (Coleoptera: Scarabaeidae) of economic importance in turfgrass in North America. *Biological Control*, 38:397–404. https://doi. org/10.1016/j.biocontrol.2005.12.01 3.

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. https://doi.org/ 10.3390/ insects 8020061.

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. https://doi.org/ 10.1007/s12639-018 -0983-1.

Meister, A. (1957): Biochemistry of the amino Acids, 2nd edition, Academic Press, New York, USA.

Mordue W, Galdsworthy G J (1973) Transaminase levels and uric acid production in adult locusts. *Insect Biochemistry*, 3: 419-427. https://doi.org/10.1016/0020-1790(73) 90075-9.

Nath, B. S. (2000): Changes in carbohydrate metabolism in hemolymph and fat body of the silkworm, *Bombyx mori* L., exposed to organophosphorus insecticides. *Pesticide Biochemistry and Physiology*, 68: 127–137. https://doi.org/10.1006/pest.2000.2509.

Oh, H.W., Lee, M. Y., Chang, Y. D. (1995): Developing periods and damage patterns of combs by the greater wax moth, *Galleria mellonelia*. Korean. *Journal of* Apiculture, 10 (1): 5-10.

Pant, R., Kumar, S. (1980): Significance of some enzymes and metabolites during aging of the dipteran fleshfly, *Sarcophaga ruficornis*. *Current Science*, 49: 10–13. https://www.jstor.org/stable/24082383.

Rahman, A., Bharali, P., Borah, L., Bathari, M., Taye, R.R. (2017): Post embryonic development of *Galleria mellonella* L. and its management strategy. *Journal of Entomology and Zoology Studies*, 5(3): 1523-1526.

Reitman, S. M. D., Frankel, S. (1957): A colorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyurvic transaminase. *American Journal of Clinical Pathology*, 28(1): 56-63. https://doi.org/ 10.1093/ajcp/28.1.56.

Stefanovska, T., Pidlishyuk, V., Kaya, H. (2008): Host range and infectivity of *Heterorhabditis bacteriophora* (Heterorhabditidae) fromUkraine.*Communications* in *Agricultural* and *Applied Biological Sciences*, 73(4): 693-698.

Surhott, B. (1976): Changes of enzyme patterns in the integument of *Locusta migratoria* during formation of adult cuticle. *Insect Biochemistry*, 6: 79-83. https://doi.org/10.1016/0020-1790(76)90062-7.

Toubarro, D., Lucena-Robles, M., Nascimento, G., Costa, G., Montiel, R., Coelho, A., Simões, N. (2009): An apoptosis-inducing serine protease secreted by the entomopathogenic nematode *Steinernema carpocapsae*. *International Journal of Parasitology*, 39(12): 1319-30. https://doi.org/10.1016/j.ijpara.2009.04.013.

Ullah, I., Khan, A. L., Ali, L., Khan, A. R., Waqaset, M., Lee, I., Shin, J. (2014) : An insecticidal compound produced by an insect-pathogenic bacterium suppresses host defenses through phenoloxidase. *Molecules*, 19: 20913–20928. https://doi.org/ 10.3390/molecules191220913.

Wojda, I. (2017): Immunity of the greater wax moth *Galleria mellonella*. *Insect Science*, 24(3): 342-357. https://doi.org/ 10.1111/1744-7917. 12325.

Zeba, M., Khan. A. (1995): Effect of fenvalerate on protein and amino acid contents and enzyme activities in the Ostracod, *Chrissica halyi*. *Pesticide Science*, 45: 279-282. https://doi.org/10.1002/ps.2780450312.

ARABIC SUMMARY

التأثيرات البيوكيميائية لهيتيروبيتيديس بكتيروفورا و و شتينيرنيما كاربوكابسي و شتينيرنيما سكابترسي وشتينيرنيما جلاسري علي يرقات فراشة الشمع الكبري جاليريا ميلونيلا (حرشفية الاجنحة: جاليريدي)

كريمه شكرى خاطر1 و سهير فيصل اللقوة2 وهند محمد عبد المنعم2 و فرج عبد الحليم أحمد1
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استهدفت الدراسة توضيح تأثير اربعة أجناس من النيماتودا الممرضة للحشرات هيتيروبيتيديس بكتيروفورا و *Heterorhabditis bacteriophora* (HP88) و شتينيرنيما كاربوكابسي (S.C) *Steinernema carpocapsae* و شتينيرنيما جلاسري *Steinernema glaseri* (S. g) و شاتينيرنيما سكابترسي *Steinernema scapterisci* (S.S)ضد العمر اليرقي الاخير لفراشة الشمع الكبري وتمت دراسة بعض التغيرات الفسيولوجية بعد فترات تعرض 6و 12 و 24 و 48 ساعة بتركيزات 20 و 50 و 100 من الاطوار المعدية. وأثبتت الدراسة وجود خلل في محتوي البروتين حيث لوحظ انخفاض معنوي في محتوي البروتين بعد المعاملة مقارنة بالتجربة الحاكمة فقد سجلت اعلي نسبة انخفاض بعد 48 ساعة من المعاملة بشتينيرنيما كاربوكابسي (58و15+ 1.5مجم/جم) بنسبة -74.6%. بينما سجلت زيادة غير معنوية بعد 24ساعة من المعاملة بـ100 طور معدي لكلا الجنسين شتينيرنيما كاربوكابسي و شتينيرنيما سكابترسي و كان هناك إختلال واضح في نشاط إنزيمات البروتييز والإنزيمات الناقلة للأمين. أثبتت كل المعاملات بـ100 طور معدي إلى إنخفاض معنوي في نشاط إنزيم البروتييز بخلاف المعاملة بهيتيروبيتيديس بكتيروفورا بعد 48 ساعه سجل إرتفاع غير معنوي في نشاط إنزيم البروتييز (82.98+2.43 ميكروجرام تيروسين/دقيقه/جرام). وأدت المعاملة إلى زيادة معنوية في نشاط إنزيم ال (AST) مقارنة بالتجربة الضابطه حيث وصلت نسبة الإرتفاع إلى أقصى قيمة 195.6% بعد 24 ساعة من المعاملة بشتينيرنيما جلاسري وسجل تذبذب في نشاط إنزيم (ALT) مقارنة بالتجربة الحاكمة حيث أظهرت النتائج زيادة معنوية في نشاط انزيم (ALT) بعد المعاملة بـ 100 طور معدي من شتينيرنيما كاربوكابسي من 6 ساعات حتى 48 ساعة بنسب 88.97% و 114.1%. ولكن على النقيض سجل إنخفاض معنوي في نشاط إنزيم (ALT) بعد 24 ساعة من المعاملة بـ 50 طور معدي من شتاينيرنيما جلاسري مقارنة بالتجربة الحاكمة.