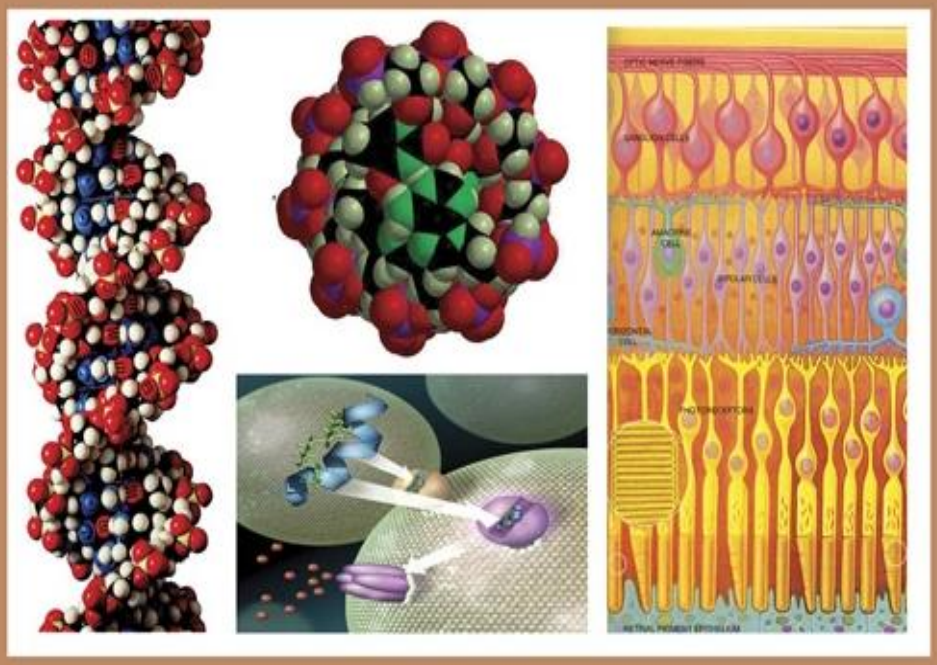
EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULARBIOLOGY

C



ISSN
2090-0767

WWW.EAJBS.EG.NET

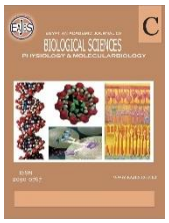
Vol. 15 No. 1 (2023)

.

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 15(1) pp121-142 (2023)*
DOI: 10.21608/EAJBSC.2023.285633

Egypt. Acad. J. Biolog. Sci., 15(1):121-142 (2023)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Enzymatic Disturbance in Larvae of The Black Cut Worm, *Agrotis ipsilon* (Lepidoptera: Noctuidae), by Infection with The Entomopathogenic Nematodes, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*

Ghoneim, K.[1*]; Hassan, H.A.[2]; Tanani, M.[1] and Bakr, N.A.[1]

1- Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Cairo, Egypt.

2- Institute of Plant Protection Research, Agricultural Research Center, Giza, Egypt

***E-mail:** karemghoneim@gmail.com

ARTICLE INFO

Article History
Received:3/12/2022
Accepted:29/1/2023
Available:6/2/2023

Keywords:
Acid phosphatase, alkaline phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, haemolymph, infectivity, larva, virulence.

ABSTRACT

The black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), is almost a cosmopolitan pest in distribution. It is a polyphagous insect attacking nearly all vegetables and many economic field crops in the world. The present study was conducted to investigate the disturbing effects of entomopathogenic nematodes (EPNs), *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* on the activities of phosphatases and transaminases in the haemolymph of *A. ipsilon* larvae. The newly moulted penultimate (5^{th}) instar larvae had been infected with LC_{50} values of *S. carpocapsae* and *H. bacteriophora* (21 IJs/ml and 62 IJs/ml, respectively) and the influenced enzymatic activities were determined in the haemolymph of last (6^{th}) instar larvae, at three-time intervals, 6, 24 & 48 hr, respectively. The most important results could be summarized as follows. The acid phosphatase (ACP) activity significantly increased in haemolymph of 6 hr-old last instar larvae of *A. ipsilon*. In contrast, ACP activity was remarkably decreased in 24 and 48 hr-old larvae. The alkaline phosphatase (ALP) activity increased in the haemolymph of 6 hr-old larvae. On the contrary, ALP activity was considerably reduced in haemolymph of 24 and 48-old larvae. Glutamic oxaloacetic transaminase (GOT) activity significantly increased in the haemolymph of 6 hr-old last instar larvae. In haemolymph of 24 hr-old larvae, GOT activity considerably increased by *H. bacteriophora* but was slightly decreased by *S. carpocapsae*. At the last time interval, GOT activity greatly decreased, regardless the EPN species. Glutamic pyruvic transaminase (GPT) activity was predominantly declined in larvae infected with *S. carpocapsae* or *H. bacteriophora*, at all-time intervals, with an exception of a slightly increased activity in 6 hr-old larvae after infection with *S. carpocapsae*. Moreover, GPT activity was increasingly reduced with the time intervals. Also, *H. bacteriophora* exhibited stronger reducing potency on the GPT activity than *S. carpocapsae.* In conclusion, the disturbances of activities of phosphatases and Transaminases, as a result of the interactions between these nematodes and their symbiotic bacteria versus the immune responses of the host insect, end in the host death. Therefore, the tested EPNs can be used as a part of the Integrated control program against *A. ipsilon*.

INTRODUCTION

The black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is globally distributed, particularly in moderate and subtropical countries (Harrison, and Lynn, 2008; Binning *et al*., 2015). It is a migrant polyphagous species known to cause serious damage to many host plants (El-Aziz *et al*., 2007; Binning *et al*., 2015; Liu *et al*., 2015; Picimbon, 2020; Moustafa *et al*., 2021). This destructive pest causes considerable economic losses in many field crops and agricultural products, especially in industrial plants and vegetables worldwide (Muştu *et al*., 2021; Wang *et al*., 2021). In Egypt, *A. ipsilon* infests about 70 plant species (Fahmy, 2014; Sharaby and El-Nojiban, 2015), including all vegetable crops and many economic field crops, especially in the seedling stage causing serious damage about 100% in some cases (Dahi *et al*., 2009; Mahmoud *et al*., 2016; Abdel-Hakim, and El-Mandarawy, 2017; Ahmed *et al*., 2022).

In Egypt, also, the control of this dangerous insect depends mainly on the application of conventional insecticides (Vattikonda and Sangam, 2017; Abd-El-Aziz *et al*., 2019; Ismail, 2021) to which the insect quickly develops resistance and cross-resistance (Yu *et al*., 2012; Fahmy, 2014; Mahmoud *et al*., 2016). Also, the conventional insecticides are often not effective and remain inadequate for the control of *A. ipsilon* because of its larval hiding behavior during the daytime and feeding actively at night, causing hidden damage in fields (Capinera, 2001; Takeda, 2008). On the other hand, the latter instar larvae of *A. ipsilon* remain hidden in cracks and crevices during most of the life cycle so chemical control is often ineffective (Kumar *et al*., 2022).

In addition, the intensive and improper uses of many currently marketed insecticides usually cause serious toxicological hazards to the environment (Haq *et al*., 2004; Tiryaki and Temur, 2010; Chowański *et al*., 2014) and drastically affect the natural enemies, allowing exponential increase of pest populations (Calvo-Agudo *et al*., 2019; Demok *et al*., 2019) as well as adverse effects on human health and domestic animals (Shahzad *et al*., 2020) beside the accumulation of pesticide residues in food (Moustafa *et al*., 2021).

These problems are an incentive to search for alternative, eco-friendly, selective, and effective control measures. Among potential control measures is the use of entomopathogenic nematodes (EPNs), especially against above-ground pests and those are spending a part of their life cycle inside the soil or cryptic habitats (Laznik and Trdan, 2011; Lacey and Georgis, 2012; Shaurub *et al*., 2016; James *et al*., 2018; Du Preez *et al*., 2022; Nouh, 2022). Therefore, EPNs have attracted much interest around the world to study their distribution, virulence, and usage in the integrated pest management (IPM) programs (Çağlayan *et al*., 2021; Ali *et al*., 2022) because they are harmless to non-target organisms, humans health and the environment (Odendaal *et al*., 2015, 2016; Gulcu *et al*., 2017; Kumar *et al*., 2022; Peçen and Kepenekci, 2022). Other major advantages of using EPNs to control insects are that they can be easily mass-produced and applied to tree trunks using common irrigation and pesticide equipment (Yağci *et al*., 2021a,b).

With regard to the EPNs steinernematids and heterorhabditids, they are widely used as biocontrol agents against diverse insect pests allover the world. They have a wide variety of host ranges, high virulence, less killing time, host-finding ability, and fast-acting generations (Lacey and Georgis, 2012; Yüksel *et al*., 2022). EPNs belong to these two families are identified by carrying symbiotic bacteria of the genera *Xenorhabdus* and *Photorhabdus* in their intestines, respectively (Boemare, 2002; Poinar and Grewal, 2012; Ferreria and Malan, 2014; Labaude and Griffin, 2018). EPNs, such as *Steinernema* and *Heterorhabditis*, have the potential to provide effective control of some economically important insect pests

belonging to orders Lepidoptera, Coleoptera, and Diptera (Burnell and Stock, 2000). They are established all over the world in diverse ecological habitats and are being developed as biological control agents against insect pests (Cranshaw and Zimmerman, 2013; Lacey *et al*., 2015). At present, about 100 *Steinernema* spp. and 19 *Heterererhabditis* spp. have been described worldwide (Bhat *et al*., 2020; Machado *et al*., 2021).

The association of EPNs *Steinernema* and *Heterorhabditis* with their symbiotic bacteria is found to be the primary cause of insect mortality (Leonar *et al*., 2022). When encountering a suitable host, the infective juveniles (IJs) of EPNs enter the haemocoel of the insect host *via* natural openings, such as the spiracles, mouth, or anus, or by penetrating the cuticle (particularly in *Heterorhabditis* spp.)(Griffin *et al*., 2005; Atwa, 2014; Gozel and Gozel, 2016). The bacteria grow rapidly in the insect haemocoel and produce toxins that it by means of inducing septicemia within 24 to 72 h of infection (Ehlers, 2001; Griffin *et al*., 2005). Recently, Ghoneim *et al*. (2023) found that the EPNs *S. carpocapsae* and *H. bacteriophora* were highly virulent against *A. ipsilon* larvae. Also, these two EPN species adversely affected the main body metabolites in haemolymph in larvae of the same insect (Ghoneim *et al*., 2022).

Acid phosphatase (ACP, E.C.3.1.3.2), known as a lysosomal marker enzyme (Csikos and Sass, 1997), is active in guts (Ferreira and Terra, 1980), Malpighian tubules (Srivastava and Saxena, 1967) and is also abundant in the disintegrating tissues and organs subjected to cytolysis (Sahota, 1975). ACP is important in biological processes that need a high level of energy, such as development, growth, gamete maturation and histolysis (Ray *et al*., 1984). Alkaline phosphatase (ALP, E.C.3.1.3.1) is a brush border membrane marker enzyme in insects (Wolfersberger, 1984) and is especially active in tissues with active membrane transport, such as intestinal epithelial cells (Caglayan, 1990), Malpighian tubules (Etebari and Matindoost, 2004 a, b) and haemolymph (Etebari *et al.*, 2007). Its primary function is to provide phosphate ions from mononucleotides and ribonucleoproteins for a variety of metabolic processes (Etebari *et al*., 2005). In insects, ALP is involved in several biological processes and responds to stress pathogenesis or infection (Miao, 2002). It is one important synthesizing enzyme of tyrosine, the precursor of dopamine and octopamine, which are known to take part in the control of levels of juvenile hormone and 20-hydroxyecdysone (Rauschenbach *et al*., 2007).

Glutamic oxaloacetic transaminase (GOT, or Alanine aminotransferase, ALT) and Glutamic pyruvic transaminase (GPT, or aspartate aminotransferase, AST) are the main enzymes that conduce to transamination within the intermediary metabolism of insects to make amino acids available for biochemical demands. These are principal enzymes in the formation of non-essential amino acids, in the metabolism of nitrogen waste, and in gluconeogenesis and are correlated with protein anabolism and catabolism (Mordue and Golworthy, 1973). GOT catalyzes the interconversion of alanine and α-ketoglutarate to pyruvate and glutamate, while GPT converts aspartate and α-ketoglutarate to oxaloacetate and glutamate (Klowden, 2007; Nation, 2008). Because these transaminases help in the production of energy and serve as a strategic link between carbohydrate and protein metabolism, they are being altered during various physiological and pathological conditions (Azmi *et al*., 1998; Etebari *et al*., 2007). The present study was conducted to investigate the disturbing effects of EPNs *S. carpocapsae* and *H. bacteriophora* on the activities of phosphatases and transaminases in the last instar larvae of *A. ipsilon*.

MATERIALS AND METHODS

I. The Experimental Insect:

A culture of the black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) was established under constant conditions (27±2°C and 65±5% R.H.) at the Department of Zoology and Entomology,

Faculty of Science, Al-Azhar University, Cairo, Egypt. It was originally started with a sample of eggs from the susceptible strain culture maintained for several generations at Plant Protection Research Institute, Doqqi, Giza, Egypt. Rearing technique was carried out according to Abdin (1979) with an improvement of El-Shershaby (2010). The eggs were kept in wide-mouth plastic jars (1000 ml) fitted with filter paper until hatching. Newly hatched larvae were kept in new jars and provided with clean castor bean leaves *Ricinus communis* as food every day. At reaching the 4th instar, larvae were reared in a few numbers, in separate jars, to avoid crowding and cannibalism. These jars were covered with pieces of cloth for preventing the escape of larvae. Sawdust and fresh castor bean leaves were renewed daily until pupation. The pupae were then placed in plastic jars (10 x 25 cm) covered with muslin and fitted with filter paper, as an oviposition site for future moths. After the adult emergence, a piece of cotton wool soaked in 10% sugar solution was suspended from the top of each jar and renewed every 48 hrs for feeding moths.

2. Entomopathogenic Nematodes (EPNs):

Imported EPN species (Nematoda: Rhabditida), *Heterorhabditis bacteriophora* (Poinar) (Steinernematidae) and *Steinernema carpocapsae* (Weiser) (Heterorhabditidae) were supplied by Dr. El-Sadawy, National Research Centre, Doqqi, Giza, Egypt. For the mass culturing of each EPN, the last (7th) instar larvae of the greater wax moth *Galleria mellonella* were used as hosts (Shamseldean *et al*., 2008). Five live *G. mellonella* larvae were placed in a Petri dish with approximately 100 live EPNs, 20 EPNs/ml, with a few drops of deionized water for each tested EPN. The infective juvenile stages (IJs) of each EPN species will enter and infect the larvae through their natural openings. Symbiotic bacteria carried within the guts of the EPNs were released after they penetrate their hosts. Toxins produced by the bacteria caused blood poisoning of the larvae usually resulting in their death within 72 hours. The EPNs complete one to three generations before they emerge from the dead larvae (cadavers). Petri dishes were stored for a week in a dark place at 20°C ±2 °C. After six days, larvae were checked for infection. The successfully infected larvae will appear beige to dark red. Upon successful infection, Petri dish containing nematodes, larvae and filter paper were placed within another larger Petri dish. The outer Petri dish halfway was filled with deionized water and covered with an opaque lid for three weeks. The IJs will emerge from the host and swim into the water within one to three weeks. The IJs were verified as still alive using a microscope. Nematodes can be stored in darkness in a container that provides a sufficient amount of air to nematodes by using shallow containers for approximately one month at 5°C.

3. Larval Infection of *A. ipsilon* with EPNs:

In a preliminary bioassay toxicity test, a series of concentrations of each EPN species was prepared as follows: *H. bacteriophora*: 200.0, 100.0, 50.0, 25.0 and 12.0 IJs/ml, and *S. carpocapsae*: 100.0, 50.0, 25.0, 12.0 and 6.0 IJs/ml. LC_{50} values of *S. carpocapsae* and *H. bacteriophora* were determined as 21 IJs/ml and 62 IJs/ml, respectively. Infection of the 5th instar larvae of *A. ipsilon* with these LC_{50} values had been carried out.

The EPN experiment was carried out in plastic cups (4 X 5 cm) filled with 50 gm of sterilized sand and moistened with 20% water (v/w). Nematodes suspensions were prepared in serial concentrations of different IJs/ml/cup. The newly moulted penultimate (5th) instar larvae of *A. ipsilon* were placed in separate plastic cups. All cups were covered and kept at 25±2^{o}C. The influenced enzymatic activities of phosphatases and transaminases were determined in the haemolymph of the last (6th) instar larvae, at three-time intervals: 6, 24 & 48 hr, respectively.

4. Sampling of Haemolymph:

Haemolymph was collected from the infected and uninfected (control) 6th instar larvae (at 6, 24 and 48 hr). The haemolymph

was obtained by amputation of one or two prothoracic legs of the larva with fine scissors. Gentle pressure was done on the thorax until a drop of haemolymph appeared at the point of amputation. Haemolymph was drawn into Eppendorff Pipetman containing a few milligrams of phenoloxidase inhibitor (Phenylthiourea) to prevent the tanning or darkening and then diluted 5× with saline solution 0.7%. The diluted haemolymph was frozen for 20 s to rupture the hemocytes. Collected haemolymph samples were then centrifuged at 2000 r.p.m. for 5 min, and only the supernatant fractions were used for assay directly or frozen until use. Three replicates were used and the haemolymph of two individuals was never mixed.

5. Phosphatase Measurements:

Acid Phosphatase (ACP) Activity:

ACP activity was determined in the larval and pupal homogenate according to the method described by Tietz (1986) and using a kit of Biodiagnostics. The enzyme was measured at a wavelength of 510 nm by spectrophotometer.

Alkaline Phosphatase (ALP) Activity:

ALP activity was determined in the larval and pupal homogenate according to the method of Klein *et al*. (1960) using a kit of Biodiagnostics. The enzyme was measured at a wavelength of 510 nm by spectrophotometer.

6. Transaminase Measurements:

Activities of the glutamic oxaloacetic transaminase (GOT) or aspartate transferase (AST) and glutamic pyruvic transaminase (GPT) or alanine transaminase (ALT) were determined in the larval tissues and pupal homogenate according to the method of Harold (1975) using a kit of Diamond diagnostics. The enzyme was measured at a wavelength of 546 nm by spectrophotometer.

7. Data Analysis:

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of the difference between means using GraphPad InStat© v. 3.01 (1998).

RESULTS

In the present study, the newly moulted 5th instar larvae of the black cutworm *Agrotis ipsilon* had been infected with LC_{50} values of EPNs, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* (21 IJs/ml and 62 IJs/ml, respectively). The influenced enzymatic activities of phosphatases and transaminases were determined in the haemolymph of 6th instar larvae, at three-time intervals, 6, 24 & 48 hr, respectively.

1. Effects of EPNs on the Activities of Phosphatases in Larval Haemolymph of *A. ipsilon*:

1.1. Effects of EPNs on the Acid Phosphatase (ACP) activity:

Data of the ACP activity in haemolymph of 6th instar larvae of *A. ipsilon* were assorted in Table (1). Depending on these data, the ACP activity gradually increased in haemolymph of uninfected (control) last instar larvae, with the age (68.15±2.10, 78.22±1.95 & 81.82±2.53 U/L, at 6, 24 and 48 hr, respectively). As clearly shown in data of the same table, the ACP activity was significantly enhanced in haemolymph of 6 hr-old instar larvae (+44.62 and +69.01% activity increments, by *S. carpocapsae* and *H. bacteriophora*, respectively). In contrast, the enzyme activity remarkably declined, at 24 and 48 hr. Moreover, the enzyme activity gradually declined with the age of the last instar larvae (-30.38 & -49.57% activity reductions by *S. carpocapsae* and -9.59 and -29.98% activity reductions by *H. bacteriophora*, at 24 and 48 hr, respectively). As obviously shown, *H. bacteriophora* exhibited a higher enhancing potency on the enzyme activity than *S. carpocapsae*, at 6 hr. On the other hand, *S. carpocapsae* exhibited a higher reducing potency on the enzyme activity than *H. bacteriophora*, at both 24 and 48 hr.

Table 1: Acid phosphatase activity in haemolymph (U/L) of last (6th) instar larvae of *A. ipsilon* as influenced by infection of the newly moulted 5th instar larvae with LC_{50} values of the tested Entomopathogenic Nematoda (EPNs).

Nematode species		Time interval		
		6 hr	24 hr	48 hr
S. carpocapsae	mean±SD	98.56±2.17 b	54.45±1.02 c	41.26±1.31 c
	Change (%)	+44.62	-30.38	-49.57
H. bacteriophora	mean±SD	115.18±1.93 c	70.72±1.21 b	57.29±1.10 b
	Change (%)	+69.01	-9.59	-29.98
Control	mean±SD	68.15±2.10	78.22±1.95	81.82±2.53

Mean±SD followed by a letter: a: insignificant (P >0.05), b: significant (P<0.05), c: highly significant (P<0.01), d: very highly significant (P<0.001).

1.2. Effects of EPNs on the Alkaline Phosphatase (ALP) Activity:

Data on the ALP activity in haemolymph of the last instar larvae of *A. ipsilon* were distributed in Table (2). As clearly shown, ALP activity gradually increased in the uninfected (control) last instar larvae with the age (27.96±0.84, 31.27±1.03 & 33.57±1.19 U/L, at 6, 24 and 48 hr, respectively). With regard to the infected last instar larvae, ALP activity was found in a similar course of ACP as previously mentioned. In some detail, ALP activity was promoted in haemolymph of 6 hr-old last instar larvae. The nematode *H. bacteriophora* exhibited a higher stimulatory potency than *S. carpocapsae* (+13.93 and +81.36% increments of ALP activity, respectively). On the other hand, ALP activity was considerably reduced in haemolymph of both 24 hr- and 48 hr-old larvae. The reduction of enzyme activity gradually increased with the age (-24.78 and -26.35% activity reduction by *S. carpocapsae* and -26.35 and -36.32% activity reduction by *H. bacteriophora*, at 24 and 48 hr, respectively). Depending on the ALP activity reduction at 48 hr, *S. carpocapsae* exhibited stronger reducing potency than *H. bacteriophora*.

Table 2: Alkaline phosphatase activity in haemolymph (U/L) of last (6th) instar larvae of *A. ipsilon* as influenced by infection of the newly moulted 5th instar larvae with LC_{50} values of the tested EPNs.

Nematode species		Time interval		
		6 hr	24 hr	48 hr
S. carpocapsae	mean±SD	31.02±1.42 a	23.52±0.60 b	20.82±0.69 b
	Change (%)	+13.93	-24.78	-37.98
H. bacteriophora	mean±SD	50.71±1.89 b	23.03±0.44 b	21.38±1.40 b
	Change (%)	+81.36	-26.35	-36.32
Control	mean±SD	27.96±0.84	31.27±1.03	33.57±1.19

a, b: see footnote of table (1).

2. Effects of EPNs on the Activities of Transaminases in Larval Haemolymph of *A. ipsilon*:

2.1. Effects of EPNs on the Glutamic Oxaloacetic Transaminase (GOT) Activity:

Data on GOT activity in haemolymph of the last instar larvae of *A. ipsilon* were summarized in Table (3). According to these data, GOT activity gradually increased in haemolymph of uninfected (control) last instar larvae

(22.66±1.79, 29.57±1.30 & 36.22±1.68 U/L, at 6, 24 and 48 hr, respectively). GOT activity in 6 hr-old larvae significantly increased in haemolymph by both EPNs (+111.91 and +137.16% increments of GOT activity by *S. carpocapsae* and *H. bacteriophora*, respectively). At 24 hr of the last instar larvae, GOT activity considerably increased by *H. bacteriophora* (+13.59% activity increment) but was slightly decreased by *S. carpocapsae* (-4.36% activity reduction). At 48 hr, GOT activity was greatly decreased (-25.32 and -15.41% reductions of activity, by *S. carpocapsae* and *H. bacteriophora*, respectively).

Table 3: Glutamic oxaloacetic transaminase activity in haemolymph (U/L) of last (6^{th}) instar larvae of *A. ipsilon* as influenced by infection of the newly moulted 5^{th} instar larvae with LC_{50} values of the tested EPNs.

Nematode species		Time interval		
		6 hr	24 hr	48 hr
S. carpocapsae	mean±SD	48.02±0.70 b	28.28±1.05a	27.05±1.84 b
	Change (%)	+111.91	-4.36	-25.32
H. bacteriophora	mean±SD	53.74±1.87 c	33.57±1.46 b	30.64±0.48 b
	Change (%)	+137.16	+13.52	-15.41
Control	mean±SD	22.66±1.79	29.57±1.30	36.22±1.68

a, b: see footnote of table (1).

2.2. Effects of EPNs on the Glutamic Pyruvic Transaminase (GPT) Activity:

Data of the GPT activity in haemolymph of the last instar larvae of *A. ipsilon* were arranged in Table (4). Depending on these data, GPT activity gradually increased in haemolymph of uninfected (control) last instar larvae with the age (34.11±2.85, 37.97±1.66 & 38.91±1.03 U/L, at 6, 24 and 48 hr, respectively). As clearly shown by these data, GPT activity was predominantly declined at all time intervals by all tested EPNs, with one exception of slightly increased activity at 6 hr by *S. carpocapsae* (+1.06% activity increment). On the other hand, the GPT activity was increasingly reduced with the age of the last instar larvae (-14.35 and -17.99% activity reduction by *S. carpocapsae*, as well as -31.92 and -37.49% activity reduction by *H. bacteriophora*, at 24 and 48 hr, respectively). Also, *H. bacteriophora* exhibited stronger reducing potency on the GPT activity than *S. carpocapsae.*

Table 4: Glutamic pyruvic transaminase activity in haemolymph (U/L) of last (6^{th}) instar larvae of *A. ipsilon* as influenced by infection of the newly moulted 5^{th} instar larvae with LC_{50} values of the tested EPNs.

Nematode species		Time interval		
		6 hr	24 hr	48 hr
S. carpocapsae	mean±SD	34.47±1.50 a	32.52±1.78 b	31.91±1.27 b
	Change (%)	+1.06	-14.35	-17.99
H. bacteriophora	mean±SD	29.16±1.88 a	25.85±1.60 c	24.32±2.17 c
	Change (%)	-14.51	-31.92	-37.49
Control	mean±SD	34.11±2.85	37.97±1.66	38.91±1.03

a, b: see footnote of table (1).

DISCUSSION

Entomopathogenic nematodes (EPNs) in the families of Steinernematidae and Heterorhabditidae are lethal endoparasites of insects, which could secrete active substances, including toxins, proteases, and so on, contributing to the lethal effect on infected host insects (Brown *et al*., 2006; Ffrench-Constant *et al*., 2007; Toubarro *et al*., 2009). The lethal processes caused by these insecticidal active substances of EPNs are often related to the activity changes of some enzymes in the host insects (Grewal *et al*., 2005; Ahmed *et al*., 2014; Ibrahim *et al*., 2019). Besides these nematode secretions, their associated symbiotic bacteria also secrete certain toxins for suppressing the immune responses of the host insect ending in the host's death. For example, *Xenorhabdus nematophila*, the symbiont bacterium of *Steinernema carpocapsae*, secretes several enzymes, including hemolysins, lipases, and proteases, which are thought to contribute to virulence or nutrient acquisition for the bacterium and its nematode host *in vivo* (Koppenhöfer, 2007; Richards and Blair, 2010; Van Damme *et al*., 2016; Askitosari *et al*., 2021; El Aalaoui *et al*., 2022).

As reported by many authors (Abdel-Razek *et al*., 2004; Shaurub *et al*., 2015; Ibrahim *et al*., 2015; Shairra *et al*., 2016; Vidhya *et al*., 2016; Shaurub *et al*., 2020; Ghoneim *et al*., 2022), some EPNs influenced certain metabolic activities of a number of insects, including some enzymes and the main body metabolites.

1. Disturbed Activities of Phosphatases in Haemolymph of *A. ipsilon* Larvae by EPNs *H. bacteriophora* and *S. carpocapsae*:

In insects, detoxifying enzymes are generally involved in the enzymatic defense against foreign materials and play a crucial role in decreasing the toxicity of xenobiotics in order to maintain the normal physiological functions in the body (Visetson and Milne, 2001; Mukanganyama *et al*., 2003; Li and Liu, 2007). The detoxification can be achieved in insects by different enzymes, such as esterases, phosphatases, glutathione S-transferase, glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase, and they have been reported as protectants against the oxidative stresses (Zibaee *et al*., 2011; Altuntaş *et al*., 2016). As clearly reported, phosphatases have been included in the category of detoxifying enzymes (Srinivas *et al*., 2004; Zheng *et al*., 2007). Phosphatase activity is related to the physiological state of the cell (Ali *et al*., 2013). As a lysosomal enzyme, acid phosphatase may have a role in autophagy and cell turnover as well as defense (Xia *et al*., 2000). In addition, acid and alkaline phosphatases are hydrolytic enzymes and are responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids in alkaline and acidic conditions, respectively under the name of dephosphorylation (Zibaee *et al*., 2011). These two phosphatases not only hydrolyze phosphate groups from the ingested toxic molecules and naturally occurring ones, but also their activities may refer to the efficiency of digestion and transportation within the insect body (Nation, 2008; Zibaee *et al*., 2011).

1.1. Disturbed Activity of Acid Phosphatase (ACP) in Haemolymph of *A. ipsilon* Larvae by EPNs:

In the present study, ACP activity significantly increased in haemolymph of 6 hr-old last instar larvae of *A. ipsilon*, after infection of penultimate instar larvae with the EPN *S. carpocapsae* or *H. bacteriophora*. *H. bacteriophora* exhibited a higher enhancing potency on the enzyme activity than *S. carpocapsae*, at this time. This result was in agreement with many reported results of increasing ACP activity in larvae of various insects after infection with different EPNs, such as larvae of the Mediterranean fruit fly *Ciratitis capitata* after infection with *Steinernema riobrave* and *H. bacteriophora* (Soliman, 2002); larvae of the Egyptian cotton leafworm *Spodoptera littoralis* after infection with *S.*

riobrave and *H. bacteriophora* (Ahmed *et al*., 2014); *A. ipsilon* 6th instar larvae at 6 hr post-infection with *Steinernema glaseri* or *H. bacteriophora* (Hassan *et al*., 2016); the 5th nymphs of the desert locust *Schistocerca gregaria* after infection with 1000 IJs of *H. bacteriophora* (Gaber *et al*., 2018); the 3rd instar larvae of *S. littoralis* after infection with EPNs *H. bacteriophora* and *S. carpocapsae* (Gomaa *et al*., 2020); and the 4th instar larvae of *S. littoralis* after infection with LD_{50} values of *S. riobrave* or *H. bacteriophora* for 24 hr (Shaurub *et al*., 2020). Also, increased ACP activity in the haemolymph of some insects after infection with entomopathogens have been reported (Xia *et al*., 2001; Mirhaghparast *et al*., 2013; Vidhya *et al*., 2016).

The increasing ACP activity in *A. ipsilon* larvae, in the present study, might indicate physiological adaptability to combat oxidative stress or might be related to an inhibition of the lipid peroxidation process and physiological response mechanism against the nematode toxic secretions. Also, the increasing activity of ACP, in the current study, might be attributed to its role in the transportation of lipids, such as monoacylglyceroles, through low-density lipoproteins to supply the energy demands of the treated larvae (Khorshidi *et al*., 2019). The increase of ACP activity in *A. ipsilon* larvae might be due to the phagocytosis of certain hemocytes to the invading nematodes, since phagocytosis is known to stimulate the production of lysosomal enzymes, and ACP acts as a lysosome marker (Xia *et al*., 2000; Hassan *et al*., 2016). In general, the increase of ACP activity, in the current study, might be an attempt by the infected insect to defend against the invading pathogens, where lysozymes are a component of humoral immunity (Mohamed *et al*., 2016; Satyavathi *et al*., 2018; Dorrah *et al*., 2019).

In contrast, ACP activity was remarkably decreased in haemolymph of 24 and 48 hr-old last instar larvae of *A. ipsilon*, after infection of the penultimate instar larvae with *S. carpocapsae* or *H. bacteriophora*, in the present study. This result was, to some extent, corroborated with a few reported results of decreasing ACP activity in larvae of some insects after infection with some EPNs, such as late 3rd instar larvae of *C. capitata* after infection with *H. bacteriophora* AS1, *H. bacteriophora* HP88, *S. carpocapsae* ALL, and *S. riobrave* ML29 (Shaurub *et al*., 2015) and 5th nymphs of *S. gregaria* after infection with 2000 IJs (high concentration) of *H. bacteriophora* (Gaber *et al*., 2018). Also, these results were in accordance with some reported results in the available literature on different host species infected with different nematodes (Żółtowska *et al*., 2006; Wu *et al*., 2013; Ahmed *et al*., 2014).

In the current investigation, the decrease of ACP activity could be due to the inability of the cell to undergo enzymatically controlled reactions under nematode infection (Soliman, 2002). In addition, decreasing ACP activity might be due to the reduced phosphorus liberation for energy metabolism and decreased rate of metabolism, as well as a decreased rate of transport of metabolites (Senthil Nathan *et al*., 2005). Also, the declination of ACP activity in *A. ipsilon* larvae, as a response to infection with the tested EPNs, might be due to strong inhibition of ecdysone which is followed by a subsequent decrease in the number of lysosomes and in turn declined level of ACP (Hassan, 2002).

1.2. Disturbed Activity of Alkaline Phosphatase (ALP) in Haemolymph of *A. ipsilon* Larvae by EPNs:

Depending on the currently available literature, there are many reported results of increasing ALP activity in several insects as a response to infection with certain EPNs. For example, Soliman (2002) recorded an increase of ALP activity in the last instar larvae of *C. capitata* after infection with *S. riobrave* and *H. bacteriophora*. Ahmed *et al*. (2014) found an increasing activity of ALP in *S. littoralis* larvae as a response to the infection with *S. riobrave* and *H. bacteriophora*. The ALP activity increased in the 3rd instar larvae of *S.*

littoralis after infection with two EPN isolates (*H. bacteriophora* and *S. carpocapsae*) and the entomopathogenic fungus, *Beauveria bassiana*, separately on in combination (Gomaa *et al*., 2020). Also, increased activity of ALP was determined in the 4th instar larvae of *S. littoralis* after incubation with LD_{50} values of *S. riobrave* and *H. bacteriophora* for 24 hr (Shaurub *et al*., 2020). Results of the present study were consistent with those reported results since ALP activity increased in the haemolymph of *A. ipsilon* at 6 hr of the last instar larvae after infection of the penultimate instar larvae with *S. carpocapsae* or *H. bacteriophora*.

On the contrary, the current investigation found that ALP activity was considerably reduced in the haemolymph of 24 and 48-old last instar larvae of *A. ipsilon*, after infection of penultimate instar larvae with *S. carpocapsae* or *H. bacteriophora*. Depending on the reduction of ALP activity at 48 hr, *S. carpocapsae* exhibited stronger reducing potency than *H. bacteriophora*. These results were, to some extent, in accordance with few reported results of decreasing ALP activity in larvae of some insects after infection with certain EPNs, such as a slight decrease of the ALP activity in *S. littoralis* larvae after infection with *S. feltiae* (Ahmed *et al*., 2014); significantly declined ALP activity in the late 3rd instar larvae of *C. capitata* due to infection with four EPNs *H. bacteriophora* AS1, *H. bacteriophora* HP88, *S. carpocapsae* ALL and *S. riobrave* ML29 (Shaurub *et al*., 2015); and decreasing activity of ALP in the 5th nymphs of *S. gregaria* at 1000 and 2000 IJs of *H. bacteriophora* (Gaber *et al*., 2018). Also, significantly declined ALP activity was recorded in some insects infected with other nematodes (Żółtowska *et al*., 2006; Wu *et al*., 2013). In the present study, the reduction of ALP activity could be attributed to the reduced enzyme synthesis and/or binding of toxicants at the active site of the enzyme (Shakoori *et al*., 1994).

2. Disturbed Activities of Transaminases In Haemolymph of *A. ipsilon* Larvae as Response to EPN Infection:

Glutamic oxaloacetic transaminase (GOT, or Alanine aminotransferase, ALT) and Glutamic pyruvic transaminase (GPT, or aspartate aminotransferase, AST) are the key enzymes of transamination within the intermediary metabolism of insects to make amino acids available for biochemical demands (Knox and Greengard, 1965; Plant and Morris, 1972). Some authors (Ender *et al*., 2005; Etebari *et al*., 2005) reported that these transaminases constitute a strategic link between carbohydrate and lipid metabolism. They are known to be disturbed under various physiological and pathological conditions. In other words, disturbance of these enzymes in insects denotes biochemical impairment and lesions of tissues and cellular function because they are involved in the detoxification processes and metabolism (Enan and Berberian, 1986).

In the current study, GOT activity significantly increased in the haemolymph of *A. ipsilon* at 6 hr of the last instar larvae after infection of penultimate instar larvae with *S. carpocapsae* or *H. bacteriophora*. At 24 hr, GOT activity considerably increased by *H. bacteriophora* while decreased by *S. carpocapsae*. At 48 hr time intervals, GOT activity was greatly decreased by both EPNs. With regard to GPT, its activity was predominantly declined after infection with *S. carpocapsae* or *H. bacteriophora*, at all-time intervals, with few exceptions. However, these predominately reduced activities of GOT and GPT in *A. ipsilon* after infection with *S. carpocapsae* or *H. bacteriophora* were in an agreement with many reported results of decreased activities of transaminases in some insects after infection with certain EPNs. For example, GOT and GPT activities decreased in *C. capitata* last instar larvae after infection with *S. riobrave* and *Heterorhabditis* sp. (Soliman, 2002). The activities of GOT and GPT were remarkably decreased at 48 hr post-infection of 4th instar larvae of *S. littoralis* with *H. bacteriophora* and *S. riobrave* (Ahmed *et al*., 2014). The activity of GOT and GPT of late 3rd instar larvae of

C. capitata infected by *H. bacteriophora* AS1 and *S. carpocapsae* ALL was significantly decreased (Shaurub *et al*., 2015). Shaurub *et al*. (2020) incubated the newly moulted 4th instar larvae of *S. littoralis* with LD_{50} values of *S. riobrave* and *H. bacteriophora* for 24 h and determined the reduction of the activities of transaminases. The activities of GOT and GPT in the 3rd instar larvae of *S. littoralis* were decreased after infection with the EPNs *H. bacteriophora* and *S. carpocapsae* and the fungus *B. bassiana*, separately on in combination (Gomaa *et al*. (2020).

To explicate the predominant reduction of GOT and GPT activities in *A. ipsilon* larvae after infection with *H. bacteriophora* and *S. carpocapsae*, in the current study, some suggestions could be provided. The decreasing activities of these enzymes might be attributed to the significant decline in the free amino acids content (Kaur *et al*., 1985), the quantum of which directly influences the activity of transaminases at the time of protein synthesis (Soliman, 2002). Also, it might be due to a disruption of the link between carbohydrate and protein metabolism (Azmi *et al*., 1998). Reduction in transaminase activities has been indicated to lead to the required reduction in the host protein synthesis since these are generalized enzymes involved in dephosphorylation and energy transfer (Shaurub *et al*., 2020). The reduction of GPT activity might be due to the interference of the tested EPNs with the hormonal regulation of protein synthesis and neurosecretory hormones involved in the regulation of transaminase levels (Abulyazid *et al*., 2005). Also, this reduction of GPT activity might due to the effects of these EPNs on the synthesis or functional levels of this enzyme directly or indirectly by altering the cytomorphology of the cells (Nath, 2000).

Conclusion:

Depending on the results of the present study, the infection of *A. ipsilon* larvae with the EPNs, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* caused considerable disturbances in the activities of phosphatases and transaminases. These disturbances were a result of interactions between these nematodes and their symbiotic bacteria *versus* the immune responses of *A. ipsilon* ending in death. Therefore, the tested EPNs can be used in the biological control program, as an effective alternative to chemical pesticides against the present dangerous insect, *A. ipsilon*.

Acknowledgement:

The authors deeply thank Dr. El-Sadawy, National Research Centre, Doqqi, Giza, Egypt, for kindly providing imported EPN species, *H. bacteriophora* and *S. carpocapsae* for achieving the present study.

REFERENCES

Abd-El-Aziz, H.S.; Abd El Mageed, E.N.I. and Salama, M.A.S. (2019): Field Evaluation of some insecticides for controlling black cutworm, *Agrotis ypsilon* and their effect on some histological aspects. *Egyptian Academic Journal of Biological Sciences*, 11(2): 57- 68. DOI: 10.21608/eajbsd.2019.92263.

Abdel-Hakim, E.A. and El-Mandarawy, M.B. (2017): Effects of juvenile hormone mimic on growth, morphogenesis and morphology of hemocytes of the black cutworm, *Agrotis ipsilon* larvae (Lepidoptera: Noctuidae). *Current Science International*, 6(3): 662-669.

Abdel-Razek, A.; Kamel, K.E. and Salama H.S. (2004): Biochemical effects of the nematode-bacteria complex on the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae). *Archives of Phytopathology and Plant Protection*, 37: 205–214. https://doi.org/10.1080/03235400420000218754

Abdin, M.I. (1979): Standard technique for mass rearing of the black cutworm, *Agrotis ipsilon*. M.Sc. Thesis, Faculty of Agriculture, Al-Azhar

University, Egypt.

Abulyazid, I.; Mahmoud, S.M.; Elshafei, A.M. and Taha, R.H. (2005): Physiological changes of irradiated and diseased mulberry silkworm. *Bombyx mori*. *Egyptian Journal of Agricultural Research*, 83(4): 1431-1445.

Ahmed, N.F.; Maklad, A.M.H.; Yassin, S.A. and Abolmaaty, S.M. (2014): Biochemical efects of *Steinernema feltiae*, *Steinernema riobrave* and *Heterorhabditis bacteriophora* on *Spodoptera littoralis* larvae. *Egyptian Academic Journal of Biological Sciences (C Physiology and Molecular Biology)*, 36(1):23–34. https://doi.org/ 10.21608/eajbsc. 2014.16044

Ahmed, A.A.I.; Khalil, S.S.H. and Sahab, A.F. (2022): Identification and evaluation of isolated entomopathogenic fungus from Egyptian soil against the black cutworm larvae of *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 32: 67. https://doi.org/10.1186/s41938-022-00564-0

Ali, N.S.; Ali, S.S. and Shakoori, A.F. (2013): Effects of sublethal doses of Malathion on biochemical components of Malathion-resistant and -susceptible adults of *Rhyzopertha dominica*. *Pakistan Journal of Zoology*, 45: 203–212.

Ali, M.; Allouf, N. and Ahmad, M. (2022): First report of entomopathogenic nematode *Steinernema affine* (Nematoda: Steinernematidae) in Syria and its virulence against *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Egyptian Journal of Biological Pest Control*, 32: 101 https://doi.org/10.1186/ s41938-022-00602-x

Altuntaş, H.; Şanal Demircl, S.N.; Duman, E. and Eegin, E. (2016): Toxicological and physiological effects of ethephon on the model organism, *Galleria mellonella* L. 1758 (Lepidoptera: Pyralidae). *Turkish Journal of Entomology*, 40 (4): 413-423. DOI: http://dx.doi. org10.16970/ted.00995

Askitosari, Th.D.; Pantjajani, T.; Nathania, S.; Wahyudi, A.F. and Sugianto, N.Ch. (2021): Identification of entomopathogenic nematode-associated bacteria originating from Mojokerto. *Indonesian Journal of Biotechnology and Biodiversity*, 5(1): 31-38. DOI: https://doi.org/10. 47007/ijobb.v5i1.76

Atwa AA (2014): Entomopathogenic nematodes as biopesticides. In: "Basic and applied aspects of biopesticides"(Sahayaraj, K., ed.). Springer, India, pp 69–98. https://doi.org/10.1007/978-81-322-1877-7_5

Azmi, M.A.; Sayed, N.H. and Khan, N.F. (1998): Comparative topological studies of RB-a (Neem Extract) and Coopex (Permethrin + Bioallethrin) against *Sitophilus oryzae*with reference to their effects on oxygen consumption and GOT and GPT activity. *Turkish Journal of Zoology*, 22: 307-310.

Bhat, A.H.; Chaubey, A.K. and Askary, T.H. (2020): Global distribution of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*. *Egyptian Journal of Biological Pest Control*, 30:31. https://doi. org/ 10. 1186/ s41938- 020- 0212-y

Binning, R.R.; Coats, J.; Kong, X. and Hellmich, R.L. (2015): Susceptibility to *Bt* proteins is not required for *Agrotis ipsilon* a version to *Bt* maize. *Pest Management Science*, 71: 601–606. doi:10.1002/ps.3901PMID: 25186105

Boemare, N.E. (2002): Biology, taxonomy and systematics of *Xenorhabdus* and *Photorhabdus*. In: "Entomopathogenic Nematology",

CABI Publishing: Wallingford, UK. Pp: 35–56.

Brown, S.E.; Cao, A.T.; Dobson, P.; Hines, E.R.; Akhurst, R.J. and East, P.D. (2006): Txp40, a ubiquitous insecticidal toxin protein from *Xenorhabdus* and *Photorhabdus* bacteria. *Applied and Environmental Microbiology*, 72:1653-1662. doi: 10.1128/AEM. 72.2.1653-1662.2006.

Burnell, A.M. and Stock, S.P. (2000): *Heterorhabditis, Steinernema* and their bacterial symbionts–lethal pathogen of insects. Nematology, 2:31–42. DOI: 10.1163/ 156854100508872

Çağlayan, S.H. (1990): Effect of 20-hydroxyecdysone on acid phosphatase activity in the larval fat body of *Manduca sexta*. *Biochemistry International*, 20(3): 511-118.

Çağlayan, A.; Atay, T. and Kepenekci, İ. (2021): Efficacy of some native Entomopathogenic nematodes against the alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), and the lucerne beetle, *Gonioctena fornicate* (Brüggemann) (Coleoptera: Chrysomelidae), adults under laboratory conditions. *Egyptian Journal of Biological Pest Control*, 31:89, 7pp. https://doi.org/10. 1186/s41938-021-00436-z

Calvo-Agudo, M.; González-Cabrera, J.; Pico, Y.; Calatayud-Vernich, P.; Urbaneja, A.; Dicke, M. and Tena, A. (2019): Neonicotinoids in excretion product of phloem-feeding insects kill beneficial insects. *Proceedings of Natural Academy of Science, USA*, 116(34): 16817-16822. doi:10.1073/pnas. 1904298116

Capinera, J.L. (2001): Hand book of vegetable pests. Academic Press, New York, 729 pp.

Chowański, S.; Kudlewska, M.; Marciniak, P. and Rosiński, G. (2014): Synthetic Insecticides- is There an Alternative? *Polish Journal of Environmental Studies*, 23(2): 291-302.

Cranshaw, W.S. and Zimmerman, R. (2013): Insect Parasitic Nematodes. Fact Sheet No 5.573. Colorado State University Extension. Available at: http://extension.colostate.edu/docs/pubs/insect/05573.

Dahi, H.F.; Ibrahem, W.G. and Ali, M.M. (2009): Heat requirements for the development of the black cutworm, *Agrotis ipsilon* (Hüfnagel) (Noctuidae: Lepidoptera). *Egyptian Academic Journal of Biological Sciences*, 2(1): 117- 124. Doi: 10.21608/EAJBSA.2009.15502

Demok, S.; Endersby-Harshman, N.; Vinit, R.; Timinao, L.; Robinson, LJ.; Susapu, M.; Makita, L.; Laman, M.; Hoffmann, A. and Karl, S. (2019): Insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* mosquitoes in Papua New Guinea. *Parasite Vectors*, 12(1): 333. doi:10.1186/s13071-019-3585-6

Dorrah, M.A.; Mohamed, A.A. and Shaurub, E.H. (2019): Immunosuppressive effects of the limonoid azadirachtin, insights on a nongenotoxic stress botanical, in flesh flies. *Pesticide Biochemistry and Physiology*, 153: 55–66. doi: 10.1016/j.pestbp. 2018. 11.004.

Du Preez, G.; Daneel, M.; De Goede, R.; Du Toit, M.J.; Ferris, H.; Fourie, H.; Geisen, S.; Kakouli-Duarte, Th.; Korthals, G.; Sanchez-Morenoh, S. and Schmidt, J.H. (2022): Nematode-based indices in soil ecology: Application, utility, and future directions. *Soil Biology and Biochemistry*, 169: 108640, 14pp. https://doi.org/10.1016/j. soilbio. 2022.108640

Ehlers, R.U. (2001): Mass production of entomopathogenic nematodes for plant protection. *Applied*

Microbiology and Biotechnology, 56(5-6): 623-633. https ://doi.org/ 10.1007/s0025 30100 711.

El Aalaoui, M.; Mokrini, F.; Dababat, A.A.; Lahlali, R. and Sbaghi, M. (2022): Moroccan entomopathogenic nematodes as potential biocontrol agents against *Dactylopius opuntiae* (Hemiptera: Dactylopiidae). *Scientific Reports*, 12, 1–17. https: // doi.org/10.1038/s41598-022-11709-4

El-Aziz, A.; Omer, E.A. and Sabra, A.S. (2007): Chemical composition of *Ocimum americanum* essential oil and its biological effects against *Agrotis ipsilon* (Lepidoptera: Noctuidae). *Research Journal of Agriculture and Biological Sciences*, 3(6): 740-747.

El-Shershaby, M.M.A. (2010): Toxicity and biological effect of *Capparis* leaves extracts to the black cutworm, *Agrotis ipsilon* (Hufn.). *Egyptian Academic Journal of Biological Sciences (F. Toxicology & Pest Control)*, 2(1): 45-51. doi: 10.21608/eajbsf.2010.17462

Enan, E.E. and Berberian, I.G. (1986): Interaction of pesticide exposure level with some biochemical enzymes among field workers. *Journal of the Egyptian Society of Parasitology*, 3: 76-90.

Ender, I.; Ferah, A.; Kemal, B. and Ahmet, G. (2005): Biochemical stress indicators of greater wax moth exposure to organophosphorus insecticides. Journal of Economic Entomology, 98: 358–366. DOI: 10.1603/0022-0493-98.2.358

Etebari, K. and Matindoost, L. (2004a): Effects of hypervitaminosis of vitamin B3 on silkworm biology. *Journal of Bioscience*, 29: 417-422.

Etebari, K. and Matindoost, L. (2004b): The study on effects of larval age and starvation stress on biochemical macromolecules abundance of haemolymph in silkworm *Bombyx mori*. In: *Proceedings of the Sixteenth Iranian Plant Protection Congress, General Entomology Symposium, August 28–September 1, University of Tabriz, Iran*,; p. 435.

Etebari, K; Mirhoseini, S.Z. and Matindoost, L. (2005): A study on interspecific biodiversity of eight groups of silkworm (*Bombyx mori*) by biochemical markers. *Insect Science*, 12: 87–94

Etebari, K.; Bizhannia, A.; Sorati, R. and Matindoost, L. (2007): Biochemical changes in haemolymph of silkworm larvae due to pyriproxyfen residue. *Pesticide Biochemistry and Physiology*, 88: 14–19. https://doi.org/10.1016/j. pestbp.2006.08.005

Fahmy, A.R. (2014): Toxicological, biological and biochemical impact of some chitin synthesis inhibitors on the black cutwom, *Agrotis ipsilon* (Lepidoptera: Noctuidae) (Hufn.). *Egyptian Academic Journal of Biological Sciences (A. Entomology)*, 7(2): 119-128. Doi: 10.21608/EAJBSA.2014.13145

Ferreira, C. and Terra, W.R. (1980): Intracellular distribution of hydrolases in midgut caeca cells from an insect with emphasis on plasma membrane-bound enzymes. *Comparative Biochemistry and Physiology (Part B: Comparative Biochemistry)*, 66: 467-473. https://doi.org/10.1016/0305-0491 (80)90235-7

Ferreira, T.; van Reenen, C.A.; Endo, A.; Tailliez, P.; Pages, S.; Spröer, C.; Malan, A.P. and Dicks, L.M. (2014): *Photorhabdus heterorhabditis* sp. nov., a symbiont of the entomopathogenic nematode *Heterorhabditis zealandica*. *International Journal of Systematic and Evolutionary Microbiology*, 64(5):1540–1545. doi: 10.1099/ ijs. 0.059840-0.

Ffrench-Constant, R.H., Dowling, A. and Waterfield, N.R. (2007): Insecticidal toxins from *Photorhabdus* bacteria and their potential use in agriculture. *Toxicon*, 49: 436-451. doi: 10.1016/ j.toxicon. 2006.11.019.

Gaber, M.A.M.; Shamseldean, M.S.M.; Ibrahim, N.M. and 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.

Ghoneim, K.; Tanani, M.; Hassan, H.A. and Bakr, N.A. (**2022**): Comparative efficiency of the entomopathogenic nematodes, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*, against the main body metabolites of *Agrotis ipsilon* (Lepidoptera: Noctuidae). *Egyptian Academic Journal of Biological Sciences (C. Physiology & Molecular Biology)*, 14(2):57-72. DOI: 10.21608/EAJBSC.2022. 260471

Ghoneim, K.; Tanani, M.; Hassan, H. and Bakr, N. (**2023**): Virulence of the entomopathogenic nematodes *Steinernema carpocapsae* (Weiser) and *Heterorhabditis bacteriophora* (Poinar) against the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae). *African Journal of Biological Sciences*, 5(1): 71-82.

Gomaa, Sh.I.; Halawa, S.M.; Shalaby, F.F. and Abdel-Hafez, H.F. (2020): Toxicological and biochemical parameters of microbial preparations on the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Egyptian Journal of Plant Protection Research Institute*, 3(1): 204-214.

Gozel, U. and Gozel, C. (2016): Entomopathogenic Nematodes in Pest Management. In "Integrated Pest Management (IPM): Environmentally Sound Pest Management"(Gill, H.K. and Goyal, G. eds.). DOI: 10.5772/63894

GraphPad InStat© v. 3.01 (1998): GraphPad Software, Inc.
7825 Fay Avenue, Suite 230 La Jolla, CA 92037 USA. Available online at: http://www.graphpad. com/scientific-software/instat/

Grewal, P.; Ehlers, R.-U. and Shapiro-Ilan, D.I. (Eds.) (2005): Nematodes as Biological Control Agents. CABI Publishing, Wallingford, UK, 505 pp.

Griffin, C.T.; Boemare, N.E. and Lewis, E.E. (2005): Biology and Behaviour. In: "Nematodes as biocontrol agents"(Grewal, P.S.; Ehlers, R.U. and Shapiro-Ilan, D. eds.). CABI, Wallingford, UK, pp. 47-64. https://doi.org/10.1079/9780851990 170.0047

Gulcu, B.; Cimen, H, Raja, R.K. and Hazir, S. (2017): Entomopathogenic nematodes and their mutualistic bacteria: their ecology and application as microbial control agents. *Biopesticides International*, 3(2): 79-112.

Haq, H.S.; Shaikh, M.A. and Khan, R.H. (2004): Protein proteinase inhibitor genes in combat against insects, pests and pathogens: natural and engineered phytoprotection. *Archives of Biochemistry and Biophysics*, 431: 145-159. doi: 10. 1016/j.abb.2004.07.022.

Harold, V. (1975): Practical Clinical Biochemistry. 4thed., Arnold-Heinemann Publishers (India) Private Limited, India, 294pp.

Harrison, R.L. and Lynn, D.E. (2008): New cell lines derived from the black cutworm, *Agrotis ipsilon*, that support replication of the *A. ipsilon* multiple nucleopolyhedrovirus and several group I nucleopolyhedroviruses. *Journal of*

Invertebrate Pathology, 99: 28–34. doi: 10.1016/j.jip.2008.02.015.

Hassan, H.A. (2002): Biological and biochemical studies on the the effect of some botanical extracts on cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). M.Sc. Thesis, Faculty of Science, Ain Shams University, Egypt, 173pp.

Hassan, H.A.; Shairra, S.A. and Ibrahim, S.S. (2016): Virulence of entomopathogenic nematodes *Steinernema glaseri* and *Heterorhabditis bacteriophora* Poinar (HP88strain) against the black Cutworm, *Agrotis ipsilon*. *Egyptian Academic Journal of Biological Sciences (A. Entomology)*, 9(1): 33-48. Doi: 10. 21608/EAJBSA. 2016.12853

Ibrahim, S.A.M.; Taha, M.A.; Salem, H.H.A. and 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(5): 111-118.

Ibrahim, S.A.M, Salem H.H.A. and Taha M.A. (2019): Dual application of entomopathogenic nematodes and fungi on immune and antioxidant enzymes of the greater wax moth, *Galleria mellonella* L. *Egyptian Journal of Biological Pest Control*, 29: 20. https://doi. org/ 10. 1186/ s41938- 019- 0125-9

Ismail, S.M. (2021): Field persistence of certain new insecticides and their efficacy against black cutworm, *Agrotis ipsilon* (Hufnagel). *Bulletin of the National Research Centre*, 45:17, 7pp. https://doi.org/10.1186/ s42269-020-00481-y

James, M., Malan, A.P. and Addison, P. (2018): Surveying, and screening South African entomopathogenic nematodes for the control of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). *Crop Protection*, 105, 41-48. https://doi. org/10.1016/j.cropro.2017.11.008

Kaur, S.P.; Sidhu, D.S.; Dhillon, S.S. and Kumar, N.K. (1985): Transaminases during development and aging of the bruchis *Zabrotes subfasiatus* (Boh.) (Coleoptera: Bruchidae). *Insect Science and its Application*, 6(5): 585-590.

Khorshidi, M.; Abad, R.F.P.; Saber, M. and Zibaee, A. (2019): Effects of hexaflumuron, lufenuron and chlorfluazuron on certain biological and physiological parameters of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Biocatalysis and Agricultural Biotechnology*, 21, 101270: 8pp. https://doi.org/10.1016/j.bcab. 2019. 101270

Klein, B.; Read, P.A. and Babson, L.A. (1960): Rapid method for quantitative determination of serum alkaline phosphatase. *Clinical Chemistry*, 6(3): 269-275. https:// doi. org/10.1093/clinchem/6.3.269

Klowden, M.J. (2007): Physiological Systems in Insects. Elservier /Academic Press, 2nd ed., 688pp.

Knox, W.E. and Greengard, O. (1965): The regulation of some enzymes of nitrogen metabolism- an introduction to enzyme physiology. *Advances in Enzyme Regulation*, 3: 247-313.

Koppenhöfer, H.S. (2007): Bacterial Symbionts of Steinernema and Heterorhabditis. In: "Entomopathogenic nematodes: systemat-ics, phylogeny and bacterial symbionts"(Nguyen, K. and Hunt, D.M., eds.). Brill, Leiden. https:// doi. org/ 10.1163/ ej. 97890 04152 939.i- 816. 43

Kumar, D.; Kumari, P.; Kamboj, R.; Kumar, A.; Banakar, P. and Kumar, V. (2022): Entomopathogenic nematodes as potential and effective biocontrol agents against cutworms,

Agrotis spp.: present and future scenario. *Egyptian Journal of Biological Pest Control*, 32:42 https://doi.org/10.1186/s41938-022-00543-5

Labaude, S. and Griffin, C.T. (2018): Transmission success of entomopathogenic nematodes used in pest control. *Insects*, 9(2): 72-79. https://doi.org/10.3390/insects9020072

Lacey L.A. and Georgis R. (2012): Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. *Journal of Nematology*, 44(2): 218–225.

Lacey, L.A.; Grzywacz, D.; Shapiro-Ilan, D.I.; Frutos, R.; Brownbridge, M. and Goettel, M.S. (2015): Insect pathogens as biological control agents: back to the future. Journal of Invertebrate Pathology, 132: 1-41. https://doi.org/10.1016/j.jip.2015.07.009

Laznik, Ž. and Trdan, S. (2011): Entomopathogenic nematodes (Nematoda: Rhabditida) in Slovenia: from tabula rasa to implementation into crop production systems. In: "Insecticides -advances in integrated pest management" (Perveen, F., ed.). InTech, Rijeka, pp: 627–656.

Leonar, A.L.; Nimkingrat, P.; Aryal, S.; Martinez, J.G.; Bhat, A.H. and Sumaya, N.H. (2022): Natural association of the entomopathogenic nematode *Heterorhabditis indica* (Rhabditida: Heterorhabditidae) from the Philippines with the non-symbiotic bacterium *Ochrobactrum anthropi* (Proteobacteria: Brucellaceae). *Egyptian Journal of Biological Pest Control*, 32:83 https://doi.org/10.1186/ s41938-022-00576-w

Li, X.Z. and Liu, Y.H. (2007): Diet influences the detoxification enzyme activity of *Bactrocera tau* (Walker) (Diptera: Tephritidae). *Acta Entomologica Sinica*, 50(10): 989-995. http://www.insect.org.cn/EN/Y2007/V50/I10/989

Liu, Y.Q.; Fu, X.W.; Feng, H.Q.; Liu, Z.F. and Wu, K.M. (2015): Trans-regional migration of *Agrotis ipsilon* (Lepidoptera: Noctuidae) in north-East Asia. *Annals of the Entomological Society of America*, 108: 519–527. https://doi.org/10.1093/aesa/sav050

Machado, R.; Bhat, A.H.; Abolafa, J.; Muller, A.; Bruno, P.; Fallet, P.; Arce, C.; Turlings, T.; Bernal, J.S.; Kajuga, J.; Waweru, B. and Toepfer, S. (2021): Multi-locus phylogenetic analyses uncover species boundaries and reveal the occurrence of two new entomopathogenic nematode species, *Heterorhabditis ruandican*. sp. and *Heterorhabditis zacatecanan*. sp. *Journak of Nematology*, 53:e2021-89. https:// doi. org/ 10. 21307/ jofnem- 2021-089

Mahmood, I.; Imadi, S.R.; Shazadi, K.; Gul, A.; Hakeem, K.H. (2016): Effects of pesticides on environment. In: "Plant, soil and microbes: implications in crop science"(Hakeem, K.H.; Akhtar, M.S. and Abdullah, S.N.A., eds). Vol. 1. Switzerland: Springer. Pp: 253–269

Miao, Y. (2002): Studies on the activity of the alkaline phosphatase in the midgut of infected silkworm, *Bombyx mori* L. *Journal of Applied Entomology*, 126: 38-142. https://doi.org/10.1046/j.1439-0418.2002. 00625.x

Mirhaghparast, S.K.; Zibaee, A. and Hajizadeh, J. (2013): Effects of *Beauveria bassiana* and *Metarhizium anisopliae* on cellular immunity and intermediary metabolism of *Spodoptera littoralis*

Boisduval (Lepidoptera: Noctuidae). *Invertebrate Survival Journal*, 10: 110–119

Mohamed, A.A.; Zhang, L.; Dorrah, M.A.; Elmogy, M. and Yousef, H.A. (2016): Molecular characterization of a *c*-type lysozyme from the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). *Developmental and Comparative Immunology*, 61: 60–69. doi: 10. 1016/j.dci.2016.03.018.

Mordue, W. and Goldsworthy, 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

Moroney, M.J. (1956): Facts from figures. (3rd ed.). Penguin Books Ltd., Harmondsworth, Middlesex, 228 pp.

Moustafa, M.A.M.; Awad, M.; Amer, A.; Hassan, N.N.; Ibrahim, E.S.; Ali, H.M.; Akrami, M. and Salem, M.Z.M. (2021): Insecticidal activity of Lemongrass essential oil as an eco-friendly agent against the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae). Insects, 12, 737.https://doi.org/10.3390/ insects12080737

Mukanganyama, S.; Figueroa, C.C.; Hasler, J.A. and Niemeyer, H.M. (2003): Effects of DIMBOA on detoxification enzymes of the aphid *Rhopalosiphum padi* (Homoptera: Aphididae). *Journal of Insect Physiology*, 49: 223-229. doi: 10. 1016/s0022-1910(02)00269-x

Muştu, M.; Aktürk, M.; Akkoyun, G. and Çakır, S. (2021): Life tables of *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) (Lepidoptera: Noctuidae) on different cultivated plants. *Phytoparasitica*, 49: 21-31. https:// doi.org/10.1007/s12600-020-00868-7

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(3): 127–137. https://doi.org/10.1006/pest.2000.25 09

Nation, J.L. (2008): Insect Physiology and Biochemistry, 2nd ed. CRC Press, London, 560pp. https://doi.org/10. 1201/9781420061789

Nouh, G.M. (2022): Effect of temperature and soil moisture on the efficacy of indigenous and imported strains of the entomopathogenic nematode, *Heterorhabditis* sp. Against the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 32:28 https://doi.org/10.1186/s41938-022-00507-9

Odendaal, D.; Addison, M.F. and Antoinette, P. (2015): Entomopathogenic nematodes for the control of the codling moth (*Cydia pomonella* L.) in field and laboratory trials. *Journal of Helminthology*, 20: 1-9. Doi: https://doi.org/10.4001/003. 023.0224

Odendaal, D.; Addison, M.F. and Malan, A.P. (2016): Control of diapausing codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), in wooden fruit bins using entomopathogenic nematodes. *Biocontrol Science and Technology*, 26: 1504-1515. DOI: https://doi.org/ 10.4001/003.023.0224

Peçen, A. and Kepenekci, İ. (2022): Efficacy of entomopathogenic nematode isolates from Turkey against wheat stink bug, *Aelia rostrata* Boheman (Hemiptera: Pentatomidae) adults under laboratory conditions. *Egyptian Journal of Biological Pest Control*, 32:91 https://doi.org/10. 1186/s41938-022-00590-y

Picimbon, J.-F. (2020): Interpopulational variations of odorant-binding

protein expression in the black cutworm moth, *Agrotis ipsilon*. *Insects*, 11(11): 798. Doi: 10.3390/ insects11110798

Plant, R. and Morris, I.D. (1972): A comparative study on the variation of aminotransferase activity and its total free amino acids in the fat body, haemolymph, intestine and haemolymph protein content in *Philosamia ricini* during larval-pupal development. *Indian Journal of Biochemistry and Biophysics*, 9: 199-202.

Poinar, G.O. and Grewal, P.S. (2012): History of entomopathogenic Nematology. *Journal of Nematology*, 44(2):153–161.

Rauschenbach, I.Y.; Bogomolova, E.V.; Gruntenko, N.E.; Adonyeva, N.V. and Chentsova, N.A. (2007): Effects of juvenile hormone and 20-hydroxyecdysone on alkaline phosphatase activity in *Drosophila* under normal and heat stress conditions. *Journal of Insect Physiology*, 53(6): 587–591. https:// doi.org/10.1016/j.jinsphys. 2007. 02.011

Ray, A.; Rao, C.G.P.; Srivevi, R. and Ramamurty, P.S. (1984): Changes in acid phosphatase activity in *Spodoptera litura* (Lepidoptera: Noctuidae) during the post embryonic and adult development. *Journal of Entomology*, 9(3): 161-167.

Richards, G.R. and Blair, H.G. (2010): Examination of *Xenorhabdus nematophila* lipases in pathogenic and mutualistic host interactions reveals a role or xlpA in nematode progeny production. *Applied and Environmental Microbiology*, 76: 221–229. Doi: 10.1128/AEM. 01715-09

Sahota, T.S. (1975): Effect of juvenile hormone on acid phosphatases in the degenerating flight muscles of the Douglas-fir beetle, *Dendroctonus pseudotsugae*. *Journal of Insect Physiology*, 21:471-478. https://doi.org/10. 1016/0022-1910(75)90152-3

Satyavathi, V.V.; Mohamed, A.A.; Kumari, S.; Mamatha, D.M. and Duvic, B. (2018): The IMD pathway regulates lysozyme-like proteins (LLPs) in the silkmoth *Antheraea mylitta*. *Journal of Invertebrate Pathology*, 154: 102–108. Doi: 10.1016/j.jip. 2018. 04.006

Senthil Nathan, S.; Kalaivani, K. and Chung, P.G. (2005): The effects of azadirachtin and nucleopolyhedrovirus on midgut enzymatic profile of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae), *Pesticide*. *Biochemistry and Physiology*, 83: 46–57. https://doi.org/10.1016/j. pestbp. 2005.03.009

Shahzad, M.; Qu, Y.; Zafar, A.; Ur Rehman, S. and Islam, T. (2020): Exploring the influence of knowledge management process on corporate sustainable performance through green innovation. *Journal of Knowledge Management*, 24(9): 2079-2106. Doi: 10.1108/JKM-11-2019-0624

Shairra, S.A.; El-Sharkawy, M.A.A.; Hassan, K.A. and Ahmed, D.A. (2016): The Efficacy of entomopathogenic nematodes on the pink bollworm, *Pectinophora gossypiella*. *Egyptian Academic Journal of Biological Sciences (F. Toxicology & Pest control)*, 8(2): 103 – 113. Doi: 10. 21608/EAJBSF.2016.17123

Shakoori, A.R.; Tufail, N. and Saleem, M.A. (1994): Response of malathion resistant and susceptible strains of *Tribolium castaneum* (Herbst) to bifenthrin toxicity. *Pakistan Journal of Zoology*, 26: 169–178.

Shamseldean, M.M.; Ibrahim, A.A.; Zohdi, N.M.; Shairra, S.A. and Ayaad, T.H. (2008): Effect of the Egyptian entomopathogenic nematode

isolates on controlling some economic insect pests. *Egyptian Journal of Biological Pest Control*, 18(1): 81-89. DOI: 10.13140/2. 1. 5146.8805

Sharaby, A. and El-Nojiban, A. (2015): Evaluation of some plant essential oils against the black cutworms *Agrotis ipsilon*. *Global Journal of Advanced Research*, 2(4): 701-711.

Shaurub, E.H. and Abd El-Aziz, N.M. (2015): Biochemical effects of lambda-cyhalothrin and lufenuron on *Culex pipiens* L. (Diptera: Culicidae). *International Journal of Mosquito Research*, 2(3): 122-126.

Shaurub, E.H.; N.F. Reyad,; H.A. Abdel-Wahab, and S.H. Ahmed, (2016): Mortality and nematode production in *Spodoptera littoralis* larvae in relation to dual infection with *Steinernema riobrave*, *Heterorhabditis bacteriophora*, and *Beauveria bassiana*, and the host plant. *Biological Control*, 103: 86-94. https://doi.org/10.1016/j. biocontrol.2016.08.007

Shaurub, E.H.; Reyad, N.F. and Mohamed, A.A. (2020): Pathogen-mediated modulation of host metabolism and trophic interactions in *Spodoptera littoralis* larvae. *Entomologia Experimentalis et Applicata*, 168: 956–966. Doi: 10.1111/eea.12998

Soliman NA (2002): Pathological and biochemical effects of some entomopathogenic nematodes on Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). PhD. Thesis, Cairo University, Giza, Egypt, 114pp.

Srinivas, R.; Udikeri, S.S.; Jayalakshmi, S.K. and Sreeramulu, K. (2004): Identification of factors responsible for insecticide resistance in *Helicoverpa armigera*. *Comparative Biochemistry and Physiology (C: Toxicological Pharmacology)*, 137: 261-269. Doi: 10.1016/j.cca. 2004. 02.002.

Srivastava, J.P. and Saxena, S.C. (1967): On the alkaline and acid phosphatases in the alimentary tract of *Periplaneta americana* L. *Applied Entomology and Zoology*, 2: 85-92.

Takeda, M. (2008): Current research of pest insects of vegetables in last decade. *Annual Report of the Kansai Plant Protectection*, 50: 39-44.

Tietz, N.W. (1986): Text Book of Clinical Chemistry. W.B. Saunders Co., London, Philadelphia. 1919pp. https://doi.org/10.1016/0307-4412 (86)90182-2

Tiryaki, D. and Temur, C. (2010): The fate of pesticide in the environment. *Journal of Biological and Environmental Sciences*, 4(10): 29-32.

Toubarro, D.; Lucena-Robles, M.; Nascimento, G.; Costa, G.; Montiel, R.; Coelho, A.V. and Simões, N. (2009): An apoptosis-inducing serine protease secreted by the entomopathogenic nematode *Steinernema carpocapsae*. *International Journal of Parasitology*, 39:1319-1330. doi: 10.1016/j.ijpara.2009.04.013.

Van Damme, V.M.; Beck, B.K.E.G.; Berckmoes, E.; Moerkens, R.; Wittemans, L.; De Vis, R.; Nyuttens, D.; Casteels, H.F.; Maes, M.; Tirry, L. and De Clerq, P. (2016): Efficacy of entomopathogenic nematodes against larvae of *Tuta absoluta* in the laboratory. *Pest Management Science*, 72: 1702-1709. DOI: 10. 1002/ps.4195

Vattikonda, S.R. and Sangam, S.R. (2017): Effect of forskolin on the growth and differentiation of the ovary of *Papilio demoleus* L. (Lepidoptera: Papilionidae). *International Research Journal of Environmental Science*, 6: 13-17.

Vidhya, D.; Rajiv, P. and Padmanabhan, N (2016): Impact of entamopathogenic fungal infection on the detoxifying

enzyme in cotton leafworm *Spodoptera litura* (Fabricius). *International Journal of Pharma and Bio Sciences*, 7: 943–948. DOI: 10.22376/ijpbs.2016. 7.4.b943-948

Visetson, S. and Milne, M. (2001): Effect of root extract from derris (*Derris elliptica* Benth) on mortality and detoxification enzyme level in diamond back moth larvae (*Plutella xylostella* Linn). Kasetsart Journal : Natural Science, 35: 157-163.

Wang, Y.; Fang, G.; Chen, X.; Cao, Y.; Wu, N.; Cui, Q.; Zhu, C.; Qian, L.; Huang, Y. and Zhan, S. (2021): The genome of the black cutworm *Agrotis ipsilon*. *Insect Biochemistry and Molecular Biology*, 139: 1–10. https:// doi. org/ 10. 1016/j. ibmb. 2021. 103665

Wolfersberger, M.G. (1984): Enzymology of plasma membranes of insect intestinal cells. *American Zoologist*, 24: 187-197. https://doi.org/10. 1093/icb/24.1.187

Wu, H.; Liu, Q.; Li, X.; Wang, Y. and Zhang, H. (2013): Activities of four enzymes in *Galleria mellonella* larvae infected with entomopathogenic nematode *Heterorhabditis beicherrianan* sp. *African Journal of Agricultural Research*, 8:3245–3250. Doi: 10. 5897/AJAR11.743

Xia, Y.; Dean, P.; Judge, A.J.; Gillespie, J.P.; Clarkson, J.M. and Charnley, A.K. (2000): Acid phosphatases in the haemolymph of the desert locust, *Schistocerca gregaria*, infected with the entomopathogenic fungus *Metarhizium anisopliae*. *Journal of Insect Physiology*, 46: 1249–1257. Doi: 10.1016/S0022-1910(00)00045-7.

Xia, Y.; Clarkson, J.M. and Charnley, K.A. (2001): Acid phosphatases of *Metarhizium anisopliae* during infection of the tobacco hornworm *Manduca sexta*. *Archives of Microbiology*, 176: 427–434. Doi: 10.1007/s002030100342.

Yağci, M.; Özdem, A.; Erdoğuş, F.D. and Ayan, E. (2021a): Efficiency of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) on the codling moth (*Cydia pomonella* L.) (Lepidoptera: Tortricidae) under controlled conditions. Egyptian *Journal of Biological Pest Control*, 31:75, 5pp. https://doi.org/10. 1186/ s41938-021-00399-1

Yağcı, M.; Fırat, T.A.; Erdoğuş, F.D. and Şahin, M. (2021b): Virulence of four entomopathogenic nematode against diferent stages of the Mediterranean fruit fy, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). *Egyptian Journal of Biological Pest Control*, 31:126, 5pp. https://doi.org/10. 1186/s41938-021-00472-9

Yu, W.; Du, J.; Hu, Y.; Shen, R. and Mu, W. (2012): Toxicity of six insecticides to black cutworm *Agrotis ypsilon* (Rottemberg) and safety evaluation to oil organisms. *Acta phytophylacica sinica*, 39: 277-282.

Yüksel, E.; Imren, M.; Özdemir, E.; Bozbuğa, R. and Canhilal, R. (2022): Insecticidal effect of entomopathogenic nematodes and the cell-free supernatants from their symbiotic bacteria against different larval instars of *Agrotis segetum* (Denis & Schifermüller) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 32:54 https://doi.org/10.1186/s41938-022-00555-1

Zheng, Y.Z.; Lan, W.S.; Qiao, C.L.; Mulchandani, A. and Chen, W. (2007): Decontamination of vegetables sprayed with organophosphate pesticides by organophosphorus hydrolase and carboxylesterase (BI). *Applied Biochemistry and Biotechnology*,

136(3): 233-242. doi: 10.1007/s12010-007-9022-x

Zibaee, A.; Zibaee, I. and Jalali Sendi, J. (2011): A juvenile hormone analog, pyriproxifen, affects some biochemical components in the hemolymph and fat bodies of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae), *Pesticide. Biochemistry and Physiology*, 100: 289–298. Doi: 10.1016/J.PESTBP.2011.05.002

Żółtowska, K.; Grochla, P. and Łopieńska-Biernat, E. (2006): Activity of superoxide dismutase in *Galleria mellonella* larvae infected with entomopathogenic nematodes *Steinernema affinis* and *S. feltiae*. *Wiad Parazitology*, 4: 283–286.