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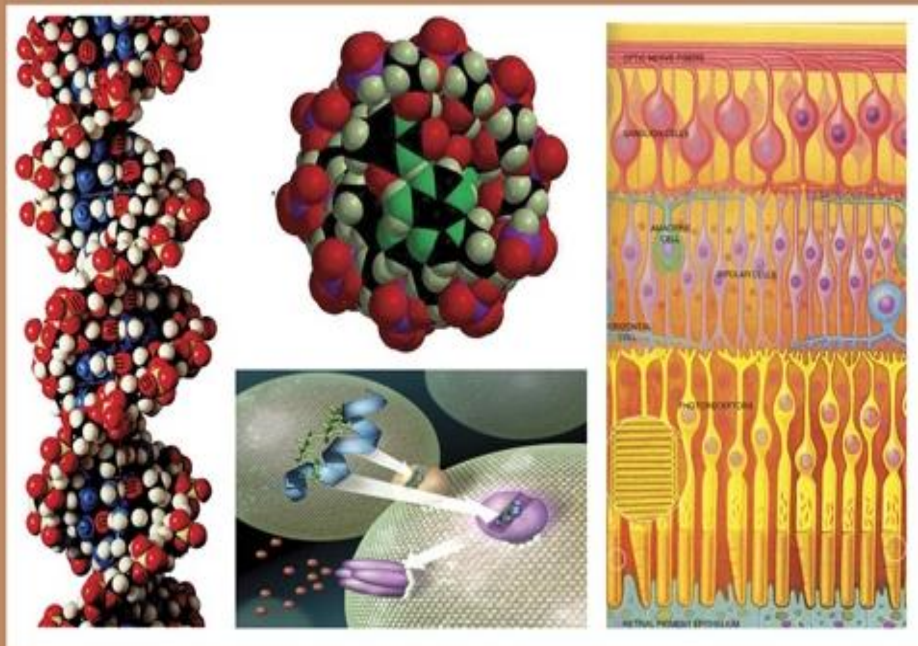

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# Comparative Efficiency of The Entomopathogenic Nematodes, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*, against the Main Body Metabolites of *Agrotis ipsilon* (Lepidoptera: Noctuidae)

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

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**ABSTRACT**

The black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), is globally distributed. It is a polyphagous insect attacking nearly all vegetables and many economic field crops in the world. The objective of the current study was to investigate the efficacy of two Entomopathogenic nematodes, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* on the main body metabolites in haemolymph of the infected larvae. The newly moulted penultimate (5th) instar larvae of *A. ipsilon* had been infected with $LC_{50}$ values of S. carpocapsae and *H. bacteriophora* (21 IJs/ml and 62 IJs/ml, respectively) and the influenced contents of the metabolites in haemolymph of last ($6^{th}$) instar larvae were determined at three-time intervals of the instar, 6, 24 & 48 hr, respectively. The most important results could be summarized as follows. The protein content in the infected larvae was predominantly reduced by both nematode species. Moreover, it was tremendously reduced at the last time interval of exposure (21.94 & 26.45% protein reductions, by *S. carpocapsae* and *H. bacteriophora*, respectively). Thus, *H. bacteriophora* exhibited stronger reducing potency than *S. carpocapsae*. Also, the lipid content in haemolymph of EPN-infected larvae was gradually reduced with the time intervals. The greatest reduction of lipids was determined at 48 hr post-infection (26.55 & 21.73% lipid reduction, by *S. carpocapsae* and *H. bacteriophora*, respectively). Thus, *S. carpocapsae* exerted greater reducing action than *H. bacteriophora*. The carbohydrate content was predominantly reduced in haemolymph of infected larvae. *S. carpocapsae* exhibited a higher reducing effect on carbohydrate content than *H. bacteriophora*, at 48 hr of the last instar. Almost, *S. carpocapsae* had greater reducing potency against the main body metabolites in haemolymph of the last instar larvae of *A. ipsilon*, leading to drastically disrupted intermediary metabolism. Therefore, *S. carpocapsae* can be applied as an effective part of the Integrated Pest Management program against this serious pest.

## INTRODUCTION

The black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is widely distributed in the world (Talpur *et al.*, 2002). It is a migrant moth with a great reproductive capacity (Santos and Shield, 1998, Mishra, 2020). It is a polyphagous insect known to feed on nearly all vegetables and many important grains worldwide (Navarro *et al.*, 2010; Fernandes *et al.*, 2013; Picimbon, 2020; Rodingpuia and Lalthanzara, 2021). It is considered one of the most destructive pests of many field crops in Egypt (Ahmed *et al.*, 2022).


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In Egypt, the control measure of *A. ipsilon* depends mainly on the application of conventional insecticides (Vattikonda and Sangam, 2017; Abd-El-Aziz *et al*., 2019; Ismail, 2021). Some authors (Capinera, 2001; Takeda, 2008, Kumar *et al*., 2022) reported that the chemical control for this pest is often not effective and remains inadequate because of its larval hiding behavior during the daylight hours causing hidden damage in fields and the fast development of resistance and cross-resistance to almost all marketed conventional insecticides (Yu *et al*., 2012; Fahmy, 2014; Mahmoud *et al*., 2016; Shaurub *et al*., 2018).

In addition, many broad-spectrum insecticides have led to several hazards to the natural enemies (like parasites, and predators), allowing an exponential increase in pest populations (Calvo-Agudo *et al*., 2019; Demok *et al*., 2019) besides the adverse impacts on human health and domestic animals (Shahzad *et al*., 2020). Therefore, alternative approaches have been encouraged recently to avoid or minimize insecticidal hazards and introduce new effective and safer compounds with negligible effects on the ecosystem (Korrat *et al*., 2012; Derbalah *et al*., 2014).

Entomopathogenic nematodes (EPNs) are good alternatives to synthetic insecticides. They are soil-dwelling multicellular organisms attacking insect pests that live in, on, or near the soil surface (Adams and Nguyen, 2002; Vashisth *et al*., 2013). The use of these EPNs is economical and eco-friendly since they are harmless to non-target organisms, human health and the environment (Georgis *et al*., 2006; Gulcu *et al*., 2017). EPNs have attracted much interest around the world to study their distribution, virulence, and usage in IPM programs (Ali *et al*., 2022).

EPNs have many advantages, such as their high reproductive potential, the ability to kill hosts quickly, high virulence, broad host range, easy mass rearing, and safety for plants and vertebrates (Kaya and Gaugler, 1993). Therefore, EPNs are usually used in different parts of the world as biological control agents against many economic insect pests (Laznik and Trdan, 2011; Belien, 2018). EPNs have been used to suppress the soil-inhabitant insects, which are applied as a successful biological control agent against *A. ipsilon* larvae and pupae (Nouh, 2022).

EPNs of the families Steinernematidae and Heterorhabditidae, and their symbiotic bacteria, are pathogenic for a wide range of insect pests and have been used successfully as a biological control agent (Yüksel *et al*., 2022). EPNs possess free-living 3rd stage infective juveniles (IJ) that can survive a long time without feeding (Koppenhöfer *et al*., 2000). These IJs invade their hosts *via* natural body openings, such as the mouth, the anus and the spiracles, or even the cuticle. Once they enter to haemocoel, the mutualistic bacteria *Xenorhabdus* in *Steinernema* and *Photorhabdus* in *Heterorhabditis* are released to kill the host within 2 days (Gaugler, 2002; Griffin, *et al*., 2005; Kaya *et al*., 2006).

In insects, the use of haemolymph as a medium for controlling insect pests has been made because the changes occurring in the haemolymph are quickly transferred to other portions of the insect's body (Pugazhvendan and Soundararajan, 2009). Hemolymph plays a vital role for insects as the place where they compete with invading microorganisms. Production of anti-microbial peptides (AMPs), activation of melanization, phagocytosis, and encapsulation are the main events against EPN infection in the insect haemolymph (Castillo *et al*., 2011). When the nematode enters the haemocoel of insect, soluble proteins in haemolymph and proteins released by hemocytes and hematopoietic organs participate in their encapsulation (Lemaitre and Hoffmann, 2007). The objective of the current study was to investigate the efficacy of two EPNs, *S. carpocapsae* and *H. bacteriophora* on the

main body metabolites (proteins, lipids and carbohydrates) in haemolymph of the infected last instar larvae of *A. ipsilon*.

## MATERIALS AND METHODS

### I. The Insect Culture:

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 in Plant Protection Research Institute, Doqqi, Giza, Egypt. Rearing technique was carried out according to Abdin (1979) with the 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 $4^{th}$ 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. Selected EPNs:

#### 2.1. Rearing and Production of 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 ($7^{th}$) 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℃±2 ℃. After six days, check larvae for infection. The successfully infected larvae will appear beige to dark red. Upon successful infection, Petri dish containing nematodes, larvae and filter paper was 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. 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 ℃.

#### 2.2. Larval Infection of *A. ipsilon*:

In a preliminary bioassay toxicity test, a series of concentrations of each EPN 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 $5^{th}$ 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 $5^{th}$ instar larvae of *A. ipsilon* were placed in separate plastic cups. Sixty cups for each instar, for each concentration and control,

were conducted for each treatment. All cups were covered and kept at 25±2ºC.

**3. Determination of the Main Body Metabolites:**

The influenced contents of the main metabolites in haemolymph of last (6th) instar larvae were determined at three-time intervals: 6, 24 & 48 hr, respectively.

**3.1. 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 Eppendorf Pipetman containing a few milligrams of phenoloxidase inhibitor (Phenylthiourea) to prevent 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.

**3.2. Protein Estimation:**

Quantitative determination of the total protein content (mg/g) was conducted according to the method of Weichselbaum (1946) and using the research kits purchased from Biodiagnostics Company (29 El-Tahrer St., Dokki, Giza, Egypt). The method depends on the protein forms of a violet complex with cupric ions in an alkaline medium and then measured the absorbance at 550 nm using a spectrophotometer.

**3.3. Lipid Estimation:**

Quantitative determination of the total lipid content was conducted in the larval and pupal homogenate according to the vanillin assay procedure (van Handel, 1985) using research kits purchased from Biodiagnostics Company (Dokki, Giza, Egypt). For the assay, 100 µL of the supernatant was transferred into a borosilicate microplate well and heated at 90ºC until complete solvent evaporation. Ten microlitres of 98% sulphuric acid were then added to each well and the microplate was incubated at 90ºC for 2 min in a water bath. After cooling the microplate on ice, 1.5 ml of vanillin reagent was added to each well. The plate was homogenized, incubated at room temperature for 15 min and its absorbance was measured spectrophotometrically at 525 nm (Foray *et al*., 2012).

**3.4. Carbohydrate Estimation:**

Quantitative determination of the total carbohydrate (as glycogen) content was conducted using the anthrone reagent according to Singh and Sinha (1977). Anthrone Reagent: 200 mg anthrone dissolved in 100 mL of ice-cold 95% $H_2SO_4$. It was prepared freshly before use. Standard Glucose: Stock –100 mg dissolved in 100 mL water. Working standard - 10 mL of stock diluted to 100 mL with distilled water. It was stored in a refrigerator after adding a few drops of toluene. The carbohydrate content was measured using the Spectrophotometer at 620 nm.

**4. Statistical Analysis of Data:**

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

**1. Effects of Entomopathogenic Nematodes (EPNs) on the Total Protein Content in Haemolymph of *A. ipsilon* Larvae**:

After infection of the newly moulted penultimate (5th) instar larvae of *A. ipsilon* with $LC_{50}$ values of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* (21 IJs/ml and 62 IJs/ml, respectively), data of the total protein content in haemolymph of last (6th) instar larvae, at three-time intervals (6, 24 & 48 hr), were assorted in Table (1). Depending on these data, the total protein content in haemolymph of control (uninfected) larvae gradually decreased with the time (7.08±0.84, 6.37±1.21 & 6.20±0.49 g/dL, at 6, 24 & 48 hr, respectively). As clearly seen

in the same table, the protein content in the infected larvae was drastically reduced, regardless of the nematode species. In some detail, the protein content in haemolymph of infected larvae was non-significantly reduced at 6 and 24 hr but tremendously reduced at the last time (21.94 & 26.45% reduction of proteins, by *S. carpocapsae* and *H. bacteriophora*, respectively). As exiguously shown by these data, *H. bacteriophora* exhibited stronger reducing potency against the protein content in the larval haemolymph than *S. carpocapsae*, at the last time interval.

**Table 1:** Total protein content in haemolymph (g/dL) of last (6$^{th}$) instar larvae of *A. ipsilon* as influenced by treatment of the newly moulted 5$^{th}$ instar larvae with $LC_{50}$ values of the tested Nematoda.

| Nematode species | | Time interval | | |
|---|---|---|---|---|
| | | **6 hr** | **24 hr** | **48 hr** |
| ***S. carpocapsae*** | mean±SD | 6.10±0.12 a | 5.78±0.33 a | 4.84±0.12 b |
| | Change (%) | -13.84 | -9.26 | -21.94 |
| ***H. bacteriophora*** | mean±SD | 5.75±0.59 a | 5.47±0.55 a | 4.56±0.37 b |
| | Change (%) | -18.78 | -14.12 | -26.45 |
| **Control** | mean±SD | 7.08±0.84 | 6.37±1.21 | 6.20±0.49 |

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

**2. Effects of Entomopathogenic Nematodes (EPNs) on the Total Lipid Content in Haemolymph of *A. ipsilon* Larvae**:

Data of the total lipid content in haemolymph of larvae at three-time intervals were distributed in Table (2). According to these data, the lipid content in haemolymph of control larvae gradually decreased with the age (3.70±0.25, 3.23±0.17 & 2.90±0.18 g/dL, at 6, 24 & 48 hr, respectively). Also, the lipid content in haemolymph of EPN-infected larvae was gradually reduced with time. The greatest reduction of lipids was determined in haemolymph of larvae at 48 hr (26.55 & 21.73% lipid reduction, by *S. carpocapsae* and *H. bacteriophora*, respectively). As clearly seen, *S. carpocapsae* exerted greater reducing action on the lipid content in haemolymph of infected larvae than *H. bacteriophora*.

**Table 2:** Total lipid content in haemolymph (g/dL) of last (6$^{th}$) instar larvae of *A. ipsilon* as influenced by treatment of the newly moulted 5$^{th}$ instar larvae with $LC_{50}$ values of the tested Nematoda.

| Nematode species | | Time interval | | |
|---|---|---|---|---|
| | | **6 hr** | **24 hr** | **48 hr** |
| ***S. carpocapsae*** | mean±SD | 2.70±0.20 b | 2.40±0.36 b | 2.13±0.21 b |
| | Change (%) | -27.03 | -25.70 | -26.55 |
| ***H. bacteriophora*** | mean±SD | 3.36±0.40 a | 2.87±0.16 a | 2.27±0.25 b |
| | Change (%) | -9.19 | -11.15 | -21.73 |
| **Control** | mean±SD | 3.70±0.25 | 3.23±0.17 | 2.90±0.18 |

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

**3. Effects of Entomopathogenic Nematodes (EPNs) on the Total Carbohydrate Content in Haemolymph of *A. ipsilon* Larvae**:

Data of the total carbohydrate content in haemolymph of larvae at three-time intervals were arranged in Table (3). As obviously shown in these data, the

carbohydrate content in haemolymph of control larvae gradually increased with the age (0.26±0.02, 0.28±0.01 & 0.29±0.02 g/dL, at 6, 24 & 48 hr, respectively). On the other hand, the carbohydrate content was predominantly reduced in haemolymph of infected larvae. Also, the reducing potency of EPN considerably increased with the time interval (41.38 & 31.03% carbohydrate reductions, at 48 hr with *S. carpocapsae* and *H. bacteriophora*, respectively). Also, these data clearly show that *S. carpocapsae* exhibited a higher reducing effect on carbohydrate content than *H. bacteriophora*, at 48 hr of the last instar.

**Table 3:** Total carbohydrate content in haemolymph (g/dL) of last (6$^{th}$) instar larvae of *A. ipsilon* as influenced by treatment of the newly moulted 5$^{th}$ instar larvae with $LC_{50}$ values of the tested Nematoda.

| Nematode species | | Time interval | | |
|---|---|---|---|---|
| | | **6 hr** | **24 hr** | **48 hr** |
| ***S. carpocapsae*** | mean±SD | 0.22±0.02 b | 0.19±0.01 c | 0.17±0.02 c |
| | Change (%) | -15.38 | -32.14 | -41.38 |
| ***H. bacteriophora*** | mean±SD | 0.23±0.01 b | 0.22±0.02 b | 0.20±0.02 b |
| | Change (%) | -11.54 | -21.43 | -31.03 |
| **Control** | mean±SD | 0.26±0.02 | 0.28±0.01 | 0.29±0.02 |

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

## DISCUSSION

In insects, different biological and physiological processes need adequate energy (Chapman, 1998; Fagan *et al*., 2002). The content of macromolecules (such as protein, lipid and carbohydrate) is a valuable indicator of the level of metabolism, after treatment with exogenous materials (Zhu *et al*., 2012). It is important to mention that protein synthesis is crucial for insect development and reproduction. Carbohydrates are the main source of energy during insect metamorphosis. Energy reserves such as proteins, lipids, and glycogen in the haemolymph are also important indicators of the level of metabolism in insects (Chowanski *et al*., 2015; Ferreira *et al*., 2014; Ismail, 2018). These energy reserves are closely related to different physiological processes in insects (Nawaz *et al*., 2017).

With regard to entomopathogenic nematodes (EPNs), it is well known that the pathological effects appear immediately after reaching the insect's haemocoel. The EPN-symbiotic bacteria when released from nematode guts into the haemocoel, rapidly multiply causing lethal septicemia to the insect host (Nickle and Welch, 1984). Therefore, biochemical changes in the haemolymph are expected, since the haemolymph is the main site of action. Also, the success of entomopathogens in insect control depends on their stress potential and ability to modulate certain physiological aspects of their insect hosts (Shaurub *et al*., 2020).

### 1. Protein Reduction in Haemolymph of *A. ipsilon* larvae by EPNs:

In this context, proteins are the most important organic constituents of animal tissues, including insects, and play an important role in energy production (Taşkın and Aksoylar, 2011). The protein synthesis in insects is a prerequisite process for development and reproduction (Taşkın and Aksoylar, 2011). As reported by many authors (Suarez *et al*., 2005; Hahn and Denlinger, 2007; Bernstein and Jervis, 2008; Sugumaran, 2010; Resmitha *et al*., 2014), proteins perform a wide variety of physiological and metabolic functions and play a key role in the production of microsomal detoxifying enzymes.

On the basis of the currently available literature, the total protein content in the haemolymph of the 4$^{th}$ instar of the mosquito *Culex pipiens* was reduced after

infection with the EPN *Romanomermis culicivorax* (Schmidt and Platzer, 1979). The haemolymph protein content of the Egyptian cotton leafworm *Spodoptera littoralis* larvae was markedly reduced 30 hrs post-infection with some EPNs (El-Bishry, 1989). Also, the total protein content of *S. littoralis* larvae was significantly decreased post-infection with the EPNs *Steinernema riobrave* and *Heterorhabditis bacteriophora* (Ahmed *et al*., 2014). Four EPNs *H. bacteriophora* AS1, *H. bacteriophora* HP88, *Steinernema carpocapsae* ALL, and *Steinernema riobrave* ML29 caused a significant decline in the total protein content in larvae of the Mediterranean fruit fly *Ceratitis capitata* (Shaurub *et al*., 2015). Hassan *et al*. (2016) studied the disturbance of the protein in the *A. ipsilon* $6^{th}$ instar larvae at different time intervals after infection with *S. glaseri* and *H. bacteriophora*. There was a significant decrease in total protein content after 24 hr of infection. Infection of the $5^{th}$ nymphs of desert locust *Schistocerca gregaria* with the nematode juvenile concentrations 1000 and 2000 IJs of *H. bacteriophora* resulted in a reduction of total protein content in nymphs (Gaber *et al*., 2018). Shaurub et al. (2020) incubated the newly moulted $4^{th}$ instar larvae of *S. littoralis* with $LD_{50}$ of *S. riobrave* and *H. bacteriophora* for 24 h. They determined decreasing protein content in the infected larvae. Gomaa *et al*. (2020) evaluated the efficacy of two EPN isolates (*H. bacteriophora* and *S. carpocapsae*) and the entomopathogenic fungus *Beauveria bassiana*, separately and in combination, on the $3^{rd}$ instar larvae of *S. littoralis*. The total protein content was reduced post-infection with all treatments.

Results of the present study were, to a great extent, in agreement with these reported results, since the protein content in haemolymph of the infected last instar larvae of *A. ipsilon* was predominantly reduced by the nematode species, *S. carpocapsae* and *H. bacteriophora*. It was tremendously reduced, especially at 48 hr of the last instar (21.94 & 26.45% protein reductions, by *S. carpocapsae* and *H. bacteriophora*, respectively). Moreover, *H. bacteriophora* exhibited stronger reducing potency against the protein content than *S. carpocapsae*, at 48 hr. In contrast, the present results were inconsistent with few reported results of increasing protein content in larvae of some insects after infection with certain ENPs, such as *C. capitata* larvae at 4 and 18 hr post-infection with *S. feltiae* Filipjev (Ghally *et al*., 1988). Total protein content significantly increased in the full-grown larvae of pink bollworm *Pectinophora gossypiella* after treatment with *S. riobrave* but slightly increased after treatment with *H. bacteriophora* (Shairra *et al*., 2016).

In the current study, the predominantly reduced protein content in haemolymph of last instar larvae of *A. ipsilon* after infection with $LC_{50}$ values of *S. carpocapsae* and *H. bacteriophora* could be understood in view of the following information. This protein reduction might be attributed to the proteolytic activity in the haemolymph of the infected larvae. This activity is suggested to be the main cause of the host's quick death (El-Bishry, 1989). According to Lee and Atkinson (1976), the high reduction in protein content could be referred to that many nematodes secrete chemicals to facilitate penetration and migration through host tissues, and for feeding and avoidance of host immunity responses. These chemicals include toxins and digestive enzymes (von Brando, 1973). The reduction in total protein content after infection with EPNs might be also attributed to the stimulation of protein catabolism in the host fat body–the major organ for metabolism, nutrient storage, and synthesis of vitellogenin, a yolk protein precursor (Kamruzzaman *et al*., 2020) to acquire a dietary supply of amino nitrogen from haemolymph (Gordon *et al*., 1973). Schmidt and Platzer (1980) reported protein degradation when *Culex pipiens* was infected with *Romanomermis culicivorax*. They suggested that the production of some proteases from the nematodes leads to this degradation of haemolymph proteins. Also, the protein reduction in haemolymph might

be due to the conversion of some proteins to fat, resulting in low protein content in the infected larvae (Abdel-Razek *et al*., 2004). Ali *et al* (2011) reported that the breakdown of protein into free amino acids would ultimately lead to a decrease in protein content. Wee *et al*. (2000) suggested the production of proteases by symbiotic bacterial cells, followed by the breakdown of insect protein and serving as nutritional resources for nematode-bacterium development (Istkhar and Chaubey, 2019). Decreased protein content might be expected to suppress the immune response of infected larvae, including encapsulation, prophenoloxidase activity, phenoloxidase activity, total haemolymph proteins, and hemocyte density (Wilson *et al*., 2019).

**2. Lipid Reduction in Haemolymph of *A. ipsilon* Larvae by EPNs**:

Lipids represent a principal source of the energy for insects. They are transferred from their synthesis site *via* the haemolymph towards the target organs for use, in particular chitin synthesis, oogenesis, vitellogenesis, embryogenesis and continuous muscular activity (Dapporto *et al*., 2008; Zhou and Miesfeld, 2009). In addition to the sites of lipid storage in the body, lipids located in the egg play a very important role in achieving the energy needed for the developing embryo (Boz and Gülel, 2012). The quantity of lipids available for the reserves seems to be the result of a balance between the obtained food and the requests for reserves by processes, such as maintenance, growth and reproduction, and this balance is disturbed by any xenobiotic stress (Canavoso *et al*., 2001). Also, impaired synthesis of lipids has resulted in adversely influenced physiology and subsequently disrupted vital functions of growth and reproduction.

In the present study, the lipid content in haemolymph of the nematode-infected *A. ipsilon* larvae was gradually reduced with the age. The greatest reduction of lipids was determined in haemolymph of larvae at 48 hr (26.55 & 21.73% lipid reduction, by *S. carpocapsae* and *H. bacteriophora*, respectively). As clearly seen, *S. carpocapsae* exerted greater reducing action on the lipid content of infected larvae than *H. bacteriophora*. The current results were found compatible with some reported results of lipid reduction in some insects as a consequence of the nematode infection. For example, the total lipids in *C. capitata* larvae had declined after 18 hr post-infection with *S. feltiae* Filipjev (Ghally *et al*., 1988). The total lipid content of the host *S. littoralis* larvae was significantly decreased post-infection with the nematodes *S. riobrave* and *H. bacteriophora* (Ahmed *et al*., 2014). The lipid content was remarkably decreased in the infected $4^{th}$ instar larvae of *S. littoralis* after incubation with $LD_{50}$ values of *S. riobrave* and *H. bacteriophora* for 24 hr (Shaurub *et al*., 2020). On the contrary, the total lipid content of the fat body of larvae of the red palm weevil *Rhynchophorus ferrugineus* increased after infection with *S. carpocapsae* and *H. bacteriophora* (Abdel-Razek *et al*., 2004).

To interpret the reduction of total lipid content in the last instar larvae of *A. ipsilon*, after infection of penultimate instar larvae with *S. carpocapsae* and *H. bacteriophora* in the current investigation, it may important to point out that the lipid turnover in insects is regulated by neuroendocrine-controlled feedback loops (Kim *et al*., 2002; Etebari *et al*., 2007). Therefore, the reduced lipid content might be due to the disrupting effects and stress of these EPNs on neurosecretion or other hormones in larvae of *A. ipsilon*. Also, this declined lipid content in larvae of *A. ipsilon* might be due to a shift in energy metabolism towards lipid catabolism as a result of physiological stress caused by the tested EPNs. In other words, these EPNs induced stress on larvae to use lipids and glucose for cell repair and increase protein catabolism which may be stimulated due to high energy demand under such stress conditions.

**3. Carbohydrate Reduction in Haemolymph of *A. ipsilon* Larvae by EPNs**:

In insects, carbohydrates represent an important energy source and perform a crucial role in the structure and function of tissues during development and metamorphosis, as well as for the maturation of reproductive organs and embryonic development (*cf*. Chippendale, 1978). In insects, also, the soluble carbohydrates are accumulated during the larval stage and utilized in metamorphosis (Pant and Kumar, 1979) and are stored in the fat body as glycogen, which is converted into trehalose before releasing into the haemolymph for utilization (Gilbert and Chino, 1974). Also, carbohydrates are reported to be disturbed by xenobiotics (Kaufmann and Brown, 2008).

In the present study, carbohydrate content was prevalently reduced in haemolymph of the EPN-infected larvae of *A. ipsilon*. Also, the reducing potency of EPNs considerably increased with the time interval of exposure (41.38 & 31.03% carbohydrate reductions by *S. carpocapsae* and *H. bacteriophora*, respectively, at 48 hr). Therefore, *S. carpocapsae* exhibited a higher reducing effect on carbohydrate content than *H. bacteriophora*, at 48 hr. These results were in accordance with some reported results of decreased carbohydrates in larvae of some insects after infection with certain EPNs. For example, the total carbohydrates in the haemolymph of 4$^{th}$ instar larvae of *Cx pipiens* were reduced after infection with the EPN *R. culicivorax* (Schmidt and Platzer, 1979). Infection with the EPN *Mermis nigrescens* resulted in the decrease of trehalose level and reduced carbohydrate metabolism in the fat body of its host, *S. gregaria* (Gordon *et al*., 1971). The total carbohydrate content of *S. littoralis* larvae was significantly decreased post-infection with the nematodes *S. riobrave* and *H. bacteriophora* (Ahmed *et al*., 2014). Shaurub *et al*. (2020) determined decreasing carbohydrate content in the 4$^{th}$ instar larvae of *S. littoralis* after infection with $LD_{50}$ of *S. riobrave* and *H. bacteriophora* for 24 hr. On the contrary, four EPNs *H. bacteriophora* AS1, *H. bacteriophora* HP88, *S. carpocapsae* ALL, and *S. riobrave* ML29 significantly enhanced the total glucose content in *C. capitata* 3$^{rd}$ instar larvae (Shaurub *et al*., 2015).

However, the prevalent reduction of the carbohydrate content in haemolymph of last instar *A. ipsilon* larvae, after infection of penultimate instar larvae with *S. carpocapsae* and *H. bacteriophora*, in the present study, might be resulted from the nematode's nutritional demands for glucose and was symptomatic of accelerated glycogenolysis and/or impaired glycogenesis. In other words, the determined decrease in carbohydrates indicated that more sugar might be metabolized to meet the energy demands of both the EPNs and the host leading to the consumption of sugar and carbohydrate contents (Sharma *et al*., 2011; Yazdani *et al*., 2014; Shaurub *et al*., 2020). The interaction between nematode and *A. ipsilon* larvae appeared to be primarily nutritional. Growth of the nematode proceeds while the nutritional status of the host larvae deteriorates, i.e., the host becomes in a state of physiological starvation (Ahmed *et al*., 2014). On the other hand, the tested EPNs, in the current study, might interfere with the hormonal regulation of carbohydrate metabolism in *A. ipsilon* (Gade, 2004; Sugumaran, 2010) or exhibited some effects on carboxylase activity (Mukherjee and Sharma, 1996).

**Conclusion**:

As clearly shown in the current study, *S. carpocapsae* almost possess greater reducing potency against the main body metabolites in the haemolymph of the last instar larvae of *A. ipsilon*, leading to drastically disrupted intermediary metabolism. Therefore, this EPN can be applied as an effective part of the Integrated Pest Management program against this serious pest.

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