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IMPORTANCE AND CHEMICAL ANALYSIS OF TRIGLYCERIDES AND FATTY ACIDS COMPOSITIONS OF THREE ENTOMOPATHOGENIC NEMATODE SPECIES

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ABSTRACT

Triglycerides and fatty acids compositions in the juveniles of two species of *Heterorhabditis* (*Heterorhabditis bacteriophora* and *H. indica*) and one species of *Steinernema* (*Steinernema riobrave*) were analysed and determined at two ages 0 and 20 days for *S. riobrave* and *H. indica* , less than 7 and 30 days for *H. bacteriophora*. Results revealed that triglycerides comprised approximately 82, 88and 85 % of the total lipids in *S. riobrave*, *H. indica* and *H. bacteriophora* respectively, Fatty acids recorded 8, 7 and 8% of the total lipid content, so, neutral lipids are the major content of total lipids. The major fatty acids found in nematode juveniles of three species were: palmitic C16:0, stearic C18:0, oleic C18:1 and linoleic C18:2. The most abundant saturated fatty acids were palmitic (C16:0) and stearic (C18:0) acids. Oleic (C18:1) and linolelic (C18:2) were the principal unsaturated fatty acids. These nematodes have relative high levels of poly unsaturated fatty acids. Triglycerides and fatty acids composition were declined with storage. This decline was quantitative rather than qualitative. These results might be helpful in increasing the efficiency of entomopathogenic nematodes (EPN) as biological control agents against insect pests and improving different nematode species or strains. Fatty acids may be play role in toxicity of entomopathogenic nematodes (EPN) against hosts.

Keywords: Entomopathogenic nematodes, *Steinernema*, *Heterorhabditis*, Energy reserves, lipids, triglycerides and fatty acids.

INTRODUCTION

The infective third-stage juvenile of the entomopathogenic species is a non-feeding phenotypically distinct survival stage, whose ability to locate and infect the host has been reported to decline with declining total lipid content in several species during storage (Lewis *et al.*, 1995). Lipids, phospholipids and sterols are essential for nematode growth, development, and reproduction (Lee & Atkinson, 1977). The role of the membrane, fatty acids and sterols is important in several physiological processes, such as desiccation survival (Patel *et al.*, 1997) and energy reserves (Patel &Wright 1997a,b; Patel *et al.*, 1997; Wright *et al.*, 1997). Fatty acids play an important role in determining the physiochemical properties of cellular and membrane lipids (Abu Hatab and Gaugler 1997a).

Stored neutral lipids (mainly triacylglycerols) provide the main source of fatty acids for locating and infecting a new insect host (Patel *et al.*, 1997). Selvan *et al.*, (1993) reported that the total lipids fatty acid of newly emerged IJs of *Steinernema. carpocapsae*, *S. feltiae*, *S. glaseri*, and *S. scapterisci* mostly contained saturated fatty acids (50–66%), while the total lipids of the

heterorhabditid nematodes, *H. bacteriophora* and *Heterorhabditis megidis* contained proportionately more unsaturated fatty acids (57 and 62%, respectively). In contrast, Wijbenga and Rodgers (1994) found unsaturated fatty acids accounted for over 70% of the total lipid composition in *S. feltiae.*

The present investigation aims to study the role of neutral lipids (triglycerides and fatty acids) of two species of *Heterorhabditis* and one species of *Steinernema* during two ages of their life. Such chemical analysis might play a vital role in improving shelf-life of nematode juveniles and establishing a simple and easy quality control method for different nematode species or strains.

MATERIALS AND METHODS

Nematode source and culturing of the nematodes

The three species *H. bacteriophora*, *H. indica* and *S. riobrave* were obtained from the Laboratory of Insect Parasitic Nematodes, Plant Protection Research Institute. Nematodes were cultured on last-instar larvae of *Galleria mellonella* (L.) as a host at 25°C according to procedures described bys Kaya and Stock, (1997). Nematode juveniles at the tested ages (0 and 20 days for *S. riobrave* and *H. indica* , less than 7 and 30 days for *H. bacteriophora*) were used for the following biochemical tests after being stored at 15°C.

Lipid extraction

Freshly harvested nematode juveniles at the tested ages were centrifuged or vacuum filtered, weighed and incubated in 15 ml ethanol 80% at 75°C for 5 minutes to inactivate degradative enzymes such as phospholipases. The suspension was cooled and stored in a tight-capped tube after flushing with N_2, and stored at -70°C (Abu Hatab, & Gaugler, 1997a). Lipids were extracted and purified according to Folch *et al.*, (1957). Total lipids were determined according to Knight *et al.*, (1972). Lipid content expressed as µg/g nematode was calculated using a standard curve (oleic and palmitic acids).

Determination of lipase enzyme in nematode juveniles

The activity of lipase enzyme was studied in entomopathogenic nematodes at different juvenile ages. Nematode enzyme solution was prepared by the extraction method of Abu Hatab *et al.*, (1995). Nematode samples (0.05 g) were homogenized in 0.1 M phosphate buffer at pH (7.6) with periods of relaxation over ice. Homogenates were centrifuged at 14,000 rpm at 4°C .The pellets were collected and dried at 90°C for 48 hr to determine the dry weight. The supernatant was used for assay. Lipase activity was determined by the method of Tahoun & Abdel Ghaffer, (1986).

Analysis and determination of lipid fractions

Triglycerides

Triglycerides in nematode lipids were calculated by the full enzymatic determination using kit of bio Mérieux (France) (Fossatip and Prencipe, 1982).

Fatty Acids Analysis

Extracted FAMEs (fatty acids methyl esters) were performed according to the method described by Abu Hatab and Gaugler(1997a). Lipid fatty acids composition was analyzed qualitative and quantitative by using gas liquid chromatography, (Hewlett Packard 6890 series gas liquid chromatography equipped with a flame ionization detector). The column used was HP-INNOWAX-cross-linked polyethlyene (25 m x 0.2 mm x 0.4 µm film thickness). The inert carrier gas was nitrogen with flow rate 1 ml/min, oven, injector and detector temperatures were set at 200, 250, 280°C, respectively.

Statistical Analysis

The data presented in percentage values in the present study were normalized using arcsine transformation. The significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($P<0.05$). All analyses were made using a software package "Costat", a product of Cohort Software Inc., Berkeley, California.

RESULTS AND DISCUSSION

Total lipids determination

Data illustrated in Table (1) showed that the percentages of total lipids were 42.55, 50.52 and 53.75 % of nematode dry weight for *S. riobrave* , *H. bacteriophora* and *H. indica* respectively, when freshly emerged from their hosts (0 day old) for *S. riobrave* and *H. indica*, and less than one week for *H. bacteriophora*. Our results are in agreement with those reported by Lewis *et al.*, 1995, Fitters *et al.*, 1997 & 1999, El-Badawy 2002, El-Assal *et al.*, 2008 and Shapiro *et al.*, 2005. These percentages declined significantly to nearly 22-45% after 30 days, and to 16-00% after 50 days of storage for *H. bacteriophora*. This is similar to the percentages reported by El-Badawy 2002& 2011 in press and supported by Lewis *et al.*, 1995 in another strain of the same species. In case of *H. indica* these percentages decreased to 37.39% after 20 days, and to 25.51% after 40 days of storage. Radwan (2002) showed that the percentage of total lipids content was declined significantly from 54.75% (0-day) to 39.39% after 20 days, and to 26.51% after 40 days of storage in *H. indica* . On the other hand the total lipid content of *S. riobrave* IJs, was nearly the same during the first 20 days of their life (41.95%). A sharp significant decrease was recorded in total lipid content after 90 days of storage, where the recorded value was 18.00%. This confirmed by Lewis *et al.*, 1995 when they reported that total lipids decline at species-specific rates. Entomopathogenic nematodes are capable of adjusting their lipid content at different periods of life (Abu Hatab and Gaugler, 1997b). This meaning that nematode juveniles of *H. bacteriophora* consumed more than 70% of lipid reserves during 50 days of their life. In other words, nematode juveniles of *H. indica* and *S. riobrave* consumed more than 50% of lipid reserves during 40 and 90 days of their life respectively. Lewis *et al.*, (1995) reported that *H. bacteriophora* IJs used approximately 60% of their total lipids during 7 weeks of storage. Fitters *et al.*, (1999)

reported the total lipids in two isolates of *Heterorhabditis* sp (.HF85 and UK21) after 5 weeks storage at 20°C, HF85 had lost 57% of its total lipid, and UK211 had lost 94%. This indicated that total lipids are the chief food reserve for infective juveniles.

Table (1): Lipid content of *S. riobrave H. indica* and *H. bacteriophora* at different juvenile ages.

Juvenile age (days)	% Lipid content (Mean ± SEM)		
	S. riobrave	*H. indica*	*H. bacteriophora*
0 or less than 7 days *	42.55±0.29 [a]	53.75±0.54 [a]	50.52±0.34 [a]
20 or 30 **	41.95±1.13 [a]	37.39±0.77 [b]	22.45±0.77 [b]
40 or 50 or 90 ***	18.00±2.00 [b]	25.51±0.34 [c]	16.00±0.34 [c]

Values are expressed as dry weight % of total lipids content

Lipid fractions analysis

Neutral lipids and triglycerides

In the present investigation, triglycerides were the most unsaturated of the individual classes analyzed and comprised approximately 82, 88 and 85% of the total lipids in *S. riobrave, H. indica* and *H. bacteriophora* respectively(Table1); while, free fatty acids were recorded 8, 7 and 8% of the total lipids in the three species respectively. Triglycerides and free fatty acids were the major neutral lipid classes in the three species, this meaning that neutral lipids are the major content of the total lipids. Neutral lipids comprised approximately 90, 94 and 93% of the total lipids. Therefore, polar lipids comprised about 10. 6 and 7% of the total lipids (Table 2).

Table (2): Triglycerides percentages at two different juvenile ages of *S. riobrave* and *H. indica* and *H. bacteriophora*.

Juvenile age (days)	% Triglycerides (Mean ± SEM)		
	S. riobrave	*H. indica*	*H. bacteriophora*
0 or Less than 7 days*	82.14±1.22 [a]	87.76±0.68 [a]	84.78±1.90 [a]
20 or 30 **	58.2±1.33 [b]	56.63±0.88 [b]	44.77±1.22 [b]

Values are expressed as dry weight % of triglycerides content

*** 0 days for *H. indica*; and *S. riobrave* and less than 7 days for *H. bacteriophora*.**

**** 20 days for *H. indica*; and *S. riobrave*, 30 days for *H. bacteriophora*.**

***** 40 days for *H. indica*; 50 days for *H. bacteriophora* and 90 days for *S. riobrave*.**

SEM = Standard error of the mean

For each nematode species, means followed by the same letter are not significantly different (P<0.05; Duncan's multiple range

The obtained results match those of Lee and Atkinson (1997) who reported that the major fraction of total lipids in entomopathogenic nematodes is triglycerides, which are the most important long-term energy storage molecules for nematodes; and Fitters *et al.*, (1999) who reported that neutral lipids in three isolates of *Heterorhabditis* sp. comprised from 70 to over 90% of the total lipids content. Similarly, triglycerides (82±3.0%) and free fatty acids (7.3±0.4) were the major neutral lipid classes in *S. riobrave* (Abu Hatab and Gaugler, 1997a). and they concluded that, among lipid reserves, triglycerols to be the major neutral lipid fraction of entomopathogenic nematodes, which emphasizes their importance for long-term energy storage.

Also, (Abu Hatab *et al*,. 1998) noted that neutral lipids were the major lipid constituents of S. *glaseri*, and triglycerides were the major neutral lipid class in S. *glaseri* produced in all 4 culture methods and the percentage of polar lipids was 6.8%. Patel & Wright (1997b) reported higher percentages of polar lipids (15-18% of the total lipids) in newly emerged IJs of four species of entomopathogenic nematodes belonging to the genus *Steinernema*.. El-Badawy (2002) reported that neutral lipids comprised 93% of the total lipid content in the nematode *H. bacteriophora* N-1 strain (85% triglycerides and 8% fatty acids).

Percentages of triglycerides are declined significantly to nearly 44.77 % after 30 days of storage for *H. bacteriophora* and to 58.2 , 56.6 after 20 days of storage for *S. riobrave* and *H. indica* respectively (Table 2). This decline in triglycerides content was mainly due to a decrease in neutral and total lipids with storage.

Fatty Acid Analysis

Fatty acid analysis of nematode juveniles (Table 3, 4) indicates that the major fatty acids found in nematode juveniles of the three species were: palmitic C16:0, stearic C18:0, oleic C18:1 and linoleic C18:2. The percentages of these acids in freshly emerged *S. riobrave* were 15.30, 7.70, 43.70 and 21.33 %, respectively. The corresponding values in *H. indica* were 14.30, 7.50, 41.42 and 29.78 % for the four acids, respectively (Table 3) while, for *H. bacteriophora*, emerged less than 7 days, were 26.18, 5.15, 51.30 and 9.00 % (Table 4).

Table (3): Pattern of fatty acids of total lipids of *S. riobrave* and *H. indica* at two different juvenile ages.

Fatty acids (FA)	Days after emergence			
	Zero day		20 days	
	S. riobrave	*H. indica*	*S. riobrave*	*H. indica*
Palmitic (C16:0)	15.30±1.00	14.30±1.00	18.47±1.00	21.01±1.00
Stearic (C18:0)	7.70±0.08	7.50±0.08	4.19±0.08	5.65±0.08
Oleic (C18:1)	43.70±1.20	41.42±1.00	32.93±0.70	30.65±0.70
Linoleic (C18:2)	21.33±0.60	29.78±0.70	10.01±0.70	18.92±0.70
Eicosatrienoic 20:3 n-6	3.20±0.09	2.00±0.08	4.30±0.02	3.00±0.02
Eicosatetraenoic20:4 n-3	2.30±0.08	1.30±0.02	3.90±0.04	3.50±0.04
Arachidonic20:4 n-6	4.10±0.20	1.30±0.02	00±0.0	00±0.0
Eicosapentaenic20:5 n-3	2.40±0.10	2.40±0.02	00±0.0	00±0.0
% of unsaturation	77.00±0.10	78.20±0.10	51.10±0.10	56.07±0.10
% of saturation	23.00±0.10	21.80±0.10	22.66±0.10	26.66±0.10
Ratio of sat.\unsat (S/U).	0.30±0.04	0.28±0.04	0.44±0.04	0.48±0.04
% PUFA	33.33±0.10	36.76±0.10	18.21±0.10	25.42±0.4
U.I	133.56	129.30	81.45	91.49

Values are expressed as dry weight % of total fatty acids.
Sat.=saturated fatty acid, Unsat= unsaturated fatty acids
PUFA= poly unsaturated fatty acids
U.I= unsaturation indices = $\sum$ (% of fatty acids x number of double bonds)
SEM = Standard error of the mean

Table (4): Pattern of constituent fatty acids of total lipids of *H. bacteriophora* at different ages

Fatty acids (FA)	Days after emergence	
	Less than 7 days	30 days
	%	%
Palmitic (C16:0)	26.18±1.22	33.47±1.52
Stearic (C18:0)	5.15±0.08	7.66±0.05
Oleic (C18:1 n-9)	51.34±1.50	44.81±2.20
Linoleic (C18:2 n-6)	9.00±0.71	1.67±0.07
Linoleic (C18:2 n-3)	0. 40±0.05	00.00
Linolenic(C18:3 n-3)	0. 62 ±0.03	00.00
Eicosatrienoic 20:3 n-6	2.47±0.10	3.07±0.20
Eicosatetraenoic20:4 n-3	1.34±0.10	3.37±0.20
Arachidonic 20:4 n-6	1.35±0.10	00.00
Eicosapentaenic20:5 n-3	2.14±0.10	00.00
% of unsaturation	68.67±1.70	52.92±1.30
% of saturation	31.33±1.09	41.13±1.54
Ratio of sat.\unsat. (S/U).	0.46±1.00	0.78±1.34
% PUFA	17.32±0.10	8.11±0.10
U.I	100.06	70.84

Values are expressed as dry weight % of total fatty acids.
Sat.=saturated fatty acid, Unsat= unsaturated fatty acids
PUFA= poly unsaturated fatty acids
U.I= unsaturation indices = $\sum$ (% of fatty acids x number of double bonds)
SEM = Standard error of the mean

The percentage of fatty acid, as a whole, declined after 20 days of storage by 26.24 % in *S. riobrave*, 17.27 % in *H. indica* and after 30 days by 6.00 % in *H. bacteriophora*. This decline was quantitative rather than qualitative. In details, the decline was much obvious in stearic (from 7.70 to 4.19% in *S. riobrave*; from 7.5 to 5.65% in *H. indica*), Oleic (from 43.7 to 32.93 % in *S. riobrave*; from 41.42 to 30.65% in *H. indica* and from 51.3 to 44.81 %. in *H. bacteriophora*) and linoleic (from 21.33 to 10.01 % in *S. riobrave*; from 29.78 to 18.92 % in *H. indica* and from 9.0 to 1.67 %. in *H. bacteriophora*). On the other hand, an increase occurred in palmitic acid (from 15.30 to 18.47% in *S. riobrave*; from 14.30 to 21.01% in *H. indica* and from 26.18 to 33.47%. in *H. bacteriophora*). Also, there is an increase in stearic acid from 5.15 to 7.66% in. *H. bacteriophora*). Generally, percentages of fatty acids of *H. bacteriophora* and *H. indica* were higher than S. *riobrave*, this may be due to the total lipid of S. *riobrave* was approximately not changed after 20 days of storage.

Similar results were reported by Patel and Wright, (1997a) who mentioned that the profile of neutral lipids fatty acid was palmitic C16:0 (18-23%), stearic C18:0 (4-8%), oleic C18:1 (43-49%), and linoleic C18:2 (8-14%) in four species of entomopathogenic nematodes of the genus *Steinernema*. Fitters *et al.* (1999) reported that the patterns of fatty acids were similar in three isolates of *Heterorhabditis* sp.; where oleic (C18:1n-9), palmitic (C16:0), and linoleic (C18:2n-6) acid predominated with 51, 13, 12%, respectively in the total lipids of fresh nematodes. Also they reported that, for both isolates (.HF85 and UK21), the percentage of palmitic acid and oleic acid in the total lipids decreased significantly between week 0 and week 5, and arachidonic

acid (C20:4n-6) increased significantly. Patel and Wright (1997a), found that during storage, the relative levels (%) of C16:0, C18:0 and C18:1in the neutral lipids were declined significantly, suggesting that they were preferentially utilized. The sharp decrease in the percentage of linoleic C18:2 in the three nematode species after 20 days for *S. riobrave*; *H. indica* and 30 day for *H. bacteriophora* was due to the oxidation of this acid by two enzymes; *i.e.*, isomerase and epimerase, losing two carbon units in the form of acetyl CoA. The produced compound is (Cis) β & γ unsaturated acyl CoA (Acyl CoA= R-C=O-CoA), this product by the action of certain enzymes is converted to (trans) α & β unsaturated compound. This might explain the increase in the ratio of palmitic (C16:0) after the same period.

Fatty acids with 16 and 18 carbons palmitic (16:0) and stearic (18:0) acids were the major saturated fatty acids. Oleic (18:1) and linoleic (18:2) fatty acids were the principal unsaturated fatty acids. Unsaturated fatty acids predominated, with about 77, 78.2 and 68.67% for newly emerged IJs of *S. riobrave*; *H. indica* and *H. bacteriophora*, respectively. Wijbenga and Rodgers, (1994) found that the unsaturated fatty acids accounted for over 70% of the total lipid composition in *S. feltiae*, and Fodor *et al.* 1994 found a similar situation in the neutral lipids of *S. carpocapsae*. The proportion of saturation increased after 20 day for *S. riobrave* and *H. indica* and after 30 day for *H. bacteriophora*, but, in general, the proportion of unsaturated fatty acids remained higher than that of saturated fatty acids. High proportion of unsaturated fatty acids was observed in all lipids fractions of *S. riobrave* grown on *G. mellonella*, (Abu Hatab and Gaugler, 1997a). In another study, the entomopathogenic nematode, *S. feltiae,* infective juveniles showed a decline in the proportion of total poly- unsaturated fatty acids relative to those of monounsaturated and saturated fatty acids after four weeks. Thus, confirming the results obtained in the present investigation. Generally, there was a general decline in the content of unsaturated fatty acids and a concomitant increase in the saturated fatty acids content in total lipids. This high proportion of unsaturated fatty acids may be tend to increase the membrane permeability, and utilization of energy reserves become easier, since, the double bonds in the unsaturated fatty acid are easier to be broken rather than the single bonds in saturated ones. However, the fluidity of biological membranes is known to be directly proportional to the number of double bonds in fatty acyl moieties (Hakomori, 1986). In this respect, Fodor *et al.* (1994) suggested that the increased unsaturation in lipids of *S. carpocapase* may facilitate membrane permeability and thus easier utilization of energy reserves, and the high proportions of dietary polyunsaturated fatty acids may increase membrane fluidity in entomopathogenic nematodes.

Levels of saturated-to-unsaturated fatty-acid (S/U) ratio of fresh emerged nematodes were an average 0.30, 0.28 and 0.46 for *S. riobrave*, *H. indica* and *H. bacteriophora*, respectively (Tables 3 & 4). These levels were increased to 0.44, 0.48 and 0.78 after storage. Fodor *et al.*, (1997) reported that the saturated-to-unsaturated fatty-acid ratio is believed to indicate the fluidity of membranes of symbiotic bacteria associated with EPN. According to this finding and the fact, each strain of EPN species is capable of changing the S/U ratio and the fluidity of their membrane (Fodor *et al.*, 1994). The

decreasing of unsaturated fatty acids give us a plausible explanation of increasing of S/U ratio of the three species during storage and fluidity of their membrane. Fitters *et al.* (1999) reported that, the unsaturation index (U.I.) for NL (neutral lipids) fraction increased during storage, suggesting a preferential use of saturated fatty acids. Our results in this investigation showed the opposite of this finding, since, the unsaturation index (U.I.) of the three EPN species were 133.56, 129.30 and 100.06 for newly emerged *S. riobrave*, *H. indica* and *H. bacteriophora*, respectively, (Tables 3 & 4). These levels were decreased to 81.45.91.49 and 70.84 after storage, this meaning that more consuming of unsaturated fatty acids. *H. bacteriophora* had a slightly lower U.I than the other two nematode species after storage, meaning, less consuming of unsaturated fatty acids.

These three nematode species showed relative high levels of poly unsaturated fatty acids (Eicosatrienoic 20:3, Eicosatetraenoic (20:4) and Eicosapentaenoic (20:5) acids). We found that, the decreasing of unsaturated fatty acid (Linoleic (C18:2) and disappearance of unsaturated fatty acid (Linoleic (C18:3) and polyunsaturated fatty acids (Arachidonic 20:4 n-6 and Eicosapentaenic 20:5n-3) was accompanied by an increase in polyunsaturated fatty acids (Eicozatrienoic 20:3 n-6 and Eicozatetraenoic 20:4 n-3). This finding confirmed by Fodor *et al.*, 1994, who suggested that S. *carpocapsea* has the ability to elongate and desaturate linolenic (18:3) acid, and found different levels of C-20 polyunsaturated fatty acids depending on the dietary lipid source and they added that, the amounts of C-20 polyunsaturated fatty acids could be regulated by at least three mechanisms: chain elongation and desaturation of linolenic acid, selective absorption or deposition, and de novo biosynthesis. Our results supported the hypothesis that de novo poly unsaturated fatty acid biosynthesis occurs in some species of nematodes, as has been proposed in microbivorous nematode species (Satouchi, *et al.*, 1993). In contrast, Lee and Atkinson, 1977 proposed that the vast majority of parasites seem incapable of synthesizing long-chain fatty acids and sterols de novo and they cannot introduce double bonds into the carbon skeleton of fatty acids.

The minor differences between the percentages of these fatty acids in the present study in comparison with those in previous ones may be due to the fact that content of fatty acids is governed by two main processes, *i.e.*, 1) production of these acids by hydrolysis of lipids; and 2) consumption by nematode juveniles. The relative speed of each process at any given time determines the percentage of these acids.

Hydrolysis of energy reserves to simple utilized substances, generally, takes place by enzymatic reactions. In the present investigation, determination of lipase activity at the tested juvenile ages (Table 5) revealed that this activity in *S. riobrave* recorded lower value (0.20 µg oleic acid) than that of *H. indica* (0.48 µg oleic acid) at 0-day old juveniles. This low activity might explain the stability of total lipid content during the first 20 days of life in the former nematode species. It also indicates that nematode IJs during this early stage do not depend upon total lipids as a main source of energy.

Table (5): Lipase activity of *S. riobrave*, *H. indica* and *H. bacteriophora* at different juvenile ages.

Juvenile age (days)	Lipase activity (Mean ± SEM)		
	S. riobrave	*H. indica*	*H. bacteriophora*
0 or less than one week*	0.20±0.03 [b]	0.48±0.01 [b]	10.50±0.58 [b]
20 or 30**	1.41±0.05 [a]	1.22±0.08 [a]	17.93±0.60 [a]

Lipase activity is expressed as µg oleic acid/g body weight.
* 0 days for *H. indica*; and *S. riobrave* and less than 7 days for *H. bacteriophora*.
** 20 days for *H. indica*; and *S. riobrave*, 30 days for *H. bacteriophora*.
For each nematode species, means followed by the same letter are not significantly different (P<0.05; Duncan's multiple range

On other hand lipase activity in *H. bacteriophora* increased significantly at 30 days than that of IJs less than 7 days. However, this finding supports the concept that total lipids (especially neutral lipids) are the main source of energy reserves in these organisms, and its consumed gradually through the period life of nematodes. None of the previous studies ever put into consideration the determination of hydrolysis enzymes. Estimation of these processes in the ecological studies of nematode species and isolates can be used as an indicator for their efficiency and quality.

Chemical analysis of nematode juveniles at the indicated ages revealed that, total lipids (mainly neutral lipids) are the main source of energy in these juveniles which they depend upon in searching and penetrating their hosts. This may be attributed to the fact that neutral lipids, especially triglycerides are easily hydrolyzed to glycerol and a mixture of fatty acids. Glycerol can enter glycolysis pathway and fatty acids are broken down (two carbons at a time) to provide units of acetyl coenzyme A for fueling citric acid cycle and the respiratory chain. Thus, adenosine-triphosphate (ATP), needed for all metabolic processes and the other activities of these juveniles, is thereby produced (Grodsky *et al.*, 1981).

Host diet sources have high influence on nematode yield and lipid content (especially triglycerides and fatty acids compositions) of EPN and subsequently their efficiency as a biological control and there are similarity between lipid content and fatty acids compositions of EPN and their bacteria and others of host or host diet. This is obvious from many studies such as Abu Hatab & Gaugler(1997 a,b) and Fodor *el al.*(1994) The role of neutral lipids (mainly fatty acids and triglycerides) is important in several processes in EPN, such as: facilitate membrane permeability and accommodation of proteins involved in thermal adaptation. (Fodor *et al.* 1994), nematode survival by affecting the fluidity of the lipids within the nematode and the amount of potential energy derived from them (Patel and Wright, 1997a), the physiochemical properties of cellular and membrane lipids. (Abu Hatab and Gaugler, 1997a), differences in shelf life of EPN species (Selvan *et al.*, 1993), to yield energy (Lee and Atkinson, 1977) increasing of shelf life of commercially produced nematodes (Fitters *et al.*, 1999). From these studies and our results in this investigation, we can improve the efficiency of EPN and their shelf life through increasing the accumulation of triglycerides and fatty acids constituents quantitatively and qualitatively by selection the suitable host or host diet sources.

Experimentally it has been shown that either the associated bacteria or the axenic nematode are able to kill insects host usually within 48 h after infection. This fact reported by Simes *et al.*, 2000. and Sergeant *et al.*, 2003. Some studies refer to these toxins produced by the associated bacteria or the axenic nematode were allelochemicals such as Grewal *et al.*, (1999 & 2005) who reported that allelochemicals produced by EPN or their symbiotic bacteria may have both repellent and toxic effects on plant-parasitic nematodes. Fatty acids might serve as allelochemicals (Chiang *et al.*, 2004) and allelochemicals can interfere with a variety of processes in organisms and act depends on the kind of compound; they can inhibit protein or enzyme activity, damage cell membranes, change physiological functions, and lyses target cells (Legrand *et al.*, 2003).

According to these studies and there is no researchers refer to the role of fatty acids in toxicity of EPN. The fatty acids may be one of the toxins produced by EPN and play role in the toxicity of nematodes against insects. This finding, can be supported by many studies indicated that fatty acids and their methyl esters or salts have growth inhibition and toxic effect against insects and other organisms, (Arita *et al.*, 2003, Barakat *et al.*, 2004 and Tsolakis & Ragusa 2008).

Conclusion

Chemical analysis of nematode juveniles at the indicated ages revealed that total lipids (mainly neutral lipids) are the main source of energy in EPN juveniles and consumption of these reserves resulted in the observed decrease in nematode efficiency. This may be attributed to the fact that neutral lipids, especially triglycerides are easily hydrolyzed to glycerol and a mixture of fatty acids. The major fatty acids found in nematode juveniles of three species were: palmitic C16:0, stearic C18:0, oleic C18:1 and linoleic C18:2. Palmitic (C16:0) and stearic (C18:0) acids were the most abundant saturated fatty acids. Oleic (C18:1) and linolelic (C18:2) were the principal unsaturated fatty acids. We recommend the use of natural or artificial media rich in linoleic (18:2), oleic (18:1), palmitic (16:0) and stearic (18:0) acids in the growth of EPN species. This tend to production of nematode juveniles which are rich of the most consumable energy reserves and thus improving their shelf-life, their quality and consequently their value as biological control agents of insect pests and establishing a simple and easy quality control method for different nematode species or strains. The free fatty acids and their ester or salt may be one of the toxins produced by EPN and play role in the toxicity of nematodes against insects.

REFERENCES

Abu Hatab, M., Selvan, S. and Gaugler, R. (1995). Role of porteases in penetration of insect gut by the entompathogenic nematode *Steinernema glaseri* (Nematoda: Steinernematidae). J. Invertebr. Pathol., 66: 125-130.

Abu Hatab, M.A. and Gaugler, R. (1997a). Influence of growth temperature on fatty acids and phospholipids of *Steinernema. riobrave* infective juveniles. J. Them. Biol., 22: 237-244.

Abu Hatab, M.A and Gaugler, R. (1997b). Fatty acids of *Xenorhabdus* sp. in response to different growth conditions. J. Appl. Microbiol., 82: 351-358.

Abu Hatab, M.A., Randy, G. and Udo Ehlers. R. (1998). Influence of culture method *Steinernema. glaseri* lipids. J. Parasitol., 82: 215-221

Arita, K., Yamamoto, Y., Takehara, Y., Utsumi, T., Kanno, T., Miyaguchi, C., Akiyama, J., Yoshioka, T. and Utsumi, K., (2003) .Mechanisms of enhanced apoptosis in HL-60 cells by UV-irradiatedn-3 and n-6 polyunsaturated fatty acids. Free Radical Biology& Medicine 15: 189–199.

Barakat, A.A., El-mahy, A. S., omaima, k., Moustafa, K. O., Mansour, F. A. and El-hadek, K. M. (2004), Biological effect of *Cassia fistula* (l.) seeds against the cotton leafworm *Spodoptera littoralis* (boisd.) with special reference to chemical constituents. Bull ent Soc. Egypt. Econ. Ser.. 30(1): 1-14.

Chiang, I.Z., Huang, W.Y. and Wu, J.T. (2004). Allelochemicals of *Botryococcus braunii* (Chlorophyceae). J. Phycol. 40: 474–480.

El-Assal, F.M.,. El-Lakwah, S.F., Hasheesh, W.S. and El-Mahdi, M. (2008). Effect of the change in energy reserves on the entomopathogenic nematode efficacy. J Egypt Soc Parasitol. 38(3):929-44

El-Badawy, S.S.M. (2002). Organic chemical studies on some natural enemies of insect pests. M.Sc. Thesis, Fac. Sci., Al-Azhar Univ., 76 pp.

El-Badawy, S.S.M. (in press). Relationship between biochemical energy reserves and some biological and ecological traits of three species of entomopathogenic nematodes Egypt. *J. Agric. Res.*

Fitters, P.F.L., Meijer, E.M.J., Wright, D.J. and Griffin, C.T. (1997). Estimation of lipid reserves in unstained living and dead nematodes by image analysis J. nematol. (USA). 29(2), 160-167.

Fitters, P.F.L., Patel, M.N., Griffin, C.T., and Wright, D.J. (1999). Fatty acid composition of Heterorhabditis sp. during storage. Comparative Biochemistry and Physiology. Part B; Biochemistry and Molecular Biology. 124 (8):.81-88.

Fodor, A., Dey, I., Farkas,T. and chitwood, D.J. (1994). Effect of temperature and dietary lipids on phospholipid fatty acids and membrane fluidity in S. *carpocapsae, J.Nematol.* 26: 278-285

Fodor, E., Szalls, E., Zsuzsanna Kiss, Z., Fodor, A., Horvath, I.., Chitwood, J., and Farkas, T. (1997). Composition and biophysical properties of lipids in *Xenorhabdus nematophilus* and *Photorhabdus luminescens*, symbiotic bacteria associated with Entomopathogenic nematodes. Applied And Environmental Microbiology, 63, (7.) 2826–2831.

Folch, J., Lees, M. and Sloane-Stanly.G. (1957). A simple method for the isolation and purifications of total lipids from animal tissues. *J. Biological Chem.* 226: 497-509.

Fossatip, P. and Prencipe, L. (1982). Enzymatic determination of triglycerides. Clin. Chem., 28: 2077.

Grewal, P.S., Lewis, E.E. and Venkatachari, S., (1999). Allelopathy: a possible mechanism of suppression of plant-parasitic nematodes by entomopathogenic nematodes. Nematology 1: 735–743

Grewal, P.S., Ehlers, R.U. and Shapiro-Ilan, I. (2005). Nematodes As Biocontrol Agents. CABI Publishing, CAB International, Oxon, UK. 505 pp.

Grodsky, G.M., Martin, D., Mayes, P.A., Nestle, M. and Rodwell, V.W. (1981). Harper's Review of Biochemistry. 18th ed. D.W. Martin, P.A. Mayes and V.W. Rodwell (Eds.). Image Medical Publications, Los Altos, California, pp. 614.

Hakomori, S. (1986). Glycophingolipids. Scientific American 254: 32-41..

Kaya, H.K. and Stock, S.P. (1997). Techniques in insect nematology. In: Lacey LA, editor. Manual of Techniques in Insect Pathology. San Diego, CA: Academic Press;. 281–324

Knight J. A., Anderson S. and Rawle J. A. (1972). Chemical basis of sulfuric-phosphovanillin reaction for estimation of total lipids. *Clinical. Chemistry.*, Vol.18: 199-202.

Lee, D.L. and Atkinson, H.J. (1977). Physiology of Nematodes. Columbia University Press, New York. 215 pp.

Legrand, C., Rengefors, K., Fistarol, G.O. and Graneli, E. (2003). Allelopathy in phytoplankton: biochemical, ecological and evolutionary aspects. Phycologia 42: 406–419.

Lewis, E.E., Selvan, S., Campbell, J.F. and Gaugler, R. (1995). Changes in foraging behaviour during the infective stage of entomopathogenic nematodes. Parasitology 110: 583–590.

Patel, M.N., Stolinski, M., and Wright, D.J. (1997). Neutral lipids and the assessment of infectivity in entomopathogenic nematodes: observations on four *Steinernema* species. Parasitology 114: 489-496.

Patel M. N., and Wright D. J. (1997a). Fatty acid composition of neutral lipid energy reserves in infective juveniles of entomopathogenic nematodes. Comp. Biochem. Physiol. Biochem Mol Biol. 118: 341-348.

Patel, M.N., and Wright, D.J. (1997b). Glycogen: its importance in the infectivity of aged juveniles of *Steinernema carpocapsae*. Parasitology 114: 591-596.

Radwan, H. M. (2002). Biochemical studies on some entomopathogenic nematodes. M.Sc. Thesis, Fac. Agri, Cairo Univ., 44-46 pp

Shapiro-Ilan, D. I., Dutcher, J. D. and Hatab, M. (2005). Recycling Potential and Fitness of Steinernematid Nematodes Cultured in *Curculio caryae* and *Galleria mellonella* J.Nematol. 37(1):12–17.

Satouchi, K., Hirano, K., Sakaguchi, M., Takehara, H. and Matsuura, F. (1993). Phospholipids from the free-living nematode *Caenorhabditis elegans*.Lipids 28:837-840.

Selvan, S., Campbell, J.F. and Gaugler, R. (1993). Density-dependent effects on entompathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host. J. Ivertebr. Pathol., 62: 278-284.

Sergeant, M., Jarrett, P., Ousley, M. and Morgan, J.A. (2003). Interactions of insecticidal toxingene products from *Xenorhabdus nematophilus* PMFI296, *Appl, Environ, Microbiol*, 69:3344–3349.

Simes, N., Caldas, C., Rosa, J.S., Bonifassi, E. and Laumond. C. (2000). Pathogenicity caused by high virulent and low virulent strains of *Steinernema carpocapsae* to *Galleria mellonella*. J Invertebr Pathol; 75:47–54

Tahoun, M.K. and Abdel-Ghaffar, M. (1986). A modified colormetric method for assay of lipase activity. Alex.Sci. Exch. Vol.7 (2) : 235-244.

Tsolakis, H. and Ragusa. S. (2008). Effects of a mixture of vegetable and essential oils and fatty acid potassium salts on *Tetranychus* urticae and *Phytoseiulus persimilis*. Ecotoxicology and Environmental Safety 70 : 276–282

Wijbenga, J. and Rodgers, P.B. (1994). Storage of *Heterorhabditis megidis* UK211: Effect on performance and the physiological state of the nematode. Proc. Vi*th* Colloq. Invertebe. Pathol. and Micorobial Control. Montpellier, France 28 Aug.- 2Sept., 1-6.

Wright, D.J., Grewal, P.S. and Stolinski, M. (1997). Relative importance of neutral lipids and glycogen as energy stores in dauer larvae of two entomopathogenic nematodes, *Steinernema carpocapsae* and *Steinernema feltiae*. Comp. Biochem Physiol Biochem. Mol Biol. 118: 269-273

التحليل الكيميائي الحيوي للجليسريدات الثلاثية والأحماض الدهنية وأهميتهم لبعض أنواع من النيماتودا

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يهدف هذا البحث إلي تقدير وتحليل الجليسيرات الثلاثية، الأحماض الدهنية لثلاث سلالات من النيماتودا الممرضة للحشرات وهي *Steinernema riobrave* التي تنتمي إلي عائلة Steinernematidae والنوعين الأخرين ينتميان إلي عائلة Heterorhabditidae وهما *Heterorhabditis bacteriophora* *Heterorhabditis indica* خلال الأعمار الآتية (صفر، ٢٠ يوم) لسلالة *S. riobravae* وسلالة *H. indica* (أقل من ٧ أيام، ٣٠ يوم) لسلالة *H. bacteriophora* وإستنتاج أهميتهم للنيماتودا. أظهرت النتائج أن الجليسريدات

الثلاثية شكلت حوالي ٨٢، ٨٨، ٨٥ % والأحماض الدهنية حوالي ٨، ٧، ٨ % من الليبيدات الكلية للثلاثة سلالات *S. riobrave H. indica* و *H. bacterophora* علي التوالي. وبناءاً علي ذلك تعتبر الليبيدات المتعادلة هي أهم وأكبر مكون من مكونات الليبيدات الكلية ، بالإضافة إلي الليبيدات القطبية في كل الأعمار للسلالات الثلاثة. هذه النسبة من الجليسيرات الثلاثية والأحماض الدهنية تقل تدريجياً في كل الأعمار للسلالات الثلاثة، وهذا الانخفاض يكون كمياً وليس كيفياً. أوضحت الدراسة أيضاً أن الأحماض الدهنية الأساسية في النيماتودا هي حمض البلمتيك(C16:0)، حمض الاستياريك (C18:0) ، حمض الأوليك (C18:1)، حمض اللينوليك(C18:2). كذلك هناك نسبة عالية نسبية من الأحماض الدهنية العديدة الغير مشبعة. وأضافت الدراسة أن حمض البلمتيك (C16:0), حمض الاستياريك (C18:0) يمثلا الغالبية العظمي من الأحماض الدهنية المشبعة بينما حمض الأوليك (C18:1), حمض اللينوليك (C18:2) يمثلا الأحماض الدهنية الأساسية الغير المشبعة، ولكن عامة نسبة الأحماض الدهنية الغير المشبعة أعلي من نسبة الأحماض الدهنية المشبعة.

وبناءاً علي هذه النتائج نخلص إلي الآتي :

- يمكننا أن نزيد من كفاءة النيماتودا كوسيلة مكافحة حيوية ضد الأفات عن طريق تحسين وإطالة عمر النيماتودا.
- إيجاد طريقة سهلة وبسيطة للتحكم في جودة سلالات النيماتودا المختلفة.
- الأحماض الدهنية يمكن أن يكون لها دوراً في التأثير السمي للنيماتودا ضد الحشرات.

قام بتحكيم البحث

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