

RELATIONSHIP BETWEEN BIOCHEMICAL ENERGY RESERVES AND SOME ECOLOGICAL TRAITS OF THREE SPECIES OF ENTOMOPATHOGENIC NEMATODES

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(Manuscript received 22 December 2010)

Abstract

The biochemical energy reserves of three species of entomopathogenic nematodes (EPN) were analysed quantitatively at three infective juvenile ages 0, 20 and 90 days for *Steinernema* (*Steinernema riobrave*), less than 7, 30 and 50 days and 0, 20 and 40 days for *Heterorhabditis* (*Heterorhabditis bacteriophora* and *Heterorhabditis indica*) respectively. Lipid, protein, carbohydrates and glycogen contents of the two species of *Heterorhabditis* and one species of *Steinernema* were determined. Results revealed that total lipids are the main component, comprising approximately 42.55 of nematode dry weight for *S. riobrave* and 50.52%, 53.75% for *H. bacteriophora* and *H. indica*, respectively, when freshly emerged from their hosts. These percentages declined significantly at the other two juvenile ages of the three species. Glycogen and protein contents in all species exhibited minor changes during the tested juvenile ages. Estimation of total carbohydrates appeared to be source of energy that only utilized early in the ambushing state of *S. riobrave* and when juveniles get older in *H. indica* and *H. bacteriophora*. Infectivity of the three nematode species at the three tested ages was not affected, where approximately 100% *Galleria mellonella* mortality was recorded except a slight insignificant decrease (97.33%) in *H. indica* at the age of 40 days. Penetration rate of *H. indica*, and *H. bacteriophora* decreased with the declining of the total lipid content at the three juvenile ages, while the penetration rate in *S. riobrave* exhibited significant decrease only at 90-day old juveniles. Significant decreases in migration rate were evident in *H. indica* and *H. bacteriophora* as the total lipid content declined in the juvenile ages. These results might be helpful in increasing the efficiency of EPN as biological control agents against insect pests by improving shelf-life of nematode juveniles and establishing a simple and easy quality control method for different nematode species or strains.

Key Words: Entomopathogenic nematodes, *Steinernema*, *Heterorhabditis*, energy reserves and ecological traits.

INTRODUCTION

Since the nematodes free-living infective juveniles are non-feeding, the stored energy reserves in these juveniles are of most important. Some energy reserves of the nonfeeding nematode juveniles have been reported to play important roles in their life. Infective juveniles (IJs) of entomopathogenic nematodes (EPN) do not feed but have ample stored energy reserves and can survive for several months in soil or in

water, (Fitters and Griffin 2006). Factors of major importance are age and lipid reserves of infective juveniles (IJs). Energy reserves, particularly neutral lipids, are an important indicator of quality (infectivity and motility) of stored nematodes. Lipids, carbohydrate (especially glycogen), and protein, accumulated during the preceding juvenile stages within the host, all provide energy for IJs during storage, (Qiu and Bedding 2000). Lipids can be used to predict nematode quality and nematode shelf-life, (Abu-Hatab and Gaugler 1997a). Lewis *et. al.* (1995) studied the relationship between the metabolic rate, energy reserves, and foraging in three species of EPN (*Steinernema carpocapsae*, *Steinernema glaseri* and *H. bacteriophora*). Their studies showed that stored lipids are the major component of nematode energy reserves and declined at species-specific rates. Fitters *et. al.* (1997) noted that, during storage, non-feeding stages of EPN (*S. carpocapsae* and two strains of *Heterohabditis*) became visibly more transparent due to depletion of energy reserves.

The present investigation aims to analyse energy reserves (Lipids, carbohydrates protein and glycogen) of the two species of *Heterorhabditis* and one species of *Steinernema* during three different ages. The correlation between the biological and ecological traits virulence, penetration rate and migration rate of EPN during the indicated ages, and the results of the chemical analysis.

MATERIALS AND METHODS

Nematode Source

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 reared on last instar *G. mellonella* (L.) as a host at 25°C according to procedures described by (Kaya and Stock 1997). Nematode juveniles at the tested ages were used for the following ecological and biochemical tests after being stored at 15°C.

Ecological Traits

Nematode infective juveniles of the three species at the three ages (0, 20 and 90 days) for *S. riobrave*; less than one week, 30, 50 days and 0, 20, 40 days for *H. bacteriophora* and *H. indica*, respectively, were tested for the following ecological activities:

1- Infectivity

Full grown larvae of *G. mellonella* kept in 1.5 ml Eppendorf tubes, lined with double layer filter paper (Whatman No. 1), were subjected to nematode infection at a dose level of 40 IJs/larva in 300 µl distilled water and kept at 25°C, in the dark. For

each of the tested nematode ages, 3-5 replicates, (each of 10 larvae), in addition to a control treatment containing only distilled water. Mortality records were taken after 48 hours and corrected according to Abbot's formula (Abbott 1925).

2- Penetration rate

At each of the tested nematode ages, *G. mellonella* larvae, having the signs of nematode infection in the virulence test, were washed twice with distilled water to remove any nematode juveniles attached to them, dried and dissected under a stereomicroscope. The number of nematodes inside each larva was counted and the penetration rate was calculated as an average in at least 20 larvae, (Abd El-Rahman 2001).

3- Dispersal rate (Migration rate)

The T-tube choice system described by Abd El-Rahman (2001) was followed to study *H. bacteriophora* and *H. indica* migration rate at the tested ages.

Determination of Main Energy Reserves in Nematode Juveniles

1-Total lipids

Nematode juveniles (0.05-0.1g) were washed by water and incubated in 15 ml ethanol 80% at 75°C for five minutes to inactivate degradative enzymes such as phospholipases. The suspension was cooled and stored in a tight-capped tube after flushing with N₂, and stored at -70°C, (Abu Hatab and 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 palmetic acids).

2- Total proteins

Proteins were extracted from nematode tissue and prepared for assay as reported by Lewis *et al.* (1995). Nematode sample (0.05 g) was homogenized with 7 ml KOH 30% and boiled for 1 hour with occasional agitation. Protein content of nematode samples was estimated spectro-photometrically by the method of (Bradford 1976). Protein content in nematode homogenate was expressed as µg protein/g body weight using bovine serum albumin standard curve.

3-Total carbohydrates

Carbohydrates were extracted from nematode tissues and prepared for assay as described by (Lewis *et. al.* 1995). Carbohydrates content was determined by phenol-sulfuric acid reaction according to (Dubois *et. al.* 1956). Total carbohydrates content were calculated according to glucose standard and are expressed as µg glucose/g body weight.

4-Glycogen content

Glycogen was precipitated from nematode homogenate (0.05 g in 7 ml 30% KOH) with 1 ml ethanol and the mixture was kept at -15°C overnight. The precipitation was sedimented by centrifugation at 15,000 rpm for 5 minutes, redispersed in 4 ml ethanol and resedimented, the washed precipitate was dried at 105°C and dissolved in 2 ml distilled water for determination, (Lewis *et. al.* 1995). Glycogen content of nematode was estimated spectrophotometrically by phenol-sulphuric acid reaction according to (Dubois *et. al.* 1956). Glycogen content was expressed as µg glucose/g body weight using glucose standard curve.

Statistical Analysis

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, U.

RESULTS AND DISCUSSION

Ecological Traits

1-Nematode virulence

Infectivity of the three species at the three tested ages did not change significantly through the experimental period for *S. riobrave* and *H. bacteriophora*, nearly 100% mortality was recorded in *G. mellonella* larvae subjected to infection at a rate of 40 IJs/larva. Insignificant decrease in *H. indica* infectivity at the age of 40 days (97.33% *G. mellonella* mortality) was evident (Table 1).

2- Penetration rate

Penetration rate exhibited significant decrease from 25.01% at 0-day old juveniles to 22.80% at 90-day old juveniles for *S. riobrave* (Table 2). The decrease in penetration of *H. indica* juveniles was much obvious, where a gradual decrease was recorded at the three ages (21.70, 13.00 and 11.50%, respectively). Significant decreases in juvenile penetration rate were evident with *H. bacteriophora* after 30 and 50 days of storage at 25°C. The recorded values of penetration rate at these ages were 8.55 and 7.05%, respectively, opposed to 40.09% for IJs less than seven days old. In the present investigation, the number of penetrating nematodes decreased as IJs go older, similar results were reported by El-Bishry *et. al.* (2002) who reported that penetration rate of four isolates of the nematode *Heterorhabditis* spp. decreased significantly as juveniles get older. Also confirmed by Corrie *et. al.* (2004) who found

that age and species effects were noted in *G. mellonella* mortality and nematode penetration during shorter time periods. Initial mortality caused by *S. carpocapsae* and *H. bacteriophora* IJs declined with nematode age. Young *S. carpocapsae* IJs penetrated *G. mellonella* larvae at higher rates than older individuals of the species (27–45% vs. 1–4%).

3- Migration rate

Migration rate and the distance traveled by *H. bacteriophora* was significantly different

in juveniles of less than seven days old and those of 30 days old (Table 3). Migration rates were 98.88 and 78.77% of the total nematode population and the distances covered by these juveniles were 9.50 cm and 8.0cm . In the contrary, these values were much reduced at 50 days old, where migration rate significantly decreased to 38.40% and the distance covered by nematode juveniles was only 1.90 cm. Similarly, results of migration rate of *H. indica* went in the same line. Percent of migrating juveniles was 96.55% in 0-day old IJs; covering a distance of over 9 cm (Table 3). This percentage was decreased significantly to less than 30% for 20-day old juveniles; and to 15.97% at 40 day old IJs with distances 2.80cm and 1.10cm respectively. Migration and host-finding ability of *Heterorhabditis* species are essential processes in their success as biological control agents. So, juvenile age is very important factor that should be taken into consideration when using these nematodes to control a certain pest.

Biochemical Analysis of Energy Reserves:

1- Total lipids

Data illustrated in table (4) showed that 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*. These results are in agreement with those reported by Lewis *et. al.* (1995) where total lipids comprised 36-40%, also they reported that stored lipids were the major component of nematode energy reserves in these members of entomopathogenic nematodes. Abu Hatab and Gaugler (1997a) reported that lipid content in the nematode *S. riboravis* was up to 43.4% per dry weight, Also, Fitters *et. al.* (1997) found that the percentage of total lipids of *S. carpocapse* and two *Heterorhabditis* isolates (UK211 and HF85) was similar for two isolates when freshly emerged from cadaver (35-39%). Lipid is the main energy reserve, constituting between 32 and 49% of IJs body weight Lewis *et. al.* (1995) and Menti *et. al.* (2003). El-Assal *et. al.* (2008) reported that total lipid content in original *S. riobrave* and *H. bacteriophora* recorded 39.1 and 41.3% respectively. Higher

percentages of lipid content were reported by Shapiro *et. al.* (2005) where they demonstrated that total lipids content of two species of EPN (*S. carpocapsae* and *S. riobrave*) ranged from 48 to 84% according to the cultured host and method. 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) total lipid percentages of *H. bacteriophora* declined significantly from 45 % when freshly emerged from their hosts (less than one week) to nearly 19-20% after 30 days, and to 11-12% after 50 days of storage and Lewis *et. al.* (1995) in another strain of the same species (18-20%) and (13-15%) after 4 weeks and 7 week respectively. In case of *H. indica*, these percentages decreased to 37.39% after 20 days, and to 25.55% after 40 days of storage. Radwan (2002) showed that the percentage of total lipids content 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 (42.0%). A sharp significant decrease was recorded after 90 days of storage, where the recorded value was 18.00%. This is confirmed by Lewis *et. al.* (1995) they reported that total lipids declined at species-specific rates. This means that infective juveniles of entomopathogenic nematodes are capable of adjusting their lipid content at different periods of life. The above results refer that the infective juveniles of *H. bacteriophora* nematodes 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. Menti *et. al.* (2003) stated that, NL (neutral lipids) of both *S. feltiae* populations decreased by ca 60% during the first 4 weeks but remained more or less stable during the next 16 weeks. In the two Heterorhabditis species, NL decreased by ca 70% during the first 4 weeks and continued to decrease, with 3-5% remaining after 12 weeks. Chemical analysis of nematode juveniles at the indicated ages suggests that, total lipids are the main source of energy. Lewis *et. al.* (1995) found that stored lipids are the major component of nematode energy reserves in members of *Steinernema* spp. Abu Hatab and Gaugler (1997a) reported that the high lipids content of *S. riobrave* suggests that these compounds are the chief food reserve for infective juveniles.

2- Total proteins

Data represented in table (5) showed that protein contents in all species exhibited minor changes during the tested juvenile ages. Protein percentage remained

constant during the three inspection periods (approximately 11% of nematode dry weight for *S. riobravae* and *H. bacteriophora* and 12% for *H. indica*). Therefore, this component might play minor role as an energy reserve in this nematode species. These results are confirmed by Lewis *et. al.* (1995) since they found that the level of protein content remained constant for all species (*S. carpocapsae*, *S. glaseri* and *H. bacteriophora*) at approximately 12.2% throughout the study.

3- Total carbohydrates

Estimation of total carbohydrates table (6) revealed the importance of this energy reserve in the nematode *S. riobrave*. In fact, they might be the only main source of energy during the first 20 days. Total carbohydrate content decreased significantly from 23.43% at 0-time to 16.33% of nematode dry weight after 20 days. Another decrease, but insignificant, was also evident at 90-day old juveniles (14.28%). Lewis *et. al.* (1997) provided some evidence that in some cases *Steinernema* species changed their foraging strategy for infecting a host from ambushers to cruisers (active seekers for host). It seems that nematode juveniles during the ambushing state depended mainly upon carbohydrates as energy reserves because they actually stayed motionless waiting for passing by hosts. When they changed their searching strategy to be cruisers where richer sources of energy were needed; they began to utilize their lipid content.

In case of *H. indica*, during the first 20 days total carbohydrates declined from 13.94 to 11.84% of nematode dry weight. At 40-day old juveniles, total carbohydrates decreased to comprise 8.25. Total carbohydrate content of *H. bacteriophora* might play minor role as energy reserves in nematode juveniles, where the recorded values were 11.97, 10.80% and 9.32% at the three tested periods, respectively; with insignificant differences among them and this result was in agreement with El-Badawy (2002) who reported that in the nematode *H. bacteriophora* (N-1 strain), during a 50-day period, total carbohydrates were of minor importance as energy reserves.

4- Glycogen content

Estimation of glycogen content as an energy reserve in nematode juveniles of the three species at the tested ages table (7) indicated that this component has minor role as energy reserve. The glycogen recorded values of *S. riobrave* were 8.30, 8.09 and 7.93% of nematode dry weight at 0, 20 and 90-day old juveniles, respectively. The four species (*S. carpocapsae*, *S. riobravensis* (Biosys 355), *S. feltiae* (UK76) and *S. glaseri* (NC) had appreciable amounts of glycogen; from ca. 8% dry weight in *S. riobravensis* to ca. 18% in *S. glaseri* (Patel and Wright 1997). Lewis *et. al.* (1995) reported that level of glycogen remained constant for *S. glaseri*, *S. carpocapsae* and *H. bacteriophora* at approximately 7.80%.

In case of *H. indica* during the first two ages, a slight insignificant decrease was found in glycogen content (from 7.35 to 7.25%), but at 40-day old juvenile, significant decrease was recorded (5.00% of nematode dry weight). The recorded values of *H. bacteriophora* exhibited an insignificant decrease after 30 days from (7.45%) to reach 7.23% and slight significant decrease was recorded at 50 days (6.17%). Infective juveniles of *S. carpocapsae* and *S. riobravisi* survived for 120-135 days and utilized ca. 90% of their glycogen reserve at an almost constant rate during a 112-day storage period (Patel and Wright 1997). On the other hand, Wright *et. al.*, (1997) demonstrated that glycogen content declined significantly between 60 and 90 days of storage in the nematode *S. carpocapsae*, this decline began after 90 days in *S. feltiae*. These obtained findings support the conclusion of (Patel & Wright 1997). *S. carpocapsae* IJs not only consumed the lipid reserve but also proteins, glycogen and trehalose. Most lipids were consumed in the first three weeks while most of the proteins, trehalose and glycogen were consumed in the next three weeks. The results of glycogen in the present study and those of Patel and Wright (1997) and Wright *et. al.* (1997) suggest that glycogen content may be utilized as an alternative energy reserve in nematode juveniles after a considerable storage period after the lipid reserves reaches a certain level

There are different relationships between infectivity, penetration, migration rates and energy utilization. Infectivity was not affected by the declining of total lipids content (Tables 1 and 4). This is in agreement with Qiu and Bedding (2000) who indicated that infectivity of *S. carpocapsae* IJs, did not significantly decrease until nearly all lipids were consumed. This finding disagree with Patel and Wright (1997) who found that, three nematodes species (*S. riobrave*, *S. feltiae* and *S. glaseri*) showed a rapid initial decline in infectivity with declining lipid levels and Lewis *et. al.* (1995) in most species of EPN, activity and infectivity decline as lipids were depleted. This may be explained by the behavior of *S. riobravisi* where, switching to ambushing with relatively high lipid levels remaining, matching its labeling as an intermediate ambusher.

Penetration rate of *H. indica*, and *H. bacteriophora* decreased with the decreasing of the total lipid content at the three tested juvenile ages. The values of penetration rate of *S. riobrave* approximately were similar at the two juvenile ages (0 day and 20 days) also, total lipid content not changed at the same two juvenile ages (Tables 2 and 4). El-Assal *et. al.* (2008) showed that the highest penetration rate of *S. riobravisi* was recorded with the highest total lipids content. Increased energy consumption will subsequently shorten the life of the non-feeding IJs. Hence, unsuccessful penetration attempts must be costly. *H. indica* was more declining in penetration rate than *S. riobrave* at the tested juvenile ages while, *H. bacteriophora* was the highest. This result may be explained by the fact that greater aggregation occurs in

Heterorhabditids than in steinernematids, decreasing oxygenation and leading to the loss of energy reserves could be a factor that maintained nematodes of the genus *Heterorhabditis* alive for a shorter time. However, the ability of the three strains of nematodes to penetrate the host decreased as the lipid content decreased throughout all tested juvenile ages. These findings of infectivity and penetration rate may support the concept that the virulence of any nematode isolate is, most probably, due to the pathogenicity of the bacterial symbiont. Entomopathogenic nematodes without bacteria did not kill insect larvae, and the infectivity of the nematode-bacterial combination was determined by the pathogenicity of the bacterium and by the interaction between them.

Results in tables (3 and 4) showed that, there was decreasing in the migration rate of the three juvenile ages of *H. bacteriophora* and *H. indica*, with reduction of total lipid content. The ability of the two nematodes to migrate decreased as lipid content decreased throughout all tested juvenile ages. Where, penetration and migration rates were affected with the values of lipid content, persistence and pathogenicity of entomopathogenic nematodes is associated with lipid content.

Carbohydrates of *S. riobravis* during the first 20 days were important sources for energy besides total lipids, (Tables 4 and 6). This result is confirmed by Kung *et. al.* (1990) who showed that energy generated of *S. carpocapsae* and *S. glaseri* by carbohydrate consumption from stored food reserves was used up rapidly, resulting in low survival, motility and pathogenicity.

Carbohydrates appeared to be source of energy only when juveniles get older in *H. indica* and *H. bacteriophora*. This conclusion is confirmed by (Patel and Wright 1997). who reported that carbohydrate might be important for infectivity in aging IJs of *S. carpocapsae*. and also, Qiu and Bedding (2000) stated that most lipids were consumed in the first 3 weeks while most proteins, glycogen and trehalose were consumed in the last 2 or 3 weeks.

The importance of glycogen and protein as energy sources has not been investigated for three EPN strains at the three tested juvenile ages. The results of glycogen in the present study and those of Patel and Wright (1997) and Wright *et. al.* (1997) suggest that glycogen content may be utilized as an alternative energy reserve in nematode juveniles after a considerable storage period after the lipid reserves reaches a certain level. Glycogen was shown to be important in maintaining infectivity in aged IJs of *S. carpocapsae* Patel and Wright (1997) and Wright *et. al.* (1997). Glycogen and trehalose levels of *S. carpocapsae* were maintained during the first 3 weeks of incubation when lipids had not been severely depleted (Qiu and Bedding 2000).

CONCLUSION

Total lipids suggested to be the main source of energy in EPN juveniles that depend upon it in searching and penetrating hosts, and the consumption of these reserves resulted in the observed decrease in nematode efficiency. Total protein and glycogen might play minor role as utilizable energy reserves, while, total carbohydrates appeared to only utilized early in the ambushing state of *S. riobrave*, and when juveniles get older in *H. indica* and *H. bacteriophora*. Obtained results in the present study might be helpful in the field nematodes production which is 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. Also, establishing a simple and easy quality control method for different nematode species or strains.

Table 1. Infectivity of *S. riobrave*, *H. indica* and *H. bacteriophora* to *G. mellonella* larvae at different juvenile ages.

Juvenile age (days)	% <i>Galleria</i> mortality (Mean ± SEM)		
	<i>S. riobrave</i>	<i>H. indica</i>	<i>H. bacteriophora</i>
0 or less than one week*	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a
20 or 30**	100±0.00 ^a	100±0.00 ^a	99.0±3.00 ^a
40 or 90***	100±0.00 ^a	97.33±3.33 ^a	100±0.00 ^a

Table 2. Penetration rate of *S. riobrave*, *H. indica* and *H. bacteriophora* to *G. mellonella* larvae at different juvenile ages.

Juvenile age (days)	% Penetration (Mean ± SEM)		
	<i>S. riobrave</i>	<i>H. indica</i>	<i>H. bacteriophora</i>
0 or less than one week*	25.10±2.02 ^a	21.70±1.80 ^a	40.09±2.46 ^a
20 or 30**	24.75±2.88 ^a	13.00±3.00 ^b	8.55±0.65 ^b
40 or 90***	22.80±2.38 ^b	11.50±2.50 ^b	7.05±1.15 ^b

Table 3. Migration rates of *H. indica* and *H. bacteriophora* at different juvenile ages

Juvenile age (days)	% Migration (Mean ± SEM)		Distance traveled (cm)	
	<i>H. indica</i>	<i>H. bacteriophora</i>	<i>H. indica</i>	<i>H. bacteriophora</i>
0 or less than one week*	96.55±2.1 ^a	98.88±4.35 ^a	9.20±0.56 ^a	9.5±0.20 ^a
20 or 30**	25.75±1.8 ^b	78.77±0.05 ^a	2.80±0.59 ^b	8.00±1.2 ^b
40 or 50***	15.97±0.59 ^c	38.4±11.00 ^b	1.10±0.59 ^c	1.90±0.4 ^c

Table 4. Lipid content of *S. riobrave*, *H. indica* and *H. bacteriophora* at different juvenile ages.

Juvenile age (days)	% Lipid content (Mean ± SEM)		
	<i>S. riobrave</i>	<i>H. indica</i>	<i>H. bacteriophora</i>
0 or less than one week*	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 lipid content

Table 5. Protein content of *S. riobrave* and *H. indica* and *H. bacteriophora* different juvenile ages.

Juvenile age (days)	% Protein content (Mean \pm SEM)		
	<i>S. riobrave</i>	<i>H. indica</i>	<i>H. bacteriophora</i>
0 or less than one week*	11.30 \pm 0.99 ^a	11.80 \pm 0.51 ^a	11.25 \pm 0.15 ^a
20 or 30**	11.20 \pm 1.00 ^a	12.00 \pm 0.31 ^a	11.00 \pm 0.01 ^a
40 or 50 or 90***	11.20 \pm 1.00 ^a	12.10 \pm 0.79 ^a	10.96 \pm 0.30 ^a

Values are expressed as dry weight % of total protein content.

Table 6. Carbohydrate content of *S. riobrave*, *H. indica* and *H. bacteriophora* at different juvenile ages

Juvenile age (days)	% Carbohydrate content (Mean \pm SEM)		
	<i>S. riobrave</i>	<i>H. indica</i>	<i>H. bacteriophora</i>
0 or less than one week*	23.43 \pm 0.30 ^a	13.94 \pm 0.29 ^a	11.97 \pm 0.06 ^a
20 or 30**	16.33 \pm 0.37 ^b	11.84 \pm 0.16 ^b	10.80 \pm 0.04 ^b
40 or 50 or 90***	14.28 \pm 0.50 ^b	8.25 \pm 0.19 ^c	9.32 \pm 0.17 ^c

Values are expressed as dry weight % of total carbohydrates content.

Table 7. Glycogen content of *S. riobrave*, *H. indica* and *H. bacteriophora* at different juvenile ages

Juvenile age (days)	% Glycogen content (Mean \pm SEM)		
	<i>S. riobrave</i>	<i>H. indica</i>	<i>H. bacteriophora</i>
0 or less than one week*	8.30 \pm 0.97 ^a	7.55 \pm 0.15 ^a	7.45 \pm 0.15 ^a
20 or 30**	8.09 \pm 1.00 ^a	7.29 \pm 0.12 ^a	7.23 \pm 0.12 ^a
40 or 50 or 90***	7.90 \pm 1.05 ^a	5.00 \pm 0.06 ^b	6.17 \pm 0.06 ^a

Values are expressed as dry weight % of total glycogen.

SEM = Standard error of the mean.

* 0 days for *H. indica*; and *S. riobrave* and less than one week 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*.

% total lipids, protein, carbohydrates, glycogen in nematode sample = $X/Y \times 100$

X=weight of lipid, protein, carbohydrates, glycogen per mg

Y= weight of nematode sample per mg

For each nematode species, means followed by the same letter are not significantly different ($P < 0.05$;

Duncan's multiple range test).

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العلاقة بين مصادر الطاقة الكيميائية الحيوية وبعض المفاهيم الإيكولوجية لثلاثة أنواع من الديدان النيماتودا

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يهدف هذا البحث إلي التحليل الكامل لمستودعات الطاقة الكيميائية الحيوية (اللبيدات ، البروتين ، الكربوهيدرات الكلية والجليكوجين) لثلاث سلالات من الديدان النيماتودا الممرضة للحشرات وهي *Steinernema riobrave* التي تنتمي إلي عائلة *Steinernema* والنوعين الآخرين ينتميان إلي عائلة *Heterorhabditis* وهما *Heterorhabditis bacterophora* و *Heterorhabditis indica* ، خلال ثلاث أعمار للديدان النيماتودا (صفر، 90، 20 يوم) لسلالة *S. riobrae* (صفر، 40، 20 يوم) لسلالة *H. indica* (أقل من 7 أيام ، 50، 30 يوم) لسلالة *H. bacterophora* وإستنتاج العلاقة بين تلك المستودعات وبعض المفاهيم الإيكولوجية التي تشمل فعالية الديدان النيماتودا ضد العائل (دودة الشمع) ، معدل الإختراق والهجرة للعائل وقد أوضحت النتائج الآتي:

- ١ - اللبيدات هي المصدر الرئيسي للطاقة للثلاث سلالات من الديدان النيماتودا خلال كل الأعمار.
- ٢ - الكربوهيدرات مصدر للطاقة بالإضافة إلي اللبيدات في الأعمار المتقدمة لسلالة *S. riobrae* وفي الأعمار المتأخرة لسلالة *H. bacterophora* وسلالة *H. indica*.
- ٣ - البروتين والجليكوجين لهما تأثير ثانوي كمصدر للطاقة في الثلاث سلالات خلال كل الأعمار.
- ٤ - فعالية الديدان النيماتودا (إصابة العائل وقتله) لم تتأثر بمحتوي اللبيدات بالزيادة أو النقصان وكانت نسبة الموت لدودة الشمع تقريباً 100% موت مع سلالات الديدان النيماتودا الثلاثة مع كل الأعمار ماعدا سلالة *H. indica* عند عمر 40 يوم كانت نسبة الموت تقريباً 97%.
- ٥ - معدل إختراق الديدان النيماتودا للعائل لسلالة *H. bacterophora* وسلالة *H. indica* يقل مع نقصان اللبيدات الكلية في كل الأعمار المختلفة بينما يحدث ذلك فقط مع سلالة *S. riobrae* عند عمر 90 يوم.
- ٦ - معدل هجرة الديدان النيماتودا للعائل تزيد كلما زاد محتوى اللبيدات في سلالة *H. bacterophora* وسلالة *H. indica* مع الأعمار الثلاثة.

أوضحت النتائج أن هناك علاقة قوية بين مستودعات الطاقة للديدان النيماتودا والصفات الإيكولوجية وهذه النتائج يمكن أن تساعد في زيادة كفاءة الديدان النيماتودا كوسيلة مكافحة حيوية ضد الآفات عن طريق تحسين وإطالة عمر الديدان النيماتودا وأيضاً إيجاد طريقة سهلة وبسيطة للتحكم في جودة سلالات الديدان النيماتودا المختلفة.