Egypt. Acad. J. biolog. Sci., 2(2): 45 – 54(2010) **C. Physiology & Molecular Biology**
Email: egyptianacademic@yahoo.com **ISSN: 2090-0767**
Received: 20/7/2010 **www.eajbs.eg.net**

Physiological and biological studies of some entomopathogenic nematode species of families (steinernematidae and heterorabditidae)

Saheir F. El-Lakwah and Ahmed M. Azazy
Plant Protection Research institute, Agricultural Research Center.
E-mail:so_ellakwah@yahoo.com

ABSTRACT

Entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema* are used as an insect biological control in agriculture. Numerous new species are being described but generally little information is provided on their ecological or physiological information. Therefore, this paper presents examination of virulence, penetration rate, reproduction, and some energy reserves (total lipids, total proteins and total carbohydrates) for six species of entomopathogenic nematodes, 3 species of *Heterorhabditids ((*HP2*), (*HP4*)* and *H. indica*) and 3 species of *Steinernema* (*(S3), S. riobrave* and *S. rarum)* are extracted from soil samples at different countries and places to refer the superlative one to unfavorable environmental conditions. The tested species differed in their penetration rate to *Galeria mellonella* larvae. *Heterorhabditis sp.* (HP2) recorded the highest penetration rate (56 %) where it recorded highest total lipids *(*35.8 %), highest total proteins (60.3%) and the highest virulence to *G. mellonella* (25%). The highest total carbohydrates were recorded to the *steinernema rarum* (26%) while the highest reproduction was recorded to *Heterorhabditis indicus* (149914 IJs/larva).

Keywords: entomopathogenic nematodes*, Heterorhabditids spp, Steinernema spp.* Penetration, virulence, reproduction, lipid, protein, carbohydrate.

INTRODUCTION

The infective juveniles (IJs) of entomopathogenic nematodes (EPNs) are currently used as biopesticides for controlling various insect pests (Hom, 1994). The infective juveniles are non-feeding and free-living in soil and so are unable to compensate for energy consumption with energy intake, and depend solely on accumulated reserves for energy supply (Gaugler, 1988). Successful establishment of nematodes in diverse environments depends upon physiological, behavioral and biochemical adaptations (Nicholas, 1984*)*. Sixty species of entomopathogenic nematodes in the families *Steinernematidae* and *Heterorhabditidae* have been described (Nguyen, 2005; Qiu *et al*., 2005; Phan *et al*., 2005; Nguyen *et al*., 2006; Uribe-Lorio *et al*., 2007; Zhang *et al*., 2008 and Nguyen *et al*., 2010). Descriptions of new entomopathogenic nematode species usually contain very little information on their biology and ecology and are not complemented by studies supplying this information (Koppenhöfer and kaya, 1999). There is little information concerning the energy metabolism and its relation to survival and infectivity of EPNs (Selevan *et al*., 1993).Entomopathogenic nematode with their associated *Xenorhabdus spp*. bacteria, lethal pathogens of soil-inhabiting insects. Infective juveniles occur naturally in the soil where they infect and kill their insect host within 2 or 3 days and produce 2 or 3 generations in the host. Resulting infective juveniles emerge from host cadaver 1or 2 weeks later and search for new hosts (Akhurst, 1995).

Entomopathogenic nematodes have been isolated from every inhabited continent and many islands (Poiner, 1990). These nematodes are faced with wide array of environmental conditions during the non-feeding infective stage. In this study, we report the differences in lipid, protein, carbohydrate contents, virulence, penetration rate and reproduction of entomopathogenic nematodes of 6 widely distributed, geographically diverse species.

MATERIALS AND METHODS

Three *Heterorhabditis* and three *steinernematid* entomopathogenic. The six species were obtained from the Laboratory of Insect Parasitic Nematodes, Plant Protection Research Institute, Egypt. *Steinernema riobrave, Steinernema rarum, Heterorhabditids indica imported from USA,* Florida, *Steinernema sp.* (S3), *Heterorhabditids sp. (*HP4*)* originally isolated from a soil sample obtained from Saudi Arabia kingdom and *Heterorhabditids sp. (*HP2*)* originally isolated from a soil sample- EL-kasasin-Ismailia-Egypt. All the used chemicals were supplied by Sigma, Berkley, California, USA, unless otherwise mentioned. Total lipid kit was obtained from El-Gomhoriya Company, Cairo, Egypt. The Equipments: Centrifuge: Beckman, J2 MC, Beckman Co., USA. Spectrophotomer: Beckman, DU 7400 Dual Spectro, Beckman, J2 MC, Beckman Co., USA, and stereomicroscope

1-Nematode Biological Activities:

In all the following experiments, *G. mellonella* larvae (last instar) were exposed to freshly emerged nematode infecting stages, at a dose level of 20 IJ/larva in 300 µl of distilled water in 1.5 ml Eppendorf tubes, lined with double layer filter paper (Whatman No. 1) and kept at 25°C, in the dark.

A. Penetration Rate:

After 4-5days of the infection, according to the species of nematode, at least 10 dead *G. mellonella* larvae were washed twice with distilled water to remove any nematode juveniles that 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

B. Reproduction rate:

At least five dead insect larvae, after 2 days of infection, were washed twice with distilled water to remove any nematode juveniles that attached to them, and placed into modified White traps. For each of the tested nematode species, 5 replicates. After 15 days of infection all IJ that emerged from the host over this period in the water were harvested and the total nematode suspension was put in a 50 ml tissue culture flask. To assess the total production during the harvest period, the contents of the flask were mixed thoroughly with air bubbles from an aquarium pump and from this suspension 5 samples of 10 µl were counted under a stereomicroscope using a counting slide.

C. Virulence (one -on -one):

G. mellonella larvae were subjected to nematode infection at a dose level of 1 IJ/larva in 300 µl of distilled water and kept at 25°C, in the dark. For each of the tested nematode species, 5 replicates, each of 6 larvae, were made, in addition to a control containing only distilled water. Mortality records were taken after 48 hours and corrected according to Abbott's formula (Abbott, 1925).

2-Energy Reserves in Nematode Juveniles

A-Total Lipids:

Nematode juveniles were washed and incubated in 15 ml of 80% ethanol at 75°C for 5 minutes to inactivate degradative enzymes such as

phospholipases. The suspension was then cooled and stored in a tight-capped tube after flushing with N_2, and stored at –70°C *(*Abu Hatab, and Gaugler 1997). Lipids were extracted and purified from frozen nematode samples (0.05-0.1g) according to Folch *et al*., (1957). Pure vanillin (1.2 g) was dissolved in 20 ml ethyl alcohol and completed to 200 ml with distilled water; 800 ml of concentrated phosphoric acid were added. The solution was stored in dark glass bottle at room temperature. The procedure described by Knight *et al*., (1972), is used for determination of Lipids and measured spectrophotometrically at 525 nm against a blank.

B- Total Protein

Protein was extracted from nematode tissues and prepared as described by Lewis *et al*., (1995). Nematode samples (0.05 g) were homogenized in 3 ml 30% potassium hydroxide (KOH) for 5 minutes. The homogenate was then washed by 4 ml 30% KOH, and boiled for 1 hour with occasional agitation; the sample was then cooled to room temperature. Protein content of nematode samples was estimated spectrophotometrically by the method of Bradford (1976).

C- Total Carbohydrate*:*

Carbohydrates were extracted from nematode tissue and prepared according to Lewis *et al*., (1995). Nematode samples (0.05 g) were homogenized with 7 ml 30% KOH and boiled for 1 hour with occasional agitation. Carbohydrates content was determined by phenol-sulfuric acid reaction according to Dubois *et al*., (1956).

Statistical Analysis

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) (Colman, 2001) according to the statistical methods of Snedecor (1956). All analyses were made using a software package "Costat", a product of Cohort Software Inc., Berkeley, California .

RESULTS

1-Nematode Biological Activities

A-Penetration Rate:

It is evident from the fig (1A) that the highest penetration rate of nematode (56%) was found in dead *G. mellonella* larvae infected with HP2. The penetration rate of S3, *S. riobrave*, *S. rarum* and *H. indica* reported (49.7%), (38.7%), (36%) and (32.7%), respectively. The least penetration rate (9.7%) was for HP4. No significant differences were found between the penetration rates of *S. rarum* and *H. indica*. Generally statistical analysis showed that there were high significant differences between the efficacy of tested EPNs species (F=13.62, df=5, P=0000).

B-Reproduction rate:

In fig (1B) it is clear that the largest cumulative production of juveniles for the *H. indica* where each cadaver produced (149914 IJs/L) through the 2 weeks after the day of infection, while S3, S. *rarum*, *S. riobrave* and HP2 were degraded in their reproduction as it recorded (41347 IJs/L), (39060 IJs/L), (28992 IJs/L) and (14066 IJs/L), respectively. (5943 IJs/L) was the least production recorded for the HP4. Statistical analysis showed that there were very high significant differences between the efficacy of entomopathogenic nematode species (F=547.2, df=5, P=0000). No significant differences were found between the reproduction rate of the tested species, S3 and *S. rarum*.

C-Virulence (one -on -one):

Fig (1C) represented the tested isolates exhibited high virulence against larvae of *G. mellonella* was 25% mortality of HP2, while the second high mortality 20% was recorded for the S3. Both *H. indica* and *S. rarum* recorded 12.5% mortality of *G. mellonella* larvae. The other two isolates were less virulent recording (8.3% and 4.2%) mortality for *S. riobrave* and HP4, respectively, with non –significant differences among them (F=0.821 df=5 P=0.5506).

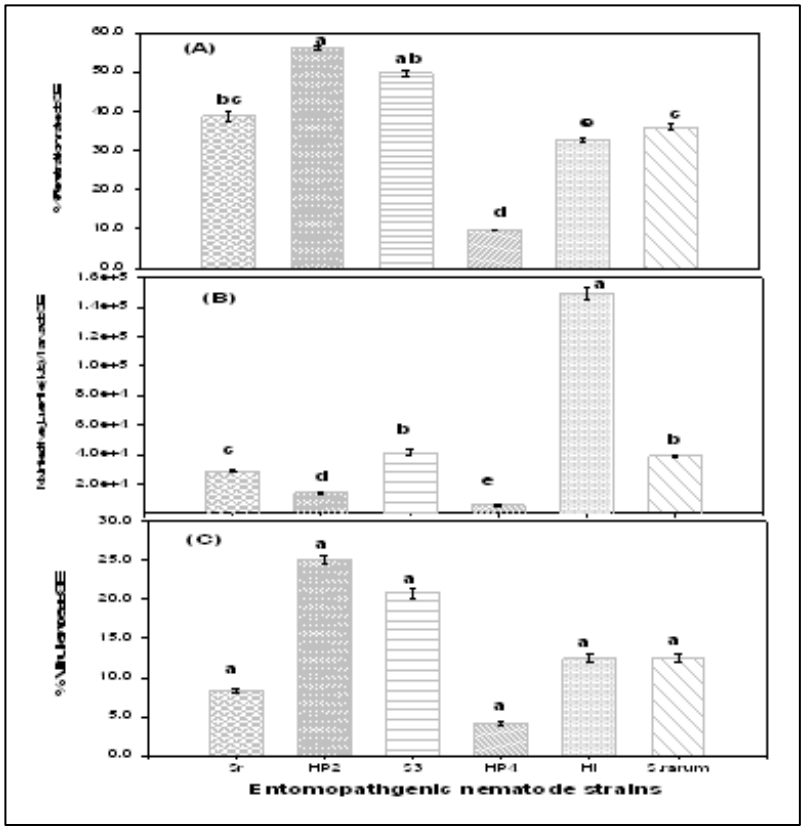

Fig.1:The biological activities (A)-Penetration rate (B)-Reproduction (C)-Virulence of *Steinernema riobrave, Heterorhabditids sp. (HP2), Steinernema sp. (S3), Heterorhabditids sp. (HP4), Heterorhabditids indica, and Steinernema rarum.* Bars indicate Standard error of mean. Columns within treatments annotated with the same letter are not significantly different (Duncan's multiple range; P<0.05).

2-Energy Reserves of Nematode Juveniles

A-Total Lipids:

The total lipids showed in fig (2A), where HP2, *H. indica, and* S3 were non-significant, recorded (35.8%), (34.5%), and (34.3%), respectively. And these three isolates were significantly different with the other three isolates, HP4, *S. riobrave*, and *S. rarum,* they recorded (30.4%), (29.2%), and (27.3%), respectively. The last three isolates were non-significantly with each other. Statistical analysis showed that there were significant differences between the total lipids of tested species (F=8.434, df=5, P=0.0013).

B-Total Protein:

Data in (fig 2B) showed that there are significant differences between the tested isolates in total protein content since 60.3, 58.5, 53.5, 52.7, 50.6 and 42.7% of HP2, *S. rarum,* HP4, *S. riobrave,* S3 *and H. indica,* respectively. Statistical analysis represented that there were highly significantly differences between the tested EPNs (F=1176.3, df=5, P=0.0000).

Total Carbohydrate:

Results of total carbohydrate contents illustrated in fig (2C), revealed that a significant different between the two isolates *S. rarum* and *S. riobrave* for this energy reserve, it recorded (26%) and (21.8%), respectively. HP2 and S3 ranked in the third and fourth places with non-significant difference between them (16.4%) and (14.9%), respectively. The lowest total carbohydrate percentage were observed in case of HP4 and *H. indica* (12.1%) and (12%), respectively. Generally, statistical analysis showed that there were significant differences between the tested species of EPNs (F=47.302, df=5, P=0.0000).

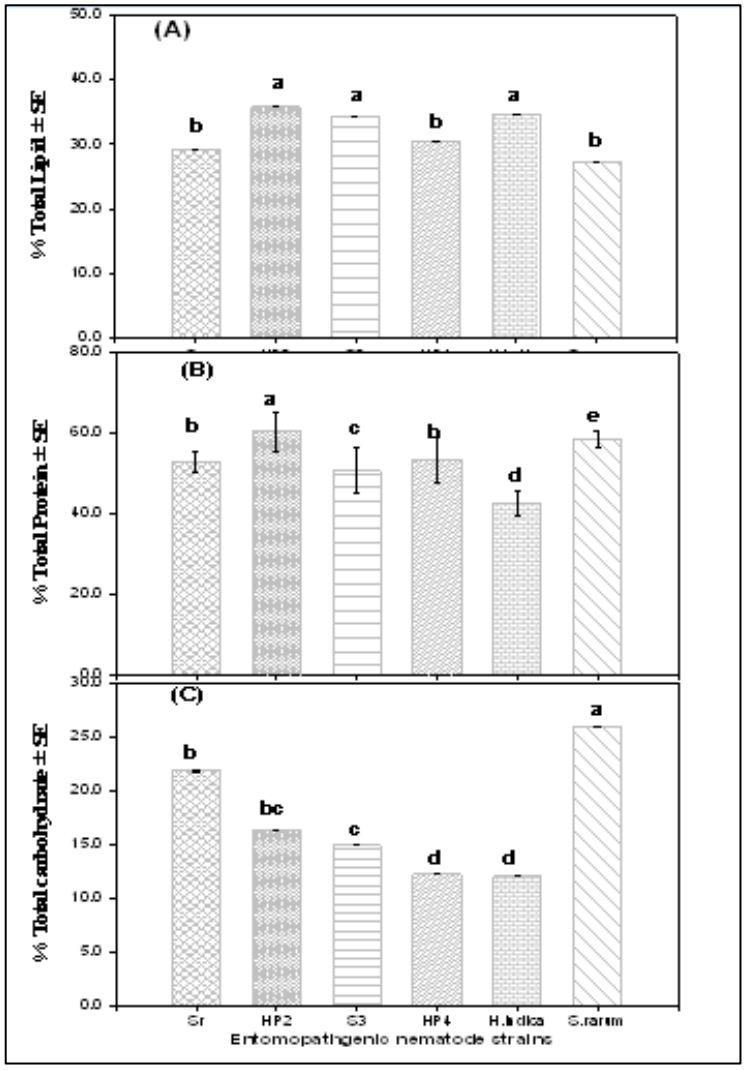

Fig. 2: The Energy reserves (A)-Total lipids (B)-Total proteins (C)-Total carbohydrates of *Steinernema riobrave, Heterorhabditids sp. (HP2), Steinernema sp. (S3), Heterorhabditids sp. (HP4), Heterorhabditids indica, and Steinernema rarum.* Bars indicate Standard error of mean. Columns within treatments annotated with the same letter are not significantly different (Duncan's multiple range; P<0.05).

The quality of nematodes that survive the rigorous of manufacturing process is analyzed by determining their shelf-life, which is predicted from storage energy reserves (Grewal and Georgois, 1995).Nematode viability and infectivity may decline at different rates during storage, and prolonged

survival of nematodes depends upon the conservation of energy reserves (Wijbenga and Rodgers, 1994). El-lakwah *et al*., (2008) used three simple methods to enhance Entomopathogenic nematodes efficacy. New progenies of *S. riobrave and H. bacteriophora*(ISk-2 strains) ,with higher penetration rate and virulence than in the original nematodes were obtained . The physiological and ecological properties are the basic information necessary for further studies of a new isolated nematode as a biological control agent.

Lipid considered as a major energy reserve. The amount of lipid varies with nematode species (Selevan *et al*., 1993). In the present study, the total lipids of the six species (*S. rarum, S. riobrave,* HP4, S3*, H. indica* and HP2) varied significantly where it recorded (27.3%, 29.2%, 30.4%, 34.3%, 34.5%, and 35.8%). Fitters *et al*., (1999) found that lipids represented 34-43% of the dry weight of three isolates of *Heterorhabditis sp*. (UK211 from England, HF85 from the Netherlands & EU17 from Estonia). Lewis *et al*., (1995), in another strain of the same species, where total lipids comprised was 36-40% for *Heterorhabditis sp*. We recorded the highest total lipid percentage (35.8%) for the HP2 and as well it obtained highest virulence and penetration rate to *G. mellonella* (25% &56%, *respectively*), but its reproduction (14065IJs/L) arranged in the fifth rank correlated to the six tested species. Pate1 *et al*., (1997) declared a decline in neutral lipids was closely connected with the observed decline in infectivity and survivorship of three steinernematids. Moreover, in the study of Lewis *et al.,* (1995) declared that stored lipids are the major component of nematode energy reserves in members of *Steinernematids sp.* This indicates the role played by lipids as an energy reserve, in these nematode species. Also, HP2 was recorded highest percentage of total protein (60.3 %). (Qiu and Bedding 2000) mentioned that, Proteins were about half dry weight of fresh IJs then asked, why IJs store large amounts of proteins instead of extra lipids which provide more energy per unit mass is still unknown but proteins might provide extra muscle for locomotion and infection for as long as possible prior to be used as an energy source. This answer may explain that, *S. rarum* was obtained the lowest total lipids percentage (27.3%), although that it did not have the lowest penetration rate or virulence but it located in the fourth rank for both and its reproduction (39060 IJs/L) arranged in third, however it recorded highest total carbohydrate (26%) and located in the second rank of protein content according to the six species studied.

We observed that *S. riboravis* has total lipids percentage (29.19%). Abu Hatab and Gaugler (1997) assured that lipid content in the nematode *S. riboravis* was up to 43.4% per dry weight. Abu Hatab *et al*., (1998), who asserted that total lipids ranged from 50.7-64.4% in *S. glaseri*, depending upon the culturing method. On the other hand, *S. riboravis* reared in the laboratory for several years and changes in its natural traits are expected. El-Assal *et al*., (2008) established progenies of *S. riboravis* and *H. bactertophora* have total lipids, total proteins and total carbohydrates higher than the origin after passing in optical laboratory condition. In our result, *S. riboravis* obtained penetration rate (38.7%), this result related to that was reported by Koppenhöfer and kaya, (1999), where they mentioned that the average number of nematodes which had penetrated during the 24-h expose at 25°C was (40.5±1.7). The reproduction of *S. riboravis* (28992IJs/L) arranged in the fourth

rank comparing to the reproduction of the other tested species. And its virulence (8.33%) to *G. mellonella* arranged in the fifth rank in correlation to the other species. *(*Qiu and Bedding 2000) affirmed that the infectivity of the different species of EPN respond differently to changes in energy reserve levels. As we show the S3 recorded penetration rate (49.7%), virulence (20.9%) and reproduction (41347 IJs/L) these arranged in the second rank according to the other studied species, even though it recorded total lipids (34.3%), total protein (50.6%) and carbohydrate (14. 9%). Lewis et al., (1995) estimated the percentage of lipid (34.53%) for *H. bacteriophora,* 32.40% for *S.carpocapsa,* and 36.13% for *S. glaseri,* even so, the author assured that the percentage of lipid was similar for all species when freshly emerged from cadaver. By looking to our result the total lipid percentage ranged from 27.30 to 35.72%, whereas the protein percentage ranged from 42.67 to 60.28%.that matched with Selvan *et al*., (1993) who projected chemical composition of inflictive juvenile entomopathogenic nematodes varied between species. Simoes *et al*., (1993) indicated to the climatic conditions of the nematode original locality, where each species has an optimum temperature for activity and reproduction of the species. Entomopathogenic nematodes isolated from diverse geographic regions and climates provide an opportunity to compare the biochemical adaptations of morphologically similar animals with similar life histories to a wide variety of environmental challenges Selvan *et al*., (1993). The 6 tested species widely distributed geographically diverse, where HP4 and S3 isolated originally from the east with a warm climate, Saudi Arabia kingdom, *S. riobrave, S. rarum and H. indica* were originally from far west, USA and HP2 from the Mediterranean climate, Egypt. The Carbohydrate percentage of HP4 and *H. indica* was (12.21% &12%, respectively). Its lipid percentage (30.4% & 34.5%, respectively) and the protein percentage (53.5% & 42.7%, respectively), whereas the reproduction for the HP4 (5943 IJs/L) was the lowest ,as well as its virulence (4.2%) and penetration rate(9.7%), all the three biological activity located in the sixth rank correlated to the same biological activity of the studied species, On the other hand, *H. indica* recorded the highest reproduction (149914 IJs/L).The rank of virulence(12.5%) for *H. indica* was the third and its penetration rate(32.66%)was the fifth according to the studied species. In the present study, the recorded penetration rate and virulence of tested nematode species were not associated with their energy reserves. This may be due to the pathogenicity of the bacterial symbiont, as the virulence was determined by the pathogenicity of the bacterium and by the interaction between the nematode and bacteria (Gerritsen and Smits, 1994).

Free living nematodes accumulate and utilize lipid as their major source of energy, whereas in the absence of oxygen, nematodes accumulate and utilize glycogen. Glycogen is critical for the IJ to survive under the anaerobic conditions where IJs are likely to encounter periodically in the soil (Qiu and Bedding 2000).Glycogen and trehalose is the principle carbohydrate energy stored in many nematode species (Behm, 1997&Qiu *et al*., 2000). Glycogen and protein play a role as alternative energy reserves which are only used on a significant scale when lipids are depleted to a low level. Proteins were about half dry weight of

fresh IJ and 40% were consumed during 6 weeks incubation at 28°C indicating that proteins may be significant energy reserves (Qiu and Bedding 2000). Lewis *et al*., (1997) provided some evidence that in some cases, *Steinernema* species change their foraging strategy for infecting a host, from ambushers to cruisers (active seekers for host). It seems that nematode juveniles during the ambushing state depend mainly upon carbohydrates as energy reserves because they, actually, stay motionless, waiting for passing by hosts. When they change their searching strategy to be cruisers, where richer sources of energy are needed, they begin to utilize their lipid content. The types of nematodes differ in their energy reserves and their biological activity, such as reproduction and penetration rate, virulence and survival alive in the soil until they find the host. The nematode, which is characterized by high levels of these properties is good nematodes for biological control, if it had some deficiencies in one of these properties, it could be enhanced and an average increase as was did by (El-lakwah *et al*., 2008),who introduced progenies of nematodes have a better ability to penetrate and virulence than their parents. Therefore, we hope in the future to offer some of these types of nematodes on one method for improvement.

REFERENCE

Abbott, W.S. (1925). A method for computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

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

Abu Hatab, M. A. and Gaugler, R. and Ehlers, R.U. (1998)**.**Influence of culture method on *Steinernema glaseri* lipids. J. of Parasitol. 84: 215-221.

Akhurst, R.J. (1995). Bacterial symbionts of entomopathogenic nematodes-the power behind the throne. In:Bedding, R., Akhurst, R. and Kaya, H.(eds) Nematodes and the Biological control of Insect Pests. CSIRO Publications, East Melboune, Australia, pp.127-135.

Behm, C.A. (1997). The role of trehalose in the physiology of nematodes. Int. J. Parasitol., 27: 215-229.

Bradford, M.N. (1976).A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle protein-dye binding. Annal. Biochem., 72: 248-54.

Colman, A.M. (2001). Duncan's multiple range test. Dictionary of Psychology. Publ Oxford University.

Dubois, M., Giles, K. A., Hamilton, J. A., Rebers, P. A., and Red, S. (1956): Colometric method for determination of sugars and related substances. Anal. Chem., 28 (3): 350-356.

EL-Assal, F.M., El-lakwah S.F., Hasheesh, W.S. and EL-mahdi, M.M. (2008). Effect of the change in energy reserves on the entomopathogenic Nematode efficacy. J Egypt .Soc. Parasitol., 38(3):929-944.

El-lakwah, S.F., EL-Assal, F.M. and EL-mahdi, M.M. (2008). Novel methods for enhancing Entomopathogenic Nematode efficacy.Egypt.J.Agric.Res., 86(1):185-197.

Fitters, P.F.L., Patel, M.N., Griffin, C.T., Wright, D.J. (1999): Fatty acid composition of *Heterorhabditis sp*. during storage. Comp. Biochem. Physiol. 124: B: 81-88.

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

Gaugler, R. (1988). Ecological consideration in the biological control of soil-inhabiting insect pests with entomopathogenic nematodes. Agric. Ecosys. Environ., 24: 351-360.

Gerritsen, L.J.M. and Smits, P.H. (1994).Pathogenicity of new combinations of *Heterorhabditis spp*. and *Photohabdus luminescens (Xenorhabdus luminescens*) against *Galleria mellonella* and *Tipula olercea*. Bull. Olib. Srop., 17: 56-61.

Grewal, P. and Georgis, R. (1995). Nematode quality Abstracts of the Second International Symposium on Entomopathogenic Nematodes and their Symbiotic Bacteria, October 15-17, Honolulu, Hawaii, USA.

Hom, A. (1994). Current status of entomopathogenic nematodes. The IPM Practitionerer 16: 1-12.

Knight, J.A.; Anderson, and Rode, J. A. (1972). Chemical basis of sulfuric-phosphovanillin reaction for

estimation of total lipids. Clin. Chem., 18: 199-202.

Koppenhöfer, A. M. and Kaya H. K. (1999): Ecological Characterization of Steinernema rarum.Journal of Invertebrate Parasitology 73:120-128.

Lewis, E.E., Campbell, J.F. and Gaugler, R. (1997). The effects of aging on the foraging behaviour of *Steinernema carpocapsae* (Rhabditida: Steinernematidae). Nematologica, 43: 355-362.

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

Nguyen, K.B.(2005). Morphology and taxonomy of entomopathogenic nematodes. Available from http://Kbn.ifas.Ufl.edu/kbnstein.htm. (Accessed November16, 2005).

Nguyen, K.B., Ginarte, C.M.A., Leite, L.G., dos Santos, J.M. and Harakava, R. (2010). *Steinernema brazilense* n. sp (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Mato Grosso, Brazil. Journal of Invertebrate Pathology. 103: 8-20.

Nguyen,K.B., Malan,A.P. and Gozel, U.(2006): *Steinernema Khoisanae n. sp.* (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa, Nematology, 8(2):157-175.

Nicholas, W. L. (1984): The biology of free-living nematodes, 2nd ed. Clarendon Press, Oxford, U.K., 251 p.

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.

Phan, L. K., Subbotin, S.A., Waeyenberge, L., Moens, M. (2005). A new entomopathogenic nematode, *Steinernema robustispiculum* n. sp. (Rhabditida: Steinernematidae), from Chumomray National Park in Vietnam. Syst Parasitol. Jan; 60(1):23-32.

Poinar, G. O., JR. (1990). Biology and taxonomy of Steinernematidae and Heterorhabditidae. In Entomopathogenic nematodes in biological control,R. Gauglar and H. K. Kaya (eds.). CRC Press, Boca Raton, Florida, p.23-62.

Qiu, L.H. and Bedding, R.A. (2000). Energy metabolism and its relation to survival and infectivity of infective juveniles of *Steinernema carpocapsae* under aerobic conditions. Nematology, 2: 551-559.

Qiu, L. H., Lacey, M.J. and Bedding, R.A. (2000). Using deuterium as an isotopic tracer to study the energy metabolism of infective juveniles of *Steinernema carpocapsae* under aerobic conditions. Comp. Biochem. Physiol., 127(B):279-288.

Qiu, L.; Yan, X.; Zhou, Y.; Nguyen, K.B. and Pang, Y. (2005). *Steinernema aciari* sp. n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from Guangdong, China. J Invertebr Pathol. Jan; 88(1): 58-69.

Selvan, S.; Gaugler, R. and Lewis, E. E. (1993). Biochemical energy reserves of entompathogenic nematodes. J. Parasitol.79: 167-172.

Simoes, N.; Laumond, C. and Bonifassi, E. (1993). Effectiveness of *Steinernema sp*. and *Heterorhabditis bacteriophora* against *Popillia japonica* in the Azores. J. Nematol, 25: 480-485.

Snedecor, G.W. (1956). Statistical Methods. 5th Ed. Iowa State University College Press, Ames, Iowa, USA..

Uribe- Lorio, L.; Mora, M. and Stock, S.P. (2007). *Steinernema Costariense* n. sp. and *S. puntaurense* n.sp.(Rhabditida: Steinematidae). two new entomopathogenic nematodes from *costa Rica*. J. systematic parasitology, 68(3):167-182.

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

Zhang, C.; Liu, J.; Xu, M.; Sun, J,.; Yang, S.; An X.; Gao G.; Lin M.; Lai R.; He Z.; Wu Y. and Zhang K. (2008). *Heterorhabdits chongmingensis* gen. nov., sp. nov. (Rhabditida: Rhabditidae), a novel member of the entomopathogenic nematodes. J Invertebr Pathol. Jun; 98(2):153-68.

ARABIC SUMMARY

دراسات فسيولوجية وبيولوجية لبعض أنواع النيماتودا الممرضة للحشرات من عائلتي (STEINERNEMATIDAE, HETRORABDITDAE)

سهير فيصل اللقوة – أحمد محمد عزازى
معهد بحوث وقاية النباتات – مركز البحوث الزراعية

يتم استخدام النيماتودا الممرضة للحشرات من أجناس Steinernema و Heterorhabditis فى المكافحة البيولوجية في الزراعة. العديد من الأنواع الجديدة يتم وصفها ولكن القليل من المعلومات البيئية أو الفسيولوجية تتوفر لدينا. لذلك يقدم هذا البحث دراسة القدره على القتل ، ومعدل الاختراق ، وومعدل التكاثر، وبعض مخزونات الطاقة (الدهون الكلية ، والبروتينات والكربوهيدرات) لستة أنواع من النيماتودا الممرضة للحشرات ثلاثه من الجنس Heterorhabditidsوهى (HP2)، (HP4)، (*H. indicus*) و3 أنواع من الجنس Steinernema وهى (S3) ، (*S. riobrave*، (*S.rarum*) تم استخراجها من التربة في بلدان وأماكن مختلفه . ترشح لنا الدراسه افضل الانواع من النيماتوده التى درسنها و الاقدر على التكيف مع الظروف البيئية غير الملائمه لها.

اوضحت الدراسه ان الانواع التى اختبرناها تختلف فى قدرتها على الاختراق ليرقه دودة الشمع الكبرى *G mellonella* ،حيث سجلت النيماتودا من النوع (HP2) أعلى معدل اختراق وكانت نسبته (56%) وأعلى نسبة للدهون الكلية (35.8 %) ، والبروتينات (60.3 %) وأعلى قدرة على القتل ليرقه دودة الشمع (25%). بينما سجلت اعلى نسبه للكربوهيدرات (26%)*فى Steinernema rarum* في حين سجل النوع *Heterorhabditis indicus* أعلى معدل للتكاثر وهو 149914 طور معدى/يرقه).