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Role of the lipids on migration rate and infectivity of nematodes.

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ABSTRACT

The present study was recorded the penetration rate, the migration% and the distance covered by the species [(*Steinernema riobrave, Steinernema sp. (S3), Steinernema rarum, Heterorhabditids sp. (Hp2), Heterorhabditids sp. (Hp4) and Heterorhabditids indica*)]. In addition, the relation between these activities and the lipid contents of each species were revealed. The authors found that, there are considerable variations in migration rate between entomopathogenic nematodes species and the migration is not affected by the original amount of lipid content in the species. Species that have high lipid contents don't show high migration rate but the infectivity was affected by the amount of lipid contents where the species contain high lipid contents recorded high penetration rate of *Galleria mellonella.*

Keywords:- Entomopathogenic nematodes, Steinernematidae, Heterorhabditidae, Infective juveniles, Migration rate, Lipid contents, Penetration rate.

INTRODUCTION

Entomopathogenic nematodes comprise two families, the Steinernematidae and Heterorhabditidae, that are not closely related phylogenetically but which share similar life histories through convergent evolution (Poinar, 1993). Nematodes have qualities that make them potentially suited to become a major factor in the control of soil insects, they actively disperse through soil, they seek out their hosts and they can be mass produced and commercialized *(Beekman et al., 1994).* Entomopathogenic nematodes associated with Xenorhabdus and Photorhabditis spp. Bacteria, lethal pathogens of soil-inhabiting insects. Nematode infective juveniles occur naturally in the soil where they infect and kill their insect host with in 2 or 3 days and produce 2 or 3 generations in the host. Resulting infective juveniles emerge from host cadaver 1or 2 week later and search for new insect hosts (Akhurst, 1995).

The infective juveniles (IJs) of entomopathogenic nematodes (EPN) are non-feeding, free-living in soil and depend solely on reserve materials for energy supply (Qiu and Bedding1999*)*. The survival and infectivity of the non- feeding larva of some plant and animal parasitic nematodes is correlated to the levels of energy reserve materials (Elliot, 1954). There is little information concerning the energy metabolism and its relation to survival and infectivity of EPN (Selevan et al., 1993). Successful establishment of nematodes in diverse environments depends upon physiological, behavioral and biochemical adaptations (Nicholas, 1984). These nematodes are faced with a wide array of environmental conditions during the non-feeding infective stage. Migration and host-finding ability are essential processes in their success as biological control agents. The present study yields new information on the relation between lipids and the migration of the entomopathogenic nematodes and highlights the difference between Steinernematidae and Heterorhabitidae and their species in the migration rate.

MATERIALS AND METHODS

Six entomopathogenic nematode species were used in the present study, three belong to the family *Heterorhabditidae* and three to the family *Steinernematidae*. The six species were obtained from regular culture in the Laboratory of Insect Parasitic Nematodes, Plant Protection Research Institute, Agriculture Research Centre, 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 and stereomicroscope.

Steinernema riobrave, Steinernema sp. (S3), Steinernema rarum, Heterorhabditids sp. (Hp2), Heterorhabditids sp. (Hp4) and *Heterorhabditids indica,* were cultured in larvae of the wax moth (*Galleria mellonella*) at 25 °C according to Woodring and Kaya (1988**)**. Upon collection, IJs were rinsed in deionised water, three times and concentrated to approximately 5000 IJs/ml, and only fresh IJs were used in all the following experiments.

Penetration Rate:-

Galleria mellonella larvae were exposed to freshly emerged nematode juveniles, at a dose level of 20 IJs/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. After 4-5 days of the infection, according to the species of nematode, at least 10 dead 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 mean of the penetration was calculated.

Dispersal and migration of nematode species.

This investigation was carried out on newly harvested nematode infective juveniles (IJs) of the six tested nematode species. The column assay (Grewal *et al*., 1994) was used, plastic tube (25 cm in length and 4cm in width), was filled with sandy soil (10% w/w) and divided into five equal sections (5 cm length for each) Figure (1). Full grown larvae of *G.mellonella* were used as bait and kept inside a wire screen cage (1 mm whole size) filled with moist sand, and placed at one end of this plastic tube (B). The prepared tubes were incubated at 25±1°C in the dark, for 24 hours to allow equilibration of any diffuses from the insects throughout the sand column before applying the nematodes. Three thousand IJs in 3 ml distilled water were, applied to the application site of each column and incubated at 25±1°C for 24 hours. After incubation period every sand soil in each section was transferred to a separate petri-dish containing four *G. mellonella* larvae and dishes were incubated at the same temperature. Mortality percent was recorded, dead larvae were dissected in the end of the 5th day after infection and numbers of adult nematodes were recorded, and migration % was calculated and the average migration (distance, cm) was determined by the following equation:

$$\text{Average migration (distance)} = \frac{R1\times 0 + R2\times 5 + R3\times 10 + R4\times 15 + R5\times 20}{R1+R2+R3+R4+R5}$$

Where R1 (point of inoculation), R2, R3, R4 and R5 are the total number of nematodes recovered from ring 1, ring 2, ring 3, ring 4 and ring 5, respectively (Chen *et al*., 2003).

Determination of Total Lipids.

Freshly emerged infection juveniles from each of the six tested nematodes were washed three times and concentrated to approximately 5000 IJs/ml. Lipid content was determined in these nematodes. Nematode juveniles were washed and incubated in 15 ml of 80% ethanol at 75°C for 5 minutes to deactivate 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), was used for the determination of Lipids and measured with spectrophotometer at 525 nm against a blank.

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.

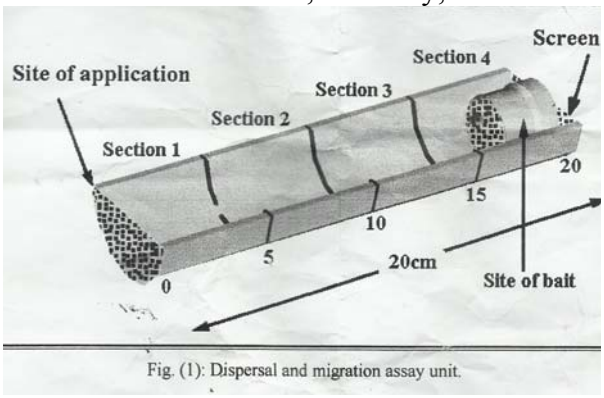


Fig. (1): Dispersal and migration assay unit.

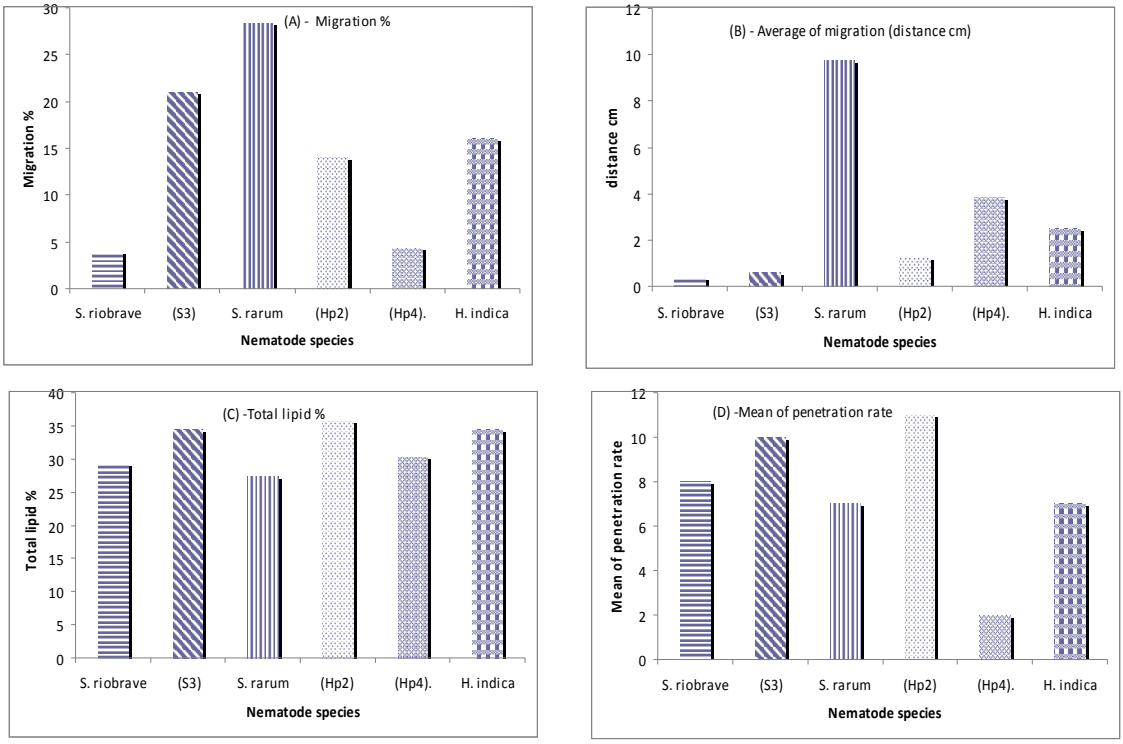
RESULTS

Data in Figure (2,A) represented the migration % of the species *Steinernema riobrave, Steinernema sp. (S3), Steinernema rarum, Heterorhabditids sp. (Hp2), Heterorhabditids sp. (Hp4)* and *Heterorhabditids indica* were 4, 21, 28.33, 14, 4.33, 16%, respectively, as we see *Steinernema rarum* recorded high migration % and the lowest was recorded by *Steinernema riobrave.* As shown in figure (2,B), the average of migration (distance cm.) covered by these species were 0.41, 0.63, 9.72, 1.19, 3.84, 2.5 cm, respectively. We found that 28.33% of the *S. rarum* migrated 9.72cm toward the *G. mellonella* (the host) and only 4% of *S. riobrave* migrated (0.41 cm) toward *G. mellonella*. In the *Heterorhabditis* Species, we noticed that, 16% of *H. indica* migrated 2.5cm, while only 4.33% of *Hp4.*migrated 3.84cm. toward the *G. mellonella*.

Results in figure (2,C) represented the percentage of total lipid for the tested species *S. riobrave, (S3), S. rarum, Hp2, Hp4* and *H. indica* were 29.19, 34.34, 27.30, 35.79, 30.36, 34.49 %, respectively. The *S. rarum* recorded the lowest total lipid percentage 27.3 %, while it covered longest distance and highest migration percentage. Where the *S. riobrave* covered shortest distance and lowest migration%, it

recorded 29.19% of total lipid content. In comparison between the *Heterorhabditis* species, the species (Hp4) covered longest distance 3.84cm and lowest migration% 4.33%, although it recorded lowest total lipids content 30.36 %.

Regarding the mean of penetration rate, presented in figure (2,D), through the *Heterorhabditis* species the highest penetration rate (11 IJs) was recorded by (Hp2) and the lowest penetration rate (2 IJs) was recorded by (Hp4). By comparing between the *Steinernema* species, the *Steinernema sp. (S3)* recorded high penetration rate (10IJs) and *S. rarum* recorded the lowest penetration rate (7 IJs). In the six tested species the species have high total lipid percentage showed high mean of penetration rate, so there is directly relationship between the mean of penetration rate and the percentage of total lipids.



Fig(2): (A)-migration% (B)- average migration (The distance cm), (C)-mean of penetration rate and (D)-total lipid% 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; $P<0.05$).

DISCUSSION

Entomopathogenic nematodes migration is an advantage for both survival and host seeking. Quick migration of nematodes into the soil is essential to escape from solar radiation and desiccation (Gaugler, 1988). Nematodes forage for hosts in several ways, but all of them involve some kind of activity, so they do not necessarily stay where they are applied. Campbell *et al.,* 1996 and Ferguson *et al.,* 1995 found that *H. bacteriophora* strains to be more evenly distributed vertically than either of the *Steinernema* species tested. Whereas, Georgis and Poinar, 1983; Schroeder and Beavers, 1987 established that *S. glaseri* and *H. bacteriophora* move primarily upwards, but also move throughout the soil column. Azazy (2001) showed that the presence of *G. mellonella* as a host, increase the migration of the IJs of *S.c* (all) and

S.c (agriotis) to (29.85 and 21.45%), respectively. and the IJs of Hp88 was found to move an average net longer of 13.07cm. than the other tested strains ranged between 3.23 to 12.62cm. As we see in our result, the distance ranges between 0.41 to 9.72 cm and the migration rate range between 4 to 28.33%. Also all the isolates which were tested by El-Assal *et al.,* (1997) could kill the host larvae up to 10 cm within 48 hrs. and can extended to 15 cm within 72 hrs. by the two tested species. Miduturi (1997) showed that *S. feltiae* and *S. affine* are able to migrate over 10 cm within a period of 3 to 7 days; the presence of a host positively affecting their migration. Where Gaugler *et al.,* (1991) mentioned that a wild strain of *S. carpocapsae* could not locate *Galleria* larvae placed farther than 3.5 cm.

Our results illustrated that there are variations in the mobile distances and the migration rates between *Heterorhabditis* and *Steinernema* species and with in the same genus. This is in agreement with Azazy (2001) who found that the dispersal and migration differ among all tested strains. All nematodes that emerge from a single cadaver are not necessarily the same (Lewis and Gaugler, 1994; Stuart *et al.,* 1996) with respect to behavior (Lewis, 2002). The same author also mentioned that differences in infective juvenile movement and infection rate are related to the presence or absence of the host cadaver. Variability of results obtained concerning persistence, dispersal and infection in soil has often been attributed to insufficient soil moisture (Kain *et al.,* 1981). In the present study, we avoided that by placing the host at the end point as bait, using IJs freshly emerged from the cadaver and keep the soil in each treatment moist during the experiment, thus the variability in the obtained results was mainly due to the differences among the tested species. This variability may be due to the depletion rate of endogenous lipid reserves in the infective juveniles which may differ among the different species (Vänninen, 1990).

Lewis *et al.,* (1995) have mentioned that *S. carpocapsae, S. glaseri* and *H, bacteriophora* became less mobile with age. Also, Vänninen (1990) found that as nematode juveniles grow older, their ability to migrate, locate a host and penetrate it significantly decreased. The shelf-life of entomopathogenic nematodes has been found to correlate with the relative abundance of stored lipids per dry weight (Bedding *et al.,* 1993; Grewal and Georgis. 1995). Lipids are ideal compounds for long term energy storage, due to their high energy content (Stryer, 1988). In the present investigation, the total lipids for the six species (*Steinernema riobrave, Steinernema sp. (S3), Steinernema rarum, Heterorhabditids sp. (Hp2), Heterorhabditids sp. (Hp4)* and *Heterorhabditids indica,*) varied significantly from 27.30% to 35.79% and the highest total lipid (35.79%) percentage was recorded for the *Heterorhabditis sp*. (HP2). Fitters *et al.,* (1999) found that lipids represented 34-43% of the dry weight of three isolates of *Heterorhabditis sp*. where total lipids percentage was 36-40% in another strain of the same species tested by Lewis *et al*., (1995), our result investigated the total lipid percentage ranged from 27.30 to 35.72%. Selvan *et al.,* (1993) have recorded that chemical composition of inflictive juvenile entomopathogenic nematodes varied between species. As so we have found that there are considerable variations in migration rate between entomopathogenic nematodes species and this rate of migration is not related to the amount of lipids in the species, in another meaning, nematode species have high amount of lipids must not show high rates of migration. Although we found that infectivity is related to the amount of lipids in the species, i.e., the species with high amount of lipids recorded high penetration to the *G. mellonella*. So we state here that migration rate of the species of entomopathogenic nematode species is not related to the lipid content but it may be

affected by the lipid content decline sharply with age than its natural amount in the same species.

These results suggest that infection strategies are likely to be much more complicated than previously known, and that each species may have a strategy that is unique. Within the species belong to *Heterorhabditis* and *Steinernema,* there is considerable variation in foraging strategies. The foraging strategies used by infective juveniles to find a host vary along a continuum between ambush and cruise foraging (Lewis *et al,* 1992, 1993; Campbell and Gaugler, 1993, 1997; Grewal *et al.,* 1994). Nematode infective juveniles are motile, and their search behavior can be divided into two broad categories, crawling, and standing on their tails (i.e. nictation) (Campbell and Gaugler, l997). Understanding the mechanics of foraging behaviors is the key to constructing predictions of how foraging strategy influences nematode biology and the mobile distance that they move. Cruiser species, such as *H. megidis*, directionally respond to host cues and can travel for long distances. Ambushers, however, lack any directional response to host cues and are less mobile. (Chen *et al,* 2003). *S. carpocapsae* and *S. scapterisci* for example, spend most of their time in prolonged bouts of motionless nictation which may last several hours, which is typical of ambushing species. Many *Steinernema* species exhibit jumping behavior, (Campbell and Gaugler, 1993). To jump, nematodes form a loop with their bodies, by contracting the loop, the nematode generates enough stored energy that when the loop is released, they are propelled through the air (Campbell and Kaya, 1999a, b). The frequency of jumping, like standing behaviour, varies among species, and is increased by mechanical contact, air movement, and volatile host cues (Campbell and Kaya, 2000). Lewis *et al.,* (1997) have mentioned that, *Steinernema* species change their foraging strategy for infecting a host, from ambushers to cruisers. During the ambushing state the nematode juveniles depend mainly upon carbohydrates as energy reserves because they stay motionless. When they change their searching strategy to be cruisers, they begin to utilize their lipid content. The present study shows that both the migration percentage and the distance traveled by entomopathogenic nematodes not related to the original amount of lipid contents but maybe related to the behavior or to the inherited traits in each species.

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ARABIC SUMMARY

دور الدهون على معدل هجرة وعدوى النيماتودا الممرضة للحشرات

احمد محمد عزازى، سهير فيصل اللقوة*، هبه يوسف
قسم فسيولوجيا الافات – معهد بحوث وقايه النباتات-مركز البحوث الزراعيه –الدقى- الجيزة

لقد تمت دراسة معدل الاختراق، ونسبه الهجرة والمسافة التي تغطيها الأنواع (*Steinernema riobrave, Steinernema sp. (S3), Steinernema rarum, Heterorhabditids sp. (Hp2), Heterorhabditids sp. (Hp4) and Heterorhabditids indica*) والعلاقة بين هذه الأنشطة والمحتوى الدهني لهذه الأنواع. وقد وجد أن هناك تباين كبير في معدل الهجرة بين انواع النيماتودا الممرضة للحشرات ولم تتأثر الهجرة بالكم الأصلي لمحتوى الدهون في الأنواع التى تم دراستها،حيث وجد ان الأنواع التى تحتوى على نسبة عالية من الدهون لم تسجل ارتفاع فى معدل الهجرة ولكن تأثرت العدوى بمقدار المحتوى الدهني حيث الأنواع التى تحتوي على نسبة عالية من الدهون سجلت اختراق عالى لدودة الشمع الكبرى.