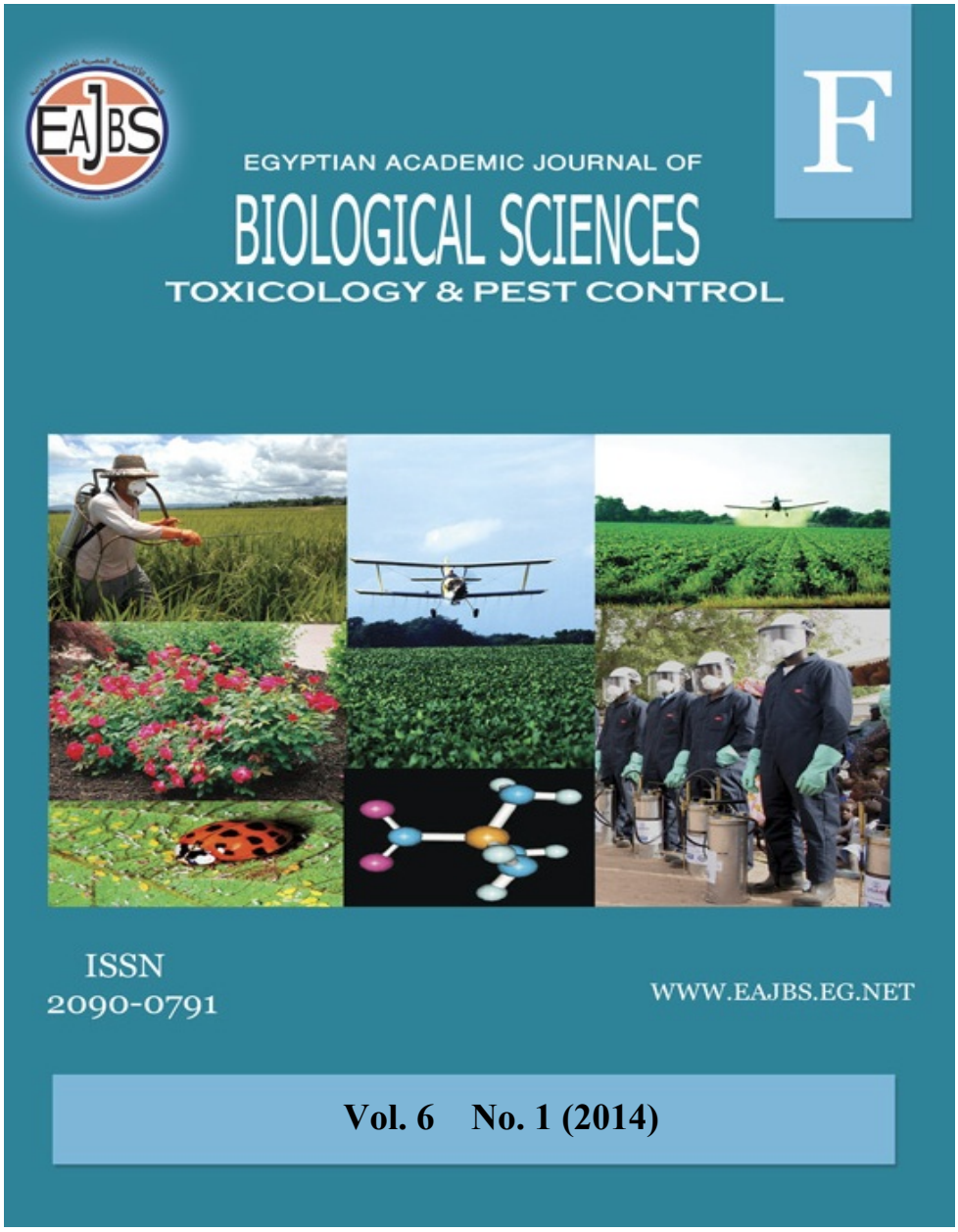

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Impact of some factors on the migration rate and the dispersal of entomopathogenic nematodes

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Migration rate
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ABSTRACT

The migration and the dispersal of five entomopathogenic nematode infective stages had been studied in column of sandy soil under laboratory condition. It was found that it differ according to some factors, these factors included comparison between the effect presence or absence of the insect host *Gallaria mellonella* and *Spodoptera littoralis*, insect feces, the nematicide (Nemacur), and host species. The nematicides improved migration and average net distance of all tested nematode strains, except the *Steinernema glaseri* strain, with which a remarkable inhibition in its mobility, was obviously, observed. Feces of *S. littoralis* increased the migration rate and the dispersal than the host itself. Most nematode strains were attracted to *S. littoralis* more than to *G. mellonella* larvae.

INTRODUCTION

Entomopathogenic nematodes (EPNs) belonging to the families Heterorhabditidae and Steinernematidae are considered excellent biocontrol organisms against numerous insect pests worldwide (Georgis *et al*., 2006). They are, symbiotically associated with bacteria of genera Photorhabdus and Xenorhabdus, respectively (Akhurst, 1993). The bacteria are carried and maintained by the EPN free-living stage, the infective juvenile (IJ) (Sugar *et al*., 2012). Once the nematodes locate the insect, they actively penetrate the body cavity and release the bacteria in the hemocoel thereby killing the insect, generally in a short time (Boemare, 2002). The digested insect tissues serve as medium for the nematode and bacterial development, and several generations are produced inside the cadaver. When the resources are depleted and excretion products become limiting, a new cohort of IJs is developed, which acquire bacteria and emerge in search of new hosts (Adams and Nguyen, 2002). The majorities of individuals of *S. carpocapsae* have a sit and wait (ambusher) strategy and tends to be near the soil surface this species was effective against larvae of *A. ipsilon,* which feeds near or at the soil surface, while *H. bacteriophora* has an active foraging (cruiser) strategy and occurs deeper in the soil profile.

It was effective against larvae of Otiorhynchus sulcatus, which occurs near roots (Kaya & Gauglar, 1993). 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. So, the aim of the work to study many factors affecting dispersal and migration of five nematode infective stages (IJs) such as presence or absence of the insect host *Gallaria mellonella* and *Spodoptera littoralis,* insect feces, the nematicide (Nemacur), and host species.

MATERIALS AND METHODS

Nematodes

Five entomopathogenic nematode species were used in the present study; two belong to the family *Heterorhabditidae* and three to the family *Steinernematidae*. The five species were obtained from regular culture in the Laboratory of Insect Parasitic Nematodes, Plant Protection Research Institute, Agriculture Research Centre, Egypt. The entomopathogenic nematode strains, *S. carpocapsae* (All) (S. c. (All)), *S. glaseri* (S. g.), *S. carpocapsae agroitis* (S. c. a), *H. bacteriophora* (HP88) imported from Florida, USA and *H. taysearae* (Ht) (from Giza) by Shamseldean *et al*., 1996.

The greater wax most, *Galleria mellonella.*

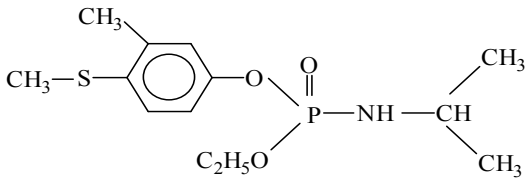
Mass rearing of *G. mellonella* larvae used in the present study was initiated from specimens of bees, heavily infested with the insect which was collected from the apiary of Plant Protection Research Institute, Agricultural Research Center, Dokki, and Giza.

The cotton leafworm, *Spodoptera littoralis*:

Some pupae were obtained from the Department of cotton Insect pests, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza.

Toxic effect of Fenamiphos (Nemacur) on nematodes:

Fenamiphos (Nemacur) 40% EC, as a nematicide



O-ethyl-O-(3-methyl-4-methyl thiophenyl)-N-isopropyl phosphoroamidate.

HP88 and Ht were used in this study. Six fold serial dilutions of (25, 50,100,200,400 and 800ppm) of Fenamiphos were prepared from the stock solution of the formulated pesticide and distilled water. Ten ml of each chemical dilution was placed in a petri dish. Nematodes were placed into dilutions at a rate of 2000 IJs per dish. Similarly the control contained 2000 IJs but maintained in only distilled water. The treatments were tri-replicated and kept at 25°C. Nematode mortality was calculated once after 48h. and were classified to "not responding to mechanical stimulation" or alive (i.e. moving in response to mechanical stimulation or actively moving). Data was adjusted according Abbott's formula.

Dispersal and migration rate of nematode species:

Such course of investigation was carried out on newly harvested nematode infective juveniles (IJs). According to the foraging strategy of the tested nematode species, the column assay (Azazy, 2001) was used, Plastic tube (20 cm in length and 4 cm in width), was filled with sandy soil (10% w/w) and divided into four equal sections (5 cm length for each) Fig.1.

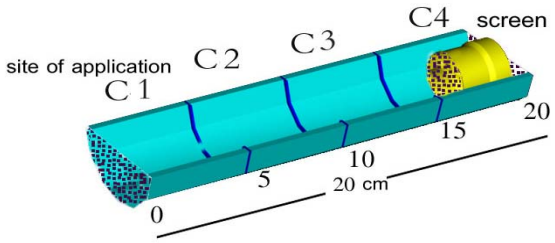


Fig. 1: Dispersal and migration assay unite.

Full grown larvae of *G. mellonella* or 6th instar larvae of *S. littoralis*, or feces of *S. littoralis* were used as a bait and kept inside a wire screen cage (1mm hole size) filled with moist sand, and placed at one end of this plastic tube (B). The prepared tubes were incubated at 25±1ºC in the dark, for 24h to allow equilibration of any diffuses from the insects and feces through the sand column before applying the nematodes. Six thousand IJs in 3 ml distilled water were, applied to the site of application of each column and incubated at 25±1ºC for 24h. After incubation period every sand soil of each section was transferred to a separate petri dish, containing four *G. mellomella* or *S. littoralis* 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. Nematode dispersal and migration rate were quantified by:

a) The migration rate "%m"; the percentage of IJs recovered outside the site of application.

b) The average net distance E (D) traveled by the IJs outside the site of application.

E (D) is calculated according to the following formula:

$$\mathbf{E\ (D)} = \frac{\sum_{i=1}^{5} n_i x_i}{\mathbf{N}}$$

Where *i* is one of five sand sections; *ni* is the number of IJs recovered in C1+C2+C3+C4+C5; xi is the distance between C1and C2 (in the center) of each section and N is the total number of nematodes recovered in the assay unit. Nematodes found in given sections, were expected to have moved in that section half of the corresponding length indicated in the figure. E (D) can range from 0 to 20 cm.

Statistical analysis:

Statistical analysis of data was carried out using a computer software package "costat", a product of Cohort Software Inc., Berkeley, California, USA. Duncan's multiple range test (Duncan, 1955) was used to differentiate between means.

RESULTS

Some factors affecting dispersal and migration of five nematode infective stages (IJs) had been studied in laboratory in column of sandy soil. These factors included the presence or absence of the insect host, host species, insect feces and the nematicide (Nemacur).

First, authors studied the toxic effect of nematicide (Nemacur) against entomopathogenic nematodes. HP88 and Ht were used as an example.

Toxic effect of Fenamiphos (Nemacur) on nematodes:

The toxicity effect of nematicide (Fenamiphos) (Nemacur) on Ht and HP88 infective stages during 48h of exposure were shown in Table (1). Results showed that, the Ht mortality, after 48h exposure to Fenamiphos ranged between 3.3 to 15.7%. Fenamiphos gave mortalities of 4.7, 3.3, 3.8, 8.5, 7.3 and 15.7% for concentrations of (25, 50,100,200,400 and 800 ppm), respectively.

Generally the average mortality was 7.3% of Fenamiphos. Statistical analysis revealed that there were no significant differences between the tested concentrations of pesticide. These pesticide achieved *H. bacteriophora* (HP88) mortality ranged between 1.1 and 6.8, successively. Statistical analysis proved that Fenamiophos were no significant differences between its concentrations.

Table 1: Mortality percent of the infective stages of *H. bacteriophora* (HP88) and *H. taysearae* (Ht) after 48h exposure to Nematicides Fenamiphos (N.)

Nematicides (Fenamiphos) (N.)				
Concentration	(HP88)		(Ht)	
25 ppm	1.1±0.6	b	4.7±2.3	b
50 ppm	3.7±0.3	ab	3.3±0.4	b
100 ppm	3.8±0.6	ab	3.8±1.9	b
200 ppm	6.8±3.5	a	8.5±5.2	ab
400 ppm	5.4±0.9	ab	7.3±2.9	ab
800 ppm	6.5±1.7	ab	15.7±3.3	a
***L.S.D**	5.04		9.4	

One way ANOVA Completely Randomized

Main Effect	**df**	**F value**	**P**
Fenamiphos on (HP88)	5	1.6	.22 ns
Fenamiphos on (Ht)	5	2.33	.10 ns

***Dunca's multiple range test**

Values in the same column followed by the same letter are not significant different (P≤0.05)

1- Comparison between the presence and absence of insect host on the dispersal and migration of nematodes.

Twenty-four hours post nematode inoculation, majority of IJs moved away from the point of application in all tested nematode strains. The proportion of migration (%m) was not the same for the different nematode species. In case of host absence (Fig. 2: A & B), results showed that the strain Ht was the best (25.5% m), whereas S.c (All) was the lowest one (9.4m %). However,

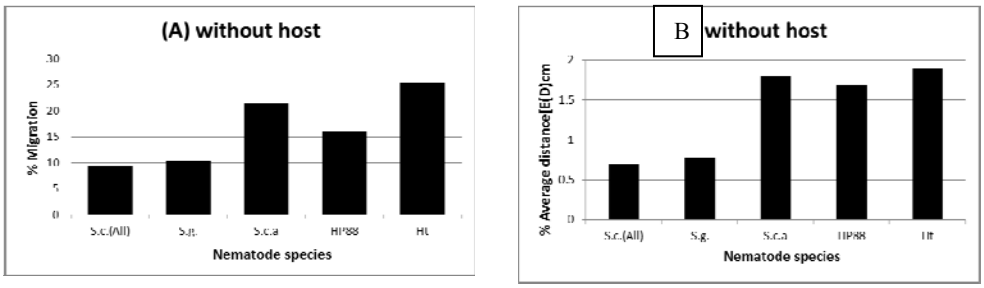


Fig. 2: The migration and dispersal of five nematode strains without hosts

In the case of host presence *G. mellonella* (Fig. 3: A), the migration of IJs of *S. g* was the highest recording 89.28%. The other strains achieved migration rates of 79.7, 76.48, 29.85 and 21.45% for Ht, HP88, S.c (All) and S.c.a, respectively. Regarding the "average net distance" [E (D)], in host presence *G. mellonella* (Fig.3: B) IJs of HP88 was found to move an "average net" longer than that of the other strains reaching the maximum distance of 13.07cm. The [E (D)] of the other tested strains were ranged between 3.23 to 12.62 cm.

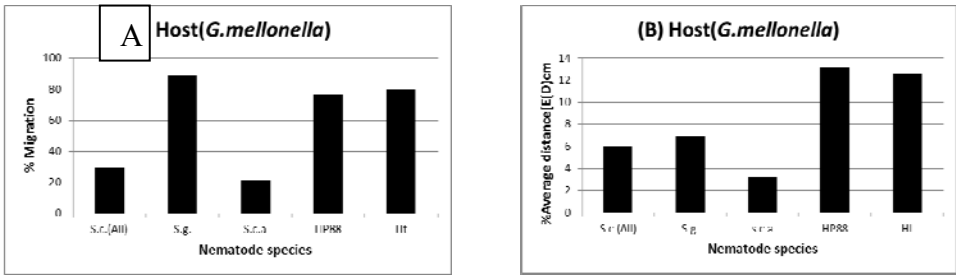


Fig. 3: The migration and dispersal of five nematode strains with *Galleria mellonella*

The presence of *S. littoralis* as bait increased the migration rates of all the tested strains (Fig.2.A and Fig.4: A). S. g showed the highest values (from 10.43 to 91.12%), followed by HP88 (from 16.1 to 80.06%), Ht (from 25.54 to34.2%), S. c. a (from21.5 to 27.3%), S.c(All) (from 9.38 to 22.14). Accordingly, as a result of the increase in % m, an increase in [E (D)] values was evident in all strains (Fig.4: .B).

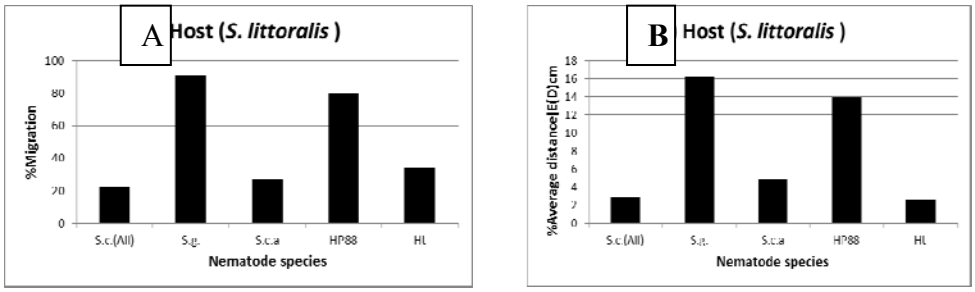


Fig. 4: The migration and dispersal of five nematode strains with host *S. littoralis*

2- Effect of Host species.

Results showed in (Figs. 3. A and 4. A), revealed that, EPNs varied in their attraction towards the two hosts. Two strains (Ht and S. c. (All)) were attracted more by *G. mellonella* larvae (79.7 and 29.9%m). While; the other three strains (S.g, S.c.a and HP88) were attracted by *S. littoralis* (91.12, 80.6 and 27.3 %m) more than *G. mellonella*.

The variations in the other strains were not too high between the two hosts as in the fore mentioned strains. Regarding E (D) values (Figs. 3: B & 4. B), it is clear that the strain S. g which was attracted by, *S. littoralis* was the superior (16.15cm) followed by the strain HP88 (13.98 cm), which against highest E (D) values (13.1cm) toward *G. mellonella* then Ht was (12.62cm) and the other two strains S.c (All) and S.c.a were the last for the both hosts.

3- Comparsion between the presence host and feces of *S. littoralis:*

As shown in Fig. (5. A), (%m) of all strains (except S. g), in presence of feces of *S. littoralis* were 83.64, 63.89, 52.3 and 42.39% for HP88, S.c.a, S.c (All) and Ht, respectively better than (%m) in presence of the host *S. littoralis* (Fig. 4.A). In contrasts S.g was the least response strain to *S. littoralis* feces

achieving 19.48% of (%m), against 91.12 % migration towards the host itself. In Fig. (5. B), the presence of feces increased values of E (D) of S.c (All), S.c.a and Ht (8.21, 10.14 and 3.96 cm, respectively), but those values were decreased, remarkably in the strains of S.g and HP88 than those of the host.

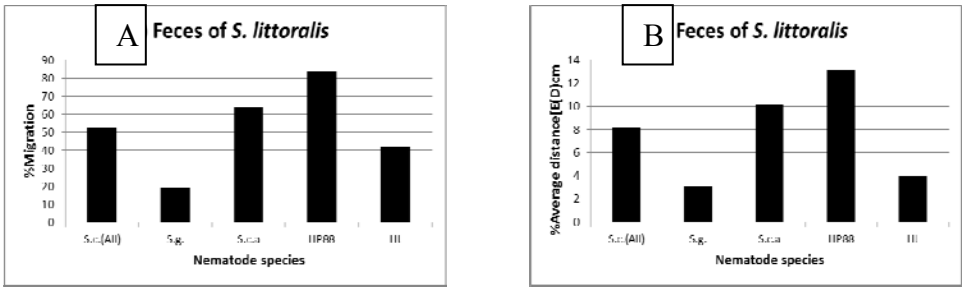


Fig.5: The migration and dispersal of five nematode strains with feces of *S. littoralis*

4-Effect of Nemacur on the dispersal and migration of nematodes:

Studying the effect of nematicide (Nemacur, 400 ppm) upon the migration of the tested strains (Fig. 6. A), compared with that of *G. mellonella* and *S. littoralis* host upon the migration of the tested strains (Figs.3.A and 4.A), it was found that the nematicide improved the performance of the nematode migration, especially in the case of HP88, Ht, S.c.a, S.c (All) showing 93.02, 81.13, 68 and 30.86%. On the other hand the nematicide caused clear inhibition in S. g migration (1.08%). Also, strains Ht, S.c.a and HP88 combined with Nemacur, moved longer distances [E (D)] of 10.16,11.9 and 17.15 cm, successively, while those of S.g and S.c (All)were the least, showing 0.1 and 7.6cm, respectively (Fig.6: B).

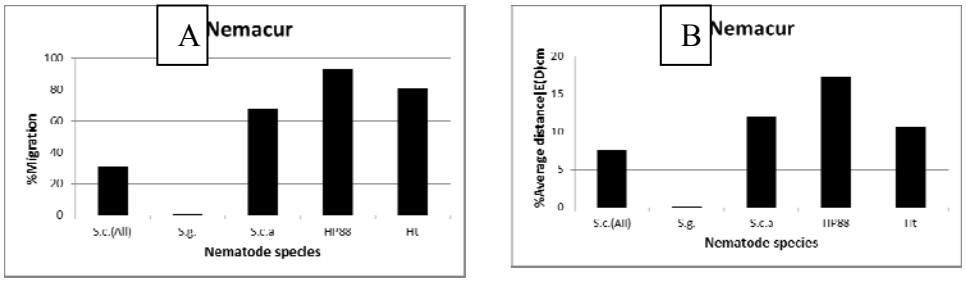


Fig. 6: The migration and dispersal of five nematode strains with Nemacur.

DISCUSSION

Entomopathogenic nematodes of the Heterorhabditidae and Steinernematidae appear to be capable of long- distance dispersal and local migration. Their transmission strategies include both, highly active seek- and destroy behaviors and ambusher strategies, and they may be sensitive to sex related factors in their own populations (Downes & Griffin, 1996). Although nematode dispersal is necessary for host –finding, they may disperse passively (Epsky *et al*., 1988; Timper *et al*., 1988), active dispersal, particularly by cruiser nematodes, appears to be the primary means for host finding, since it increases the likelihood of encountering sedentary host larvae. On the other hand, high mobility without sensitivity to host cues leads to quickly depleted food reserves (Molyneux, 1984 & Vänninen, 1990). Both locomotion and migration ability of the nematodes are thus critical factors in the control of sedentary insect soil. The sand column technique used in the present investigation enabled us to study the dispersal and migration of the tested nematode species, as well as some

factors that may affect them. Also, complying with the situation of sessile root pests in the filed, and host larvae in the assay unit were immobile (Fig.1), allowed the formation of chemical gradient around them (Steiner, 1996). From an ecological perspective, foraging strategies and motility are important variables in population dynamic models (Campos-Herrera *et al*., 2012). These variables are influenced by abiotic and biotic environmental factors (Lewis *et al*., 2006) and by intrinsic nematode traits (Campos-Herrera and Gutiérrez, 2014).

In the present investigation, it was found that dispersal and migration differ among the tested strains. These differences could be related to the inherited characters of each species, in addition to the reaction between these features and the other factors. Factors affecting active nematode dispersal and host finding in soil include small pore spaces (Blackshaw and Senthamizheselvan, 1991), moisture, temperature and plant roots (Choo *et al*., 1989). Finer particle sizes in soil inhibit nematode movement (Noosidum *et al*., 2010).

Active dispersal of entomopathogenic nematodes is short range and may be influenced by host cues. Host derived compounds such as Carbon dioxide (Co_2) (Gaugler *et al*., 1980) and fecal components (Schmidt and All, 1979) have been shown to be attractive to these nematodes. Migration rate may be directly related to the host finding strategy of tested nematode species. The low migration rate of *S. c.* (All) and *S. c .a*. in most experiments typical of an ambusher or a sit and wait strategy (Kaya & Gaugler 1993) and they tended to remain near the point of application limits contact with sedentary hosts (Georgis &Poinar, 1983a; Gaugler *et al*., 1989). As shown in our result the presence of the host increased the dispersal and migration of the tested nematode species. This finding is in a full agreement with Georgis & Poinar (1983b) who stated that the presence of the host increased dispersal of *H. bacteriophora*, but the majority were still found near the placement site. The pattern of dispersal and migration in *S.c.*a. and *S. g.* respectively were not affected by changing the host. Also the present results are in accordance with the finding of Grewal *et al*. (1995) who reported that entomopathogenic nematode species are different in their response to host chemical cues. 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).The frequency of jumping, like standing behavior, varies among species, and is increased by mechanical contact, air movement, and volatile host cues (Campbell and Kaya, 2000).

The present work proved that the nematicide (Nemacur) is an important factor affecting migration and dispersal of nematode species. This finding agrees with the previous studies, since Ishibashi and Shingi, 1993 stated that the insecticides stimulated the entomopathogenic nematodes to move actively. On other hand (Kamionek 1979) reported that the compatibility between some herbicides and insecticides can diminish host seeking ability and diminish reproductive potential without causing significant mortality in exposed nematode populations. The nematicides

can, probably be used at concentrations of no more than 800 ppm, but insecticides could be used at concentrations more than 1600 ppm with nematodes. Overall, results indicate the feasibility of an IPM use of these nematode species and chemical pesticides in crop protection (Rovesti & Doeso 1990). Further, it is possible that the movement/migration was not only modulated by host attraction but by more selected chemical attractions such as those reported by Choe *et al*., 2012 and Kaplan *et al*., 2012. *S. feltiae* was attracted to both insect and slug associated cues and its strong attraction to slug cadavers suggests that EPNs could also scavenge on carcasses other than those of insects (Nermut *et al.*, 2012).

S. littoralis feces attracted more portions of the tested nematode populations than the host it self and accordingly caused an increase in the average net distances except in the case of *S.g*. strain , since the host attracted the nematode more than feces . This finding confirmed by the work of Schmidt and All (1978, 1979). In the laboratories they found that *S. feltiae* positively responds various stimuli such as Co_2, thermal gradient, and excretory products in insect feces.

Comparing between *S. littoralis* larvae and *G. mellonella* larvae in attracting various nematode strains, it was found that *G. mellonella* attracted about 79.7%m of Ht population while 34.2%m were directed towards *S. littoralis* larvae. Also the infective stages of the strain S.c (All) preferred the same host with a lees degree. On the other hand the strains S.g, S.c.a and HP88, somewhat preferred larvae of *S. littoralis* than the other host. Klein-Beekman *et al*. (1994) found that dispersal of *S. g.* juveniles was enhanced in the presence of *Melolontha melolontha* larvae; clear response direction towards the host was not observed. Steiner (1996) reported that unidentified *Steinernema* species and *S. kraussei* exhibited negative migration when using *G. mellonella* larvae as a host. He suggested that *G. mellonella* was repellent to those nematodes. Also, he found that the host finding ability of a strain of *S. feltiae* was smaller for *M. melolontha* than for *G. mellonella*. He attributed this decrease; to that *M. melolontha* feces were repellent to the nematode juveniles.

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ARABIC SUMMERY

تأثير بعض العوامل على معدل الهجرة و الأنتشار للنيماتودا الممرضة للحشرات

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تم اختبار معدل الهجرة والأنتشار للأطوار المعدية لبعض أنواع النيماتودا في حالة وجود العائل أو عدم وجوده باستخدام عمود التربة الذي يبلغ طوله ٢٠سم في وجود يرقات دودةالشمع ودودة ورق القطن كطعم حشري،وذلك في التربة الرملية(١٠%رطوبة) ،وقد وجدت هناك اختلافات متنوعة في قدرة الأفراد المعدية لكل نوع و كانت السلالة ***S. glaseri*** الأعلى فى معدل الهجرة (٨٩,٢٨%) قاطعة مسافة مقدارها ٦.٩ سم خلال زمن قدره ٢٤ ساعة وذلك عند استخدام دودة الشمع كطعم، أما عند استخدام دودة ورق القطن كطعم فقد كان أعلى معدل للهجرة لها ٩١.١٢%وتم قطع مسافة ١٦,١٥سم خلا ل نفس الزمن.

و فى حالة عدم وجود عائل فقد كانت السلالة Ht الأعلى فى معدل الهجرة (٢٥.٥٤%) و قطعت مسافة (١.٩٢ سم) خلال نفس الزمن.

وبقياس القدرة على الانتشار والهجرة وجد أن السلالات المختبرة كانت أفضل فى حالة وجود المخلفات البرازية لدودة ورق القطن عن وجود العائل كطعم حشري وقد كانت النتائج كما هي : ٦٣.٨٩،٥٢.٣،٤٢.٣٩ ٨٣.٦٤ % للأنواع (HP88, S.c.a, , S.c(All), Ht) على الترتيب و معدل الأنتشار للأنواع , Ht ,S.c.a S.c (All), هو (٣.٩٦، ١٠.١٤، ٨٠٢١ سم) على الترتيب.

وكذلك وجد أن قدرة النيماتودا على الانتشار والهجرة كانت أفضل فى حالة استخدام دودة ورق القطن كطعم حشري عن استخدام دودة الشمع حيث كانت ٩١.١٣ ، ٨٠.٠٦، ٣٤.٢ % للأنواع S.g ، HP88 ، Htو المسافة التى قطعتها كانت هى ١٦.١٥ ، ١٣.٩٨ ، ٤.٩٣ للأنواع S.g ، HP88، S.c.a على الترتيب.