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FORAGING BEHAVIOR OF CERTAIN SPECIES OF HETERORHABDITID AND STEINERNEMATID NEMATODES FOR THE MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA* (WIEDEMANN), (DIPTERA: TEPHRITIDAE)

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ABSTARCT: Investigation on the foraging behavior of 5 different species of entomopathogenic nematodes against The Mediterranean fruit fly *Ceratitis capitata* (Weidemann) larvae were carried out for both horizontal and vertical distribution. *Steinernema carpocapsae, S. riobrave* and *S. feltiae* concentrated in 10 cm horizontal distance, while *S. glaseri* and *Heterorhabditis bacteriophora* were distributed in 30 cm distance. For vertical distribution *S. carpocapsae, S. riobrave and S, feltiae* condensed in 6 cm vertical depth while *S. glaseri* and *H. bacteriophora* distributed until 10 cm depth.

INTRODUCTION

Members of Family Tephritidae are considered as most noxious pests that invade the horticultural products. Different species of this family have been accidentally introduced into Egypt. Mediterranean fruit fly (MFF), *Ceratitis capitata* (Weidemann) was reported in Egypt early of the 19th century (Compere, 1912, Adair, 1920 and El-ghwabi, 1928) among 38 Egyptian trypanieds (Efflatoun, 1924). It causes a considerable damage and economic losses to different plant hosts (Awadallah *et al.*, 1974, Saafan, 1986, Saafan *et al.*, 1987 and Hashem *et al.*, 2001) which extend to be more than 350 plant hosts round the world (Liquido *et al.*, 1991) and its damage is increasing annually (Hafez *et al.*, 1973). Different methods of control have been used against this pest, but some of them have a negative effect against the ecosystem causing different hazardous effects.

Entomopathogenic nematodes (EPN) from the Steinernematidae and Heterorhabditidae are promising biological alternatives to chemical insecticides (Kaya, 1985, Poinar, 1986 and Ishibashi and Choi, 1991). These nematodes can penetrate and kill many economically important pests within 24-48 hours (Ishibashi and Takii, 1993), with the help of specific symbiotically associated bacteria (*Xenorhabdus* spp. for

Steinernema spp and *Photorhabdus luminscens* for *Heterorhabditis* spp.) (Akhurst and Boemare, 1990, Boemare *et al.*, 1993). The only endoparastic stage is the infective third-stage Juvenile (IJ). It emerges from the depleted cadaver into the soil environment where it may persist for months until it locates and infect a new host (Kung *et al.*, 1991). IJs of different Entomopathogenic nematode species differ in their ecological and behavioral traits. *Steinernema carpocapsae* dictates (Kondo and Ishibashi, 1986), tends to stay near the soil surface, doesn't disperse far (Moyle and Kaya, 1981, Georgis and Poinar, 1983), is unresponsive to host cues (Lewis *et al.*, 1992, 1993) and is adopyted to infecting mobile hosts on the soil surface (Campbell and Gaugler, 1993, Kaya and Gaugler, 1993). *Steinernema glaseri* occurs deeper in the soil and travel much farther (Georgis and Poinar, 1983, Shrewder and Beavers, 1987) responds strongly to host cues (Lewis *et al.*, 1992, 1993) and is adapted to infecting sedentary hosts (Campbell and Gaugler, 1993, Kaya and Gaugler, 1993).

Many factors in soil affect nematode survival, movement and infectivity: Moisture, which is probably the most important physical factor affecting survival (Laumond *et al.*, 1979, Poinar, 1979 and Moyle and Kaya, 1981), soil texture (Georgis and Poinar 1983), presence or absence of a host (Byers and Poinar, 1982) and Behavior of the species (Wallace, 1958 and Simons and Poinar, 1973).

Utilization of the EPN as a method of control needs more information about the ability of them to distribute in the soil. The present study defines the migration of 5 different EPN spp in either vertical or horizontal direction searching the Medfly larvae under the laboratory conditions.

MATERIALS AND METHODS

Fly and Nematode cultures:

The experiment was conducted using the laboratory strain of Mediterranean fruit fly (Medfly) (MFF), *Ceratitis capitata* (Weidemann) reared in Plant Protection Research Institute on wheat bran diet of Mohamed (2003) at 25 °C and 65% RH. Third larval instar was obtained by receiving them in water for 2 hours during their jumping out for pupation.

Different entomopathogenic nematode (EPN) species were reared using late larval instar of greater wax moths, *Galleria mellonella* (L.) (Lepidoptera: Pyrallidae) as described by Dutky *et al.* (1964). Nematodes were harvested daily with White traps (White, 1927, Woodring and Kaya, 1988) and stored in tissue culture flasks at 4°C except the *Heterorhabditis* spp which kept in 10°C. The EPN mixtures were moved to the room temperature for acclimation 24 hours before the experiment. Five isolates of entomopathogenic nematodes, *Steinernema carpocapsae* (Weiser) (All strain), *S. riobrave* Cbanillas (Texas strain), *S. feltiae* (Filipjev), *S. glaseri* (Steiner) and *Heterorhabditis bacteriophora* Poinar (HP88) were used for comparison in vertical and horizontal migration against the last larval instar of Medfly. Nematodes were quantified by counting number of nematodes in 100 µl of homogenous solution for five samples using the average to dilute the solutions to the needed concentration.

Entomopathogenic nematode migration:

Two types of EPN migration have been evaluated, vertical and horizontal (lateral) migration. Vertical migration of EPN was determined by using 8 cm diameter and 15 cm height plastic container, while the horizontal migration experiment was conducted using 20X30X13 cm plastic container. The evaluation was conducted using sand of <0.5 mm diameter. Vertical migration was evaluated by adding the EPN species on the surface of sand and then the sand was completed to the desired level to be at 0, 3, 6 and 10 cm deep from the sand surface (one depth per cup). For horizontal (lateral) migration, the EPN species were added at the margin of the container. Referring to previous data (Under publication), 50 Ijs/ cm^2 concentration was chosen for comparison in both vertical and horizontal experiments. According to the surface area of the container, the EPN specie was counted and distributed on sand surface. For both experiments, soil was treated with water volumetrically to be 10% of sand volume. On the fifth day and after the EPN species were distributed on the surface of sand, the MFF larvae were applied by placing them on the soil surface at 0, 10, 20 and 30 cm from the nematode application point for the horizontal evaluation experiment and haphazardly for the vertical experiment. Water was completed to be 15 % of Sand volume. 10 larvae were placed in each arena and 5 replicates were evaluated for each treatment. The containers were kept at 25°C and 65%RH. Nematode infectivity was determined by the ability of juvenile to reach and infest larvae of MFF placed at various distances (Vertical and horizontal) in

soil. After 9 days of the larval application, the sand was sifted and larvae, pupae and adults were collected, dissected and examined for nematode infection.

Data analysis:

A One-way analysis of variance (ANOVA) with a complete block design test of Dunnest Alpha ≤ 0.05 (SPSS) was used to analyze larval mortality. Mortality data are presented as percentage mortality after correction in comparison to the control of the experiment. LSD was used for multiple mean comparisons between each treatment and the other (Green and Salkind, 2003).

RESULTS

Five nematode species were established against Mediterranean fruit fly larvae, where they were distributed throughout the soil profile. Mortality percentage of control treatment was calculated and accordingly, the mortality percentages of treatments were corrected. When insects larvae were placed on soil surface (0.0 cm distance), The results represented the highest percentages of mortality (Table 1 and 2), where the highest was represented in *Steinernema carpocapsae* (Weiser) (84.4 ±0.5) followed by *S. riobrave* Cbanillas (78.7 ±0.5), *S. feltiae* (Filipjev) (55.1 ±0.5), *Heterorhabditis bacteriophora* Poinar (50.1 ±0.6) and the lowest was for *S. glaseri* (Steiner) (39.9 ±0.3).

Horizontal (Lateral) movement of the Entomopathogenic nematode (EPN) species showed different responses for the EPN species under investigation (Table 1). Significant differences were observed for *S. carpocapsae*, where the percentages of mortality of distances 10, 20 and 30 cm were 19.7 ±0.7, 6.0 ±0.4 and 1.5 ±0.2 respectively. When the difference between each treatment and the other was calculated, the results indicated that there was no significant difference between each treatment and the other. The same results were concluded in case of *S. riobrave*, where there was no significant difference between each treatment and the other where the percentages of mortality were 17.7 ±0.6, 12 ±0.6 and 4.2 ±0.3 respectively. *S. feltiae* showed significant difference among treatment, where the mortality percentages were 20 ±0.6, 4.0 ±0.2 and 0.0 ±0.0 for 10, 20 and 30 cm distances respectively. There was significant difference between each treatment and the other except between 20 and 30 cm distance treatments. For both *S. glaseri* and *H. bacteriophora*, there were no significant difference among treatments,

where the percentages of mortalities of *S. glaseri* at 10, 20 and 30 cm distances were 49.1 ±0.7, 24.2 ±0.5 and 29.8 ±0.5 respectively and for *H. bacteriophora*, they were 30 ±0.5, 25.5 ±0.7 and 30.9 ±0.7 respectively too.

Table (1): Mortality of the Mediterranean fruit fly larvae when exposed to different entomopathogenic nematodes (EPN) at different horizontal distances.

EPN	Distance (CM)	% of Mortality	±S.E.	Significance*
Steinernema carpocapsae (Weiser) (All strain)	0	84.4	0.5	
	10	19.7	0.7	a
	20	6.0	0.4	a
	30	1.5	0.2	a
Steinernema riobrave Cbanillas (Texas strain)	0	78.7	0.5	
	10	17.7	0.6	a
	20	12.0	0.6	a
	30	4.2	0.3	a
Steinernema feltiae (Filipjev)	0	55.1	0.5	
	10	20.0	0.6	
	20	4.0	0.2	a
	30	0.0	0.0	a
Steinernema glaseri (Steiner)	0	39.9	0.3	ab
	10	49.1	0.7	a
	20	24.2	0.5	c
	30	29.8	0.5	cb
Heterorhabditis bacteriophora Poinar (HP88 strain)	0	50.1	0.6	a
	10	30.0	0.5	b
	20	25.5	0.7	b
	30	30.9	0.7	ab

*Means followed by the same letter for each EPN are not significantly different according to Anova test and using Dunnett Alpha P≤0.05.

When the EPN species were injected vertically in sand, the percentages of mortality was decreased by increasing the depths. Whereas *S. carpocapsae* introduced at 3, 6 and 10 cm depths the mortality percentages were 56.2 ±0.6, 51.7 ±0.5 and 9 ±0.5 respectively. There were significant difference among treatments and between each treatment and the other except between distance 3 and 6 cm depths. In case of *S. riobrave* percentages of mortality were 58.1 ±1.1, 44 ±0.8 and 13 ±0.5 respectively. Difference among treatments was significant and when the difference between each treatment and the other was calculated, there were a significant difference except between 3 and 6 cm depths. When *S. feltiae* was compared, there were significant differences among the treatments and also between each treatment and the other except between

depths 0, 3 and 6 cm depths, where the percentages of mortality were 55.1 ±0.5, 53.1 ±0.5 and 51.7 ±0.6 respectively, while for 10 cm depth the percentage was 19 ±0.5. For *S. glaseri* treatments, there was a significant difference among different treatments, while in case of *H. bacteriophora*, there was no significant difference. For both *S. glaseri* and *H. bacteriophora* there was no significant difference between each treatment and the other, where the percentage of mortalities were 48.9 ±0.7, 45 ±0.7 and 60 ±1.1 for *S. glaseri* and 47± 0.8, 50.6 ± 0.5 and 38.6 ±0.4 for *H. bacteriophora* respectively.

Table (2): Mortality of the Mediterranean fruit fly larvae when exposed to different entomopathogenic nematodes (EPN) at different vertical distances.

EPN	Distance (CM)	% of Mortality	±S.E.	Significance*
Steinernema carpocapsae (Weiser) (All strain)	0	84.4	0.5	
	3	56.2	0.6	a
	6	51.7	0.5	a
	10	9	0.5	
Steinernema riobrave Cbanillas (Texas strain)	0	78.7	0.5	a
	3	58.1	1.1	ab
	6	44.0	0.8	b
	10	13.0	0.5	
Steinernema feltiae (Filipjev)	0	55.1	0.5	a
	3	53.1	0.5	a
	6	51.7	0.6	a
	10	19	0.5	
Steinernema glaseri (Steiner)	0	39.9	0.3	a
	3	48.9	0.7	a
	6	45.0	0.7	a
	10	60.0	1.1	a
Heterorhabditis bacteriophora Poinar (HP88 strain)	0	50.1	0.6	a
	3	47.0	0.8	a
	6	50.6	0.5	a
	10	38.6	0.4	a

*Means followed by the same letter for each EPN are not significantly different according to Anova test and using Dunnett Alpha P≤0.05.

DISCUSSION

Members of Steinernematid and Heterorhabditid and their associated bacteria have been tested against a number of insects including the Mediterranean fruit fly larvae with some encouraging results (Lindegren and Vail, 1986, Lindegren 1990, Lindegren *et al.*, 1990, Gazit *et al.*, 2000, Laborda *et al.*, 2003). As research has focused on the use of Entomopathogenic nematodes (EPN), as biological insecticides, little is known about many basic aspects of their ecology in the soil (Ehler, 1990, Hominik and Reid, 1990, Kaya, 1990 and Kaya and Gaugler, 1993). There is interspecific variation in the host foraging strategy used by EPN infective juveniles (IJs). Referring to the results of the horizontal distribution of EPN, it was found that S. *carpocapsae, S. riobrave* and *S. feltiae* concentrated in the 10 cm from the inoculation point and by the 20 cm horizontal distance, the distribution decreased, where the average percentage of mortality was 7.3 %. For both *S. glaseri* and *H. bacteriophora,* the condition reversed, where the EPN Ijs could distribute and arrived to 30 cm distance where the average percentage of mortality was 30.4% indicating that both EPN spp have strong foraging strategy. Horizontal movement of neoplectanids in soil was previously measured under laboratory conditions by Georgis and Hague (1981), Moyle and Kaya, (1981) and Poinar and Hom (1984). Georgis and Hague (1981) noted that in 5 days IJs of *Neoplectana carpocapsae* moved 7 cm in sterilized forest soil, providing a dispersal rate of 1.4 cm/day, whereas Poinar and Hom (1984) found that the Ijs dispersal rate was 4.35 cm/day in field clay loam soil and Moyle and Kaya (1981) observed that IJs moved up to 14 cm in 2 days in sand with a dispersal rate of 7 cm/day

Comparing vertical distribution of different EPN, *S. carpocapsae, S. riobrave* and *S. feltiae* concentrated in the upper layer of sand near the soil surface (6 cm depth) and these results coincide with Georgis and Poinar (1983) and this may reflect the host searching strategy of these nematodes (Kaya *et al.*, 1993, Campbell and Gaugler, 1993, Kaya and Gaugler, 1993, and Ferguson *et al.*, 1995), which is valuable to infest the larvae of Mediterranean fruit fly before their pupation. These results coincide also with Moyle and Kaya (1981) and Georgis and Poinar (1983), who concluded that more than 78% of *S. carpocapsae* IJs concentrated in 0-2 cm depth. In adverse *S. glaseri* and *H. bacteriophora* infected higher proportion of Mediterranean fruit fly larvae at soil depth of 10 cm indicating their greater vertical distribution.

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سلوك البحث لبعض أنواع من الهيتيرورهابتيد والستينرنيماتيد عن يرقات ذبابة فاكهة البحر المتوسط سيراتيتيس كابياتاتا (ثنائيات الأجنحة: تيفرتيدى)

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أجريت الفحوص لدراسة سلوك بحث بعض أنواع النيماتودا الممرضة للحشرات عن يرقات ذبابة فاكهة البحر المتوسط فى التربة الرملية فى كلا الاتجاهين الرأسى والأفقى. وجد أنه تركزت كل من ستينرنيما كربوكابسا، وستينرنيما ريوبرافا وستينرنيما فيلتاى فى مسافة ١٠ سم من نقاط التوزيع الأفقية بينما انتشرت ستينرنيما جلاسيرى والهيتيرورهابتيدس باكتيريوفورا وحتى مسافة ٣٠ سم أفقيا. وعند دراسة بحث الخمسة أنواع عن يرقات ذبابة فاكهة البحر المتوسط رأسيا وجد أن ستينرنيماتيد كاربوكابسا وستينرنيما ريوبرافا وستينرنيما فيلتاى تجمعت فى عمق ٦ سم بينما توزع الاثنان الآخران فى عمق ١٠ سم.