

## ENTOMOPATHOGENIC NEMATODES FOR BIOLOGICAL CONTROL OF HOUSE FLY, *MUSCA DOMESTICA* L. IN EGYPT

Abd El-Raheem, A.M. \* and M.E. Sweelam

Economic Entomology and Agric. Zoology Department, Fac. of Agric., Menoufia University, Egypt

### ABSTRACT

Two species of entomopathogenic nematodes *Heterorhabditis bacteriophora* (Filipjev) and *Steinernema feltiae* (Poinar) at five doses (50, 100, 500, 1000 and 2000 IJs / treatment), were examined against house fly, *Musca domestica* L. larvae under laboratory and field conditions. Under laboratory conditions, *S. feltiae* gave the highest mortality percentages with the nematode doses of 2000 and 1000 IJs / treatment, giving 67.50% and 54.97%, respectively, while the doses of 50 and 100 IJs gave unsatisfactory control results estimating by 20% and 32.50%, respectively. In addition, *H. bacteriophora* nematode gave the highest mortality percentages with the nematode doses of 2000 IJs and 1000 IJs / treatment, giving 55.85% and 45.83%, respectively, while the doses of 50 IJs and 100 IJs gave the least control results estimating by 14.15% and 15%, respectively. Meanwhile, under field conditions the highest reduction percentages of house fly larvae were recorded with the treatment of *H. bacteriophora* (100.000 IJs / treatment) giving only 8 live larvae after one month of treating 3000 house fly larvae resulted 91.8% mortality, while the treatment of *S. feltiae* (100.000 IJs / treatment) gave 14.38 live larvae from 3000 treated equaling 85.2% mortality without significant differences between them.

**Key words:** Biological control, *Heterorhabditis bacteriophora*, *Steinernema feltiae*, House fly.

### INTRODUCTION

There are an international direction to use the biological agents in the control of different pests instead of chemical pesticides which cause dangerous environmental problems by the pollution of many of natural products, besides this harmful effects against the healthy of human, as well as, destroy the natural balance of the fauna and flora of the world. It was noted that tested nematodes of the genera *Heterorhabditis* and *Steinernema* under laboratory conditions as potential control agents of *Musca domestica* maggots in poultry manure, the nematodes survived only a few days in moist manure and, therefore, had little potential for fly control (Belton *et al.*, 1987). Survival, infectivity, and movement of three insect parasitic nematodes (*S. feltiae*, *S. bibionis*, & *H. heliothidis*) were tested in poultry manure under laboratory conditions. Results confirmed that Poor survival and limited movement of nematodes in poultry manure appear to make them unlikely

candidates for biocontrol of filth flies in this habitat (Georgis *et al.*, 1987).

It was found that the effects of different dosages of *S. feltiae* IJs on the larval tissues of *M. domestica*, these nematode larvae invade the fat tissue, gut, cuticle and muscular tissues of the host and the tissues of the gut and the fat body are most severely damaged, also the damage depends largely on the timing and intensity of the infection (Ghally *et al.*, 1991). There is a positive correlation between the reproductive rate of invasive nematode *S. feltiae* IJs and the initial dosages, host species and host stages, no reproduction was obtained from the pupal stages of *M. domestica* (Ghally *et al.*, 1992). Entomopathogenic nematodes *S. feltiae* and *H. megidis* were encapsulated in calcium alginate and tested their efficacy against immature house flies in artificial diet and chicken manure. It was noticed that the mortality percentages of both two genera of Entomopathogenic nematodes at dosage of 1000.000 were high by day 2

\*corresponding author e-mail: am\_raheem@yahoo.com

and reached this highest effect by day 6 (Renn 1995). The efficacy of *S. feltiae* and *H. megidis* were compared after formulation into a house fly bait with a commercial bait formulation of methomyl for the control of *M. domestica* on a pig farm in the UK. One house was baited with the nematode species and the other with methomyl. Significantly fewer flies ( $P < 0.05$ ) were counted in the houses baited with either *S. feltiae* or *H. megidis* than those baited with methomyl. The efficacy of *S. feltiae* sprayed on to the manure was also compared with methomyl bait, the efficacy of encapsulated *S. feltiae* was also compared with methomyl bait and no significant difference was observed (Renn 1998). Two strains of *S. feltiae*, SN and UNK-36, and two strains of *H. bacteriophora* Poinar and *H. megidis* Poinar, Jackson & Klein HF-85 were tested in a fresh bovine manure substrate. All four strains produced significant fly mortality in the manure substrate, although the *S. feltiae* strains had significantly lower LC50 values than did the *Heterorhabditis* spp. (Taylor *et al.*, 1998). Infectivity of four isolates (BA1, ES1, GF and SA2) belonging to the insect nematode species *H. bacteriophora* were tested against the house fly larvae. Data revealed that the most infective isolate at a dosage level of 5 IJs / ml per larva was SA2 isolate, followed by ES1, GF and BA1 isolates, respectively (El-Sooud *et al.*, 2001). *S. carpocapsae* and *H. bacteriophora* were tested against the red palm weevil, *Rhynchophorus ferrugineus* stages at the concentration of 1000, 2000, 3000, 4000, 5000 infective juveniles per ten individuals of the weevil stages in Saudi Arabia under laboratory conditions. It was noticed that mortality percentages were increased by increasing the number of nematode infective stages for the two nematode species (Sweelam *et al.*, 2010). It was found that entomopathogenic nematodes *S. feltiae* and *H. bacteriophora* registered high efficacy against four stored product adult insects under laboratory conditions (Mousa 2012). Therefore, the objective of this study was to investigate the effectiveness of some entomopathogenic nematodes as a biological control agents against *M. domestica*.

## MATERIALS AND METHODS

### Rearing of entomopathogenic nematodes:

Two species of entomopathogenic nematodes (*H. bacteriophora* and *S. feltiae*) were isolated from the soil of the mango trees of the Experimental Station of the Faculty of Agriculture Shebin El-Kom, Menoufia Governorate by the method of Southey (1970). To rearing and harvest emerging infective juveniles nematodes (IJs) the method of White (1927) and Woodring & Kaya (1988) were used, where larvae of the greater wax, *Galleria melonella* were used as a suitable host for both nematode species.

Keys of inhomogeneous nematodes have been used according to (Pionar & Brill, 1975; Pionar, 1979; 1986). Keys of characters are normally visible under a light microscope (70-280x) and all required measurements for the identification process were carried out as proposed by (Nguyen & Smart, 1992 & Nguyen, 1993)

**House fly rearing:** The larvae of house fly, *M. domestica*, were collected from manure piles at the poultry farms of the Faculty of Agriculture, Menoufia University, Egypt. The house fly larvae were provided with nutrient compound to feed and complete life cycle. The nutrient compound was introduced in plastic cups, 10 cm diameter and 10 cm deep, the nutrient compound consisted of 9 g powder milk and 5 g yeast dissolved in 100 ml water then added to 100 g fine bran according to Wilkins & Khalequzzaman (1993). The mixture was then thoroughly stirred and put into the cups leaving 3 cm from the top. The cups were transferred to glass cages (60 × 35 × 40 cm) which used for rearing house fly under laboratory conditions ( $25 \pm 5^\circ\text{C}$  &  $60 \pm 5\%$  RH), and under a 12:12 light: dark cycle according to Palacios Sara *et al.*, (2009). These cages were covered with mesh screen with cloth sleeve opening at top and provided with electric lamps 20 watt to control temperature in cages during winter months. When adult house fly emerged, granulated sugar and milk soaked cotton wool balls were provided in Petri dishes as food to house fly adults. The emerged flies were also fed with full fat fresh milk in Petri dishes. After two days of

fly emergency, the beakers containing larval food was placed for egg laying process, then beakers were removed from cages after 2 - 3 days when eggs were visible and attached to food along the sides of beakers. The food was changed after 2 - 4 days depending upon the numbers of larvae per beaker. The beakers were kept in separate cage for fly emergency according to Ahmed & Irfanullah (2007).

#### **Application of nematodes on the second instar larvae of house fly:**

Second instars of the house fly larvae were subjected to different concentrations of 50, 100, 500, 1000 and 2000 IJs per treatment of *H. bacteriophora* and *S. feltiae* nematodes to investigate the susceptibility house fly larvae to nematodes.

Ten larvae of house fly were kept in Petri dishes, each of five cm diameter containing two moist filter papers were house fly larvae between them, and exposed to entomopathogenic nematodes. The nematode genera was sprayed on the larvae with two ml. distilled water for each concentration. The house fly larvae in control treatment was sprayed with two ml. distilled water without nematodes. Each treatment was replicated three times. Mortality was checked after three days for the two genera of nematodes and percentage of mortality was calculated for each nematode at different concentration. Mortality percentage was modified by Abbott's formula.

#### **Efficacy of two entomopathogenic nematode genera against house fly larvae under field conditions:**

To study the effect of two entomopathogenic nematode genera against the larvae of the house fly under field conditions, two nematode species were reared in the laboratory (*H. bacteriophora* and *S. feltiae*) for these experiments, as previous mentioned.

Experiments were done in the poultry farm of the Faculty of Agriculture, Menoufia University starting 23 October, 2009. samples of chicken feces (each 1000 g) were collected and examined for house fly larvae. The average numbers of larvae per one pile were 3000 individuals per sample (250 g). Nine samples for each nematode

species served as replicates. Each sample was put in the bottom of yellow house fly food traps treated with 100,000 Infective Juveniles IJs / trap and enclosed. Control treatment, was left without nematodes. Thirty days after treatment, emerged house fly adults were counted and the mortality percentages of adults were calculated.

**Statistical analysis:** All obtained data were analyzed by one way analysis of variance (ANOVA) and the means were separated using Duncan's multiple range test and the LSD 5% ( least significant difference ) according to the software computer program, Costat 22 (1998), Norman and Streiner (1994).

Reduction percentages were counted according to Abbott's formula (1925) and Henderson and Tilton formula (1955).

## **RESULTS**

#### **Effect of *S. feltiae* doses on the mortality of house fly larvae:**

Data presented in (Table 1&2) showed the effect of different entomopathogenic nematode, *S. feltiae* doses on the mortality of house fly larvae under laboratory conditions ( $25 \pm 5^\circ\text{C}$  &  $60 \pm 5\%$  RH).

Statistical analysis of the data in (Table 1) indicated that there were significant differences in the average numbers of alive and dead house fly larvae as influenced by different nematode doses along four days of exposure under laboratory conditions. In addition, there were significant differences in the numbers of alive or dead larvae between the doses.

The highest mean of alive house fly larvae was recorded with the nematodes dose (50 IJs) giving 8 / 10 house fly larvae, followed by the nematode dose (100 IJs) which resulted 6.75 house fly larvae, while the highest mean of dead house fly larvae was recorded with the nematode dose (2000 IJs) giving 2.34 house fly larvae, followed by the nematode dose (1000 IJs) which resulted 2.08 house fly larvae without significant differences between them.

Regarding to the average mortality of each dose of *S. feltiae* nematode against house fly larvae (Table 2) it could be

concluded that the highest mortality percentages were recorded with the nematode doses of 1000 & 2000 IJs / treatment, giving 54.97% and 67.50%, respectively, followed by the treatment of

500 IJs which resulted 35.83%, while the doses of 50 and 100 IJs gave unsatisfactory control results estimating by 20% and 32.50%, respectively

Table 1. Effect of entomopathogenic nematode, *S. feltiae* doses on the mortality of house fly larvae under laboratory conditions ( $25 \pm 5^\circ\text{C}$  &  $60 \pm 5\%$  RH).

Dose of IJs	Average numbers of house fly larvae											
	Alive					Dead						
	Day 1	Day 2	Day 3	Day 4	Mean	Day 1	Day 2	Day 3	Day 4	Mean		
50	9.67	9.33	7.67	5.33	08.00 ab	0.33	0.33	1.67	2.33	1.17 ab		
100	9.67	8.33	5.00	4.00	06.75 bc	0.33	1.33	3.33	1.00	1.50 ab		
500	8.00	7.00	6.00	4.67	06.42 bcd	2.00	1.00	1.00	1.33	1.33 ab		
1000	6.67	5.67	4.00	1.67	04.50 cd	3.33	1.00	1.67	2.33	2.08 a		
2000	6.00	5.00	1.33	0.67	03.25 d	4.00	1.00	3.67	0.67	2.34 a		
Cont.	10.0	10.0	10.0	10.0	10.0 a	0.00	0.00	0.00	0.00	0.00 b		
	LSD 5%					3.03						1.59

Means in column followed by the same letter (s) are not significantly different at 5% level.

Table 2. Mortality of entomopathogenic nematode, *S. feltiae* doses on the house fly larvae under laboratory conditions ( $25 \pm 5^\circ\text{C}$  &  $60 \pm 5\%$  RH).

Dose of IJs	Mortality %					
	Day 1	Day 2	Day 3	Day 4	Average mortality	
50	3.30	6.70	23.30	46.70	20.00 cd	
100	3.30	16.70	50.00	60.00	32.50 bc	
500	20.00	30.00	40.00	53.30	35.83 abc	
1000	33.30	43.30	60.00	83.30	54.97 ab	
2000	40.00	50.00	86.70	93.30	67.50 a	
Cont.	0.00	0.00	0.00	0.00	0.00 d	
	LSD 5%					30.29

Means in column followed by the same letter (s) are not significantly different at 5% level.

**Effect of *H.bacteriophora* doses on the mortality of house fly larvae:** Data in (Table 3&4) showed that the effect of different entomopathogenic nematode, *Heterorhabditis bacteriophora* doses on the mortality of house fly larvae under laboratory conditions ( $25 \pm 5^\circ\text{C}$  &  $60 \pm 5\%$  RH).

Statistical analysis of the data in (Table 3) indicated that there were significant

differences in the average numbers of alive and dead house fly larvae as influenced by different nematode doses along four days of exposure under laboratory conditions, in addition, there were significant differences in the numbers of alive or dead larvae among the doses of 50 IJs & 100 IJs nematodes house fly larvae, and the other three doses (500 IJs, 1000 IJs, 2000 IJs).

The highest mean of alive house fly larvae was recorded with the nematode dose (50 IJs) giving 8.59 house fly larvae, followed by the dose (100 IJs) which resulted 8.50 house fly larvae, then (500 IJs) giving 7.25 house fly larvae, without significant differences among them, while the highest mean of dead house fly larvae was recorded with the nematode dose (2000 IJs) giving 2.17 house fly larvae, followed by the nematode dose (500 IJs), (1000 IJs) which resulted 2, 1.75 house fly larvae, respectively, without significant differences among them.

Regarding to the average mortality of each dose of *H. bacteriophora* nematode against house fly larvae (Table 4) it could be concluded that the highest mortality percentages were recorded with the nematode doses of 2000 IJs & 1000 IJs / treatment, giving 55.85% & 45.83%, respectively, followed by the treatment of 500 IJs resulted 27.50 %, while the doses of 50 IJs and 100 IJs gave unsatisfactory control results estimating by 14.15% and 15%, respectively.

Table 3: Effect of entomopathogenic nematode, *H. bacteriophora* doses on the mortality of house fly larvae under laboratory conditions ( $25 \pm 5^\circ\text{C}$  &  $60 \pm 5\%$  RH).

Dose of IJs	Average numbers of house fly larvae									
	Alive					Dead				
	Day 1	Day 2	Day 3	Day 4	Mean	Day 1	Day 2	Day 3	Day4	Mean
50	10.00	8.67	8.00	7.67	08.59 a	0.00	1.33	0.67	0.33	0.58 bc
100	10.00	9.33	7.00	7.67	08.50 a	0.00	0.67	1.33	0.33	0.58 bc
500	9.67	8.00	6.00	5.33	07.25 ab	0.33	1.33	2.67	3.67	2.00 a
1000	8.67	5.67	4.33	3.00	05.42 bc	1.33	3.00	1.33	1.33	1.75 ab
2000	7.33	5.00	4.00	1.33	04.42 bc	2.67	2.33	1.00	2.67	2.17 a
Cont.	10.0	10.0	10.0	10.0	10.0 a	0.00	0.00	0.00	0.00	00.0 c
	LSD 5%				2.64					1.23

Means in column followed by the same letter (s) are not significantly different at 5% level.

Table 4: Mortality of entomopathogenic nematode, *H. bacteriophora* doses on the house fly larvae under laboratory conditions ( $25 \pm 5^\circ\text{C}$  &  $60 \pm 5\%$  RH).

Dose of IJs	Mortality %				
	Day 1	Day 2	Day 3	Day 4	Average mortality
50	0.00	13.30	20.00	23.30	14.15 c
100	0.00	6.70	30.00	23.30	15.00 c
500	3.30	20.00	40.00	46.70	27.50 bc
1000	13.30	43.30	56.70	70.00	45.83 ab
2000	26.70	50.00	60.00	86.70	55.85 a
Cont.	0.00	0.00	0.00	0.00	0.00 c
	LSD 5%				26.42

Means in column followed by the same letter (s) are not significantly different at 5% level.

**Efficacy of two entomopathogenic nematode genera against house fly larvae under field conditions:** Results in Table 5 showed that the lethal effect of two genera of entomopathogenic nematodes, *S. feltiae* and *H. bacteriophora* on the mortality percentages of house fly larvae under field conditions (  $27 \pm 5^\circ\text{C}$ ,  $70 \pm 5\%$  RH ).

Statistical analysis of the data in Table 5 revealed that there were no significant differences in the average mortality of house fly larvae between the tested two

genera of entomopathogenic nematodes, while there was significant differences of the average numbers of house fly larvae per sample, between the treatments of the two nematode genera and the treatment of the control. The highest reduction percentages of house fly larvae were recorded with *H. bacteriophora* treatment giving only 8 live larvae after one month of treating 3000 larvae resulted 91.8% mortality, while the treatment of *S. feltiae* giving 14.38 live larvae from 3000 treated larvae causing 85.2% mortality.

Table 5: Average numbers of house fly larvae and mortality%, one month after application of two entomopathogenic nematode genera to manure pills under field conditions.

Nematode Genera	Dose of IJs / pile	Average no. of house fly larvae		% Mortality
		Pre treatment	Post treatment	
<i>S. feltiae</i>	100.000 IJs	3000	14.38 b	85.2
<i>H. bacteriophora</i>	100.000 IJs	3000	8.00 b	91.8
Control	-	3000	97.25 a	-
LSD 5 %			13.79	

Means in each column followed by the same letter (s) are not significantly different at 5% level.

## DISCUSSION

The recorded data revealed that, both of the tested Entomopathogenic nematode species, *S. feltiae*, *H. bacteriophora* (Egyptian strains) successfully controlled the house fly, *M. domestica* under laboratory and field conditions. Mortality percentages of house fly larvae were over than 50% under laboratory conditions, while the mortality percentages were very high (more than 85%) under field conditions, this may be due to the suitable conditions for the activity of the entomopathogenic nematodes where the natural requirements of optimum temperature degrees and relative humidity, which encourage nematodes to produce new generations in short times.

The obtained results are in agreement with those of (Ghally *et al.*, 1991; 1992 & Renn, 1998) who successfully applied entomopathogenic nematodes in the

control programs of house fly insects, in addition there were no differences in the pathogenicity between entomopathogenic nematode species.

In addition, results are in harmony with Taylor *et al.*, (1998) who found that *S. feltiae* was the most virulent species toward house fly maggots, while there was a disagreement with the results of the other nematode species, *H. spp.* who pointed that it was not perfect against house fly maggots. Also, the obtained results are in disagreement with Belton *et al.*, (1987) who reported that *Steinernema* & *Heterorhabditis* species had less potential for house fly control, moreover, Mullens *et al.*, (1987) observed the same trend with *Heterorhabditis* spp.

Regarding to the phenomenon of the increase of mortality percentages under field conditions than that of laboratory, this may be due to the suitability of

environmental conditions which encourage nematode individuals to move and feed on different stages of house fly larvae, this result are in agree with Geden *et al.*, (1986) who reported higher virulence of *H. bacteriophora* than *S. feltiae* in the control of house fly maggot in manure, also Bednarek & Gauglar (1997) found that the population of *S. feltiae* was increased with the addition of chicken manure, this result was in contrast with that obtained by (Geogris *et al.*, 1987 & Shapiro *et al.*, 1996) who found that the addition of chicken manure reduced nematode virulence and decreased their survival and pathogenicity of house fly.

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