

EFFICACY OF THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA FELTIAE* CROSS N 33 AGAINST LARVAE AND PUPAE OF FOUR FLY SPECIES IN THE LABORATORY

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Summary. The susceptibility of larval, pupal and adult stages of the flies *Lucilia sericata*, *Calliphora vicina*, *Musca domestica* and *Stomoxys calcitrans* to the entomopathogenic nematode, *Steinernema feltiae* Cross N 33, was studied under laboratory conditions of 25 ± 1 °C, $60 \pm 10\%$ RH. and 12:12 L/D h photoperiod. Bioassays of second and third instar larvae of the flies were conducted in Petri dish and pupae were bioassayed in soil and manure. Mortality of second and third instar maggots in all fly species increased significantly with the increase of *S. feltiae* concentration and time post-application. *Lucilia sericata* was the most susceptible second instar to *S. feltiae* with LC₅₀ and LC₉₀ of 47.04 and 193.8 IJs, respectively. *Musca domestica* had the most susceptible third instar maggots with LC₅₀ and LC₉₀ of 75.1 and 292 IJs, respectively. In soil and manure, the rates of infected pupae and fly emergence differed significantly among the tested concentrations within each fly species. Rates of infected pupae and LC₅₀ and LC₉₀ were consistently higher in soil than in manure. The reproduction of *S. feltiae* differed significantly among the tested fly species and was greatest in *Galleria mellonella* (control) followed by *L. sericata*.

Key words: *Calliphora vicina*, *Lucilia sericata*, *Musca domestica*, *Stomoxys calcitrans*, virulence.

Entomopathogenic nematodes (EPNs) belonging to families Steinernematidae and Heterorhabditidae are obligate parasitic nematodes for many insect species (Kaya and Gaugler, 1993). Several of them are promising bio-agents for controlling many soil-dwelling insects (Grewal, 1999) as they do not seem to have adverse effects on non-target beneficial arthropods such as insect parasitoids and predators. The infective juveniles (IJs) of EPNs usually enter their hosts via body orificies (e.g. spiracles – Steinernematidae) and less often penetrate host cuticle (Heterorhabditidae). They do not kill their hosts directly. Instead, certain mutualistic bacteria, e.g. *Xenorhabdus* spp. in steinernematids and *Photorhabdus* spp. in heterorhabditids, associated with the EPNs release toxins, metabolites or proteases that kill the host within 2-3 days (Poinar, 1983). Due to this association, no insect resistance against EPNs has reported to date (Abdel-Razik, 2006).

Sheep, poultry and cattle farms suffer tremendously from attacks by a number of insect pests (Axtell and Arends, 1990). Of these, *Lucilia sericata* Meigen and *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae), *Stomoxys calcitrans* L. and *Musca domestica* L. (Diptera: Muscidae) are the most common species in farm animals (Axtell and Arends, 1990). These pests may cause myiasis (*C. vicina* and *L. sericata*), reduce

growth and milk production of affected animals (*S. calcitrans*), and transmit scores of diseases, i.e. cholera, tuberculosis, dysentery, ulcers, gastroenteritis, etc. (Olsen and Hammack, 2000).

Current control of insects of veterinary and medical importance has relied for a long time on the extensive use of traditional pesticides. Unfortunately, insects developed resistance to pesticides after several generations of exposure (Campbell *et al.*, 2001). Also, the use of pesticides causes concerns because of environmental pollution and toxicity to humans and animals (Campbell *et al.*, 2001). Hence, there is growing importance to develop a biological control strategy as an alternative to chemicals in pest control. In this context, the use of EPNs appears to be a sound solution. Renn (1995, 1998) reported that the use of *Steinernema feltiae* Filipjev and *Heterorhabditis megidis* Poinar in large numbers was very effective against house-fly maggots and, after formulation in housefly baits, EPNs were as effective as spraying carbamate pesticides in pig units.

Therefore, the objective of our study was to test the virulence of *S. feltiae* Cross N 33 to second and third instar larvae, pupae and adults of *Lucilia sericata*, *Calliphora vicina*, *Musca domestica* and *Stomoxys calcitrans* under different laboratory conditions.

MATERIAL AND METHODS

Insect maintenance

Larvae of *L. sericata* and *C. vicina* were obtained from infested sheep reared in the Faculty of Agriculture

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experimental farm, Suez Canal University (SCU), Ismailia, Egypt. Larvae were grouped according to their instars and used directly in the experiments (Toth *et al.*, 2005). The specimens of *M. domestica* and *S. calcitrans* were obtained from the stock culture kept in the Plant Protection Department, SCU, at 25 ± 1 °C, $65 \pm 10\%$ RH, and a 14 h photophase. The rearing technique was similar to that described by Mara and Angelo (2001) and adults were fed on 1:1:1 mixture of yeast, powdered milk and sugar. Larvae of *Galleria mellonella* L. were taken from a stock culture reared on wax combs in the insectary, SCU, at 25 ± 1 °C and $65 \pm 10\%$ RH.

Source of *S. feltiae*

Steinernema feltiae was obtained from the Peoples' Friendship University (RODN), Moscow, Russia and cultured *in vitro* using a foam substrate. Nematode infective juveniles (IJs) were extracted by the Baermann funnel technique and stored in sterilized distilled water at 5 °C for less than three weeks before use (Grewal, 1999).

Experiments

Virulence of S. feltiae to fly larvae in Petri dishes. The susceptibility of second and third instar larvae of the four tested fly species was determined in Petri dishes (9 cm diameter) lined with a 7 cm diameter Whatman No. 1 filter paper disc. Inoculum of 50, 100, 200, 300, 400 and 500 IJs in 1 ml distilled water was applied onto the filter paper. Five larvae of each instar were gently transferred to the Petri dish and incubated at 25 ± 1 °C, $65 \pm 10\%$ RH, and 14:10 h L/D photoperiod. Fly larvae were not fed during the treatment (Renn *et al.*, 1985). Each nematode concentration was replicated four times. Larval mortality was recorded 24, 48 and 72 h after application. Controls were Petri dishes receiving 1 ml distilled water and no EPN juveniles.

Virulence of S. feltiae to fly pupae in soil. This experiment was conducted in sixteen plastic containers (500 cm³ in volume) containing 250 g of sterilized soil (sandy-loam soil, 67.3% sand, 15.4% silt and 17.3% clay) to a depth of 7 cm. Ten pupae (1-2 days old) of each tested fly species were placed in each container and covered with a 2 cm layer of soil. Then, 1 ml aliquots of nematode suspensions containing 0, 500, 1000, 2000 or 3000 IJs were applied. Containers were covered with piece of fabric and incubated at 25 °C. Fly adults emerging from the pupae were collected every 24 h, kept in Petri dishes (11 cm diameter) and dissected when they were 2 days old to observe the pathogenicity and check the development of nematode progeny. Each treatment was replicated four-fold.

Virulence of S. feltiae to fly pupae in manure. A similar procedure to that described for the soil bioassay was used in this experiment except that the plastic containers were of 1000 cm³. Fresh bovine manure was collected from the Faculty of Agriculture experimental farm,

SCU, and each container received 700 g of it (9 cm deep), covered with a fine layer of manure (1-2 cm) and kept at 25 °C. Preliminary trials indicated that the concentrations of IJs used in soil bioassays gave low rates of mortality in manure trials. Therefore, the IJ concentrations tested in this bioassay were 2000, 3000, 4000 and 5000 IJs in 1 ml water, added to the containers. Each treatment, including controls receiving distilled water only, was replicated four times.

Reproductive capacity of S. feltiae. The reproductive capacity of *S. feltiae* was estimated on the four tested fly species and compared to that on *G. mellonella* used as control. Maggot cadavers were placed on White traps 7 days after infection. The white traps were then kept at 25 °C and checked daily for up to 21 days for the emergence of IJs. Emerging nematode juveniles were collected from the White traps and stored in tissue culture flasks at 17 °C.

Statistical analysis

Mortality rates in each nematode dose were analyzed by ANOVA, using the SAS package (SAS Institute 1999). If ANOVA indicated significant differences ($P \leq 0.05$), a subsequent LSD test was used. No mortality was recorded in control treatments in the second and third instar maggot bioassays or in the pupae in soil and manure bioassays. Therefore, the controls were excluded from these analyses. Data from the controls were included in analysis of fly emergence in the soil and manure bioassays. A standard probit analysis was used to calculate LC₅₀ and LC₉₀ of the tested insects (SAS Institute, 1999).

RESULTS

The mortality of second instar maggots increased significantly with increasing nematode concentration and time of exposure in all tested species (Table I). *Steinernema feltiae* was very virulent and parasitized 100% of second instar larvae of *L. sericata* after 48 h at 400 and 500 IJs/ml and after 72 h also at 300 IJs/ml. Mortality rates were intermediate for *C. vicina* and least for *M. domestica* and *S. calcitrans* for which the temporal increase of the mortality was less pronounced with maxima of only 58.3 and 41.6% 72 h after treatment, respectively.

In Petri dishes (Table II) the mortality patterns for third instar maggots were completely the reverse of those of second instar maggots. In general, mortality increased significantly with the increase of *S. feltiae* concentration. The mortality rates in *L. sericata* maggots were the least among the species tested and a maximum mortality of only 50% was achieved after 72 h exposure to 500 IJs/ml. In *M. domestica* and *S. calcitrans* third instar maggots, the mortality was the greatest and reached 100% with 400 and 500 IJs/ml after 72 h. In *C. vicina* the mortality rates were intermediate with a maximum

of 75% 72 h after inoculation of 500 IJs/ml.

In the soil bioassay (Table III), the percentage of infected pupae increased significantly with the increase in IJ dose in *L. sericata* (df = 3,12; F = 10.71; P = 0.0010) and *S. calcitrans* (df = 3,12; F = 10.01; P = 0.0014), but not in *C. vicina* (df = 3,12; F = 1.94; P = 0.1777) or *M. domestica* (df = 3,12; F = 1.33; P = 0.3096). The highest rate of infected pupae (70%) was observed in *L. sericata* at 3000 IJs/ml (Table III). Significant reductions in rates of fly emergence, compared to that in the control treatment, were observed in *L. sericata* (df = 4,15; F = 12.81; P = 0.0001), *C. vicina* (df = 4,12; F = 3.86; P = 0.0238), *S. calcitrans* (df = 4,12; F = 10.82; P = 0.0002) and *M. domestica* (df = 4,12; F = 1.47; P = 0.2608).

In the manure bioassay (Table III), percentages of infected pupae differed significantly among the species (df = 3,12; F = 2.31, P = 0.1281 for *L. sericata*; df = 3,12 F = 2.45; P = 0.1134 for *C. vicina*; df = 3,12; F = 3.07; P = 0.0668 for *M. domestica*; df = 3,12; F = 2.36; P = 0.1229 for *S. calcitrans*). The highest percentage of infected pupae (62.5%) was recorded in *M. domestica* at 5000 IJs. The rates of fly emergence differed significant-

ly from those of controls in all tested fly species (df = 4,15; F = 4.78, P = 0.0109 for *L. sericata*; df = 4,15, F = 1.47; P = 0.2601 for *C. vicina*; df = 4,15; F = 9.65; P = 0.0005 for *M. domestica*; df = 4,15; F = 7.24; P = 0.0019 for *S. calcitrans*).

The LC₅₀ and LC₉₀ values of the EPN *S. feltiae* estimated for second and third instar maggots and pupae were not consistent in the bioassays in Petri dishes, soil and manure. The fly *L. sericata* had the lowest LC₅₀ and LC₉₀ of 47.04 and 193.8 IJs, respectively, for second instar maggots (Table IV) and *M. domestica*, had the lowest LC₅₀ and LC₉₀ of 75.1 and 292 IJs, respectively, for third instar fly maggots. Pupae of *L. sericata* had the lowest LC₅₀ and LC₉₀ values (2148 and 4118 IJs, respectively) in the soil bioassay and *M. domestica* pupae had the lowest LC₅₀ and LC₉₀ values (3789 and 9038 IJs, respectively) in the manure bioassay. *Calliphora vicina* was the fly species least susceptible to *S. feltiae* and, with the exception of second instar maggots, it had the highest LC₅₀ and LC₉₀ values (Table IV).

The reproductive capacity of *S. feltiae* varied significantly (df = 4, 20; F = 43.82; P = 0.0001) among the fly

Table I. Effects of different concentrations of *Steinernema feltiae* on second instar maggots of four fly species in Petri dishes.

IJs/ml	Mortality (%)											
	<i>C. vicina</i>			<i>L. sericata</i>			<i>M. domestica</i>			<i>S. calcitrans</i>		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
50	0a	0a	0a	0a	0 a	16.6a	0a	0a	0a	0a	0a	0a
100	8.3b	16.6b	25.0b	33.3b	41.6b	58.3b	0a	8.3b	16.6b	0a	0a	0a
200	16.6c	16.6b	33.3c	41.6c	66.6c	83.3c	8.3b	25.0c	25.0c	0a	0a	0a
300	33.3d	50.0c	50.0d	75.0d	83.3d	100d	16.6c	25.0c	25.0c	0a	8.3b	16.6b
400	25.0e	41.6d	66.6e	91.6e	100e	100d	16.6c	25.0c	41.6d	25.0b	25.0c	33.3c
500	41.6f	58.3e	66.6e	91.6e	100e	100d	33.3d	33.3d	58.3e	33.3c	33.3d	41.6d

Means followed by the same letters in the same column are not significantly different (LSD at P ≤ 0.05).

Table II. Effects of different concentrations of *Steinernema feltiae* on third instar maggots of four fly species in Petri dishes.

IJs/ml	Mortality (%)											
	<i>C. vicina</i>			<i>L. sericata</i>			<i>M. domestica</i>			<i>S. calcitrans</i>		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
50	0a	8.3a	8.3a	0a	0a	0a	0a	8.3 a	33.3a	8.3a	8.3a	33.3a
100	8.3b	16.6b	25.0b	0a	0a	0a	8.3b	33.3b	58.3b	16.6b	16.6b	33.3a
200	25.0c	33.3c	33.3c	16.6b	16.6b	16.6b	16.6c	33.3b	66.6c	16.6b	25.0c	41.6b
300	25.0c	50.0d	58.3d	16.6b	25.0c	25.0c	41.6d	66.6c	91.6d	33.3c	41.6d	83.3c
400	33.3d	58.3e	66.6e	25.0c	25.0c	25.0c	75.0e	91.6d	100e	50.0d	66.6e	100d
500	50.0e	66.6f	75.0f	41.6d	50.0d	50.0d	75.0e	91.6d	100e	75.0e	100f	100d

Means followed by the same letters in the same column are not significantly different (LSD at P ≤ 0.05).

Table III. Effects of different concentrations of *Steinernema feltiae* on percentages of infected pupae and fly emergence of four fly species in soil and manure bioassays.

Fly species	Soil Bioassay			Manure bioassay		
	IJs/ml	% Infected pupae (Mean±SE)	% Fly emergence (Mean±SE)	IJs/ml	% Infected pupae (Mean±SE)	% Fly emergence (Mean±SE)
<i>L. sericata</i>	0	--	85.0±6.45a	0	--	92.5±2.50a
	500	7.5±2.50a	62.5±9.46a	2000	5.0±2.88a	85.0±6.45a
	1000	15.0±6.45ab	62.5±8.53a	3000	17.5±10.30ab	75.0±11.90ab
	2000	40.0±12.90b	27.5±8.53b	4000	30.0±4.08b	50.0±9.12b
	3000	70.0±9.12c	15.0±6.45b	5000	37.5±10.80b	50.0±11.54b
<i>C. vicina</i>	0	--	92.0±7.50a	0	--	87.5±7.50a
	500	0.0a	82.5±8.53ab	2000	0.0a	85.0±2.88ab
	1000	2.5±2.50a	75.0±10.40abc	3000	0.0a	85.0±6.45ab
	2000	10.0±7.07a	65.0±6.45ab	4000	0.0a	82.5±8.53ab
	3000	17.5±8.53a	50.0±8.16c	5000	7.5±4.78b	67.5±6.29b
<i>M. domestica</i>	0	--	80.0±8.16a	0	--	90.0±5.77a
	500	0.0a	80.0±10.80a	2000	30.0±14.71a	40.0±12.90b
	1000	0.0a	80.0±5.77ab	3000	37.5±4.78ab	40.0±9.12b
	2000	7.5±4.78a	75.0±11.90bc	4000	60.0±6.45b	17.5±10.3b
	3000	12.5±9.46a	55.0±6.45c	5000	62.5±11.08b	20.0±7.07b
<i>S. calcitrans</i>	0	--	82.5±7.50a	0	--	85.0±6.45a
	500	5.0±2.88a	82.5±10.30a	2000	0.0a	87.5±6.29a
	1000	12.5±6.29a	60.0±10.80ab	3000	10.0±7.07ab	70.0±4.08ab
	2000	37.5±11.08b	37.5±6.29bc	4000	17.5±8.53ab	47.0±11.81bc
	3000	52.5±4.78b	17.5±7.50c	5000	22.5±6.29b	45.0±6.45c

-- indicates that control was not included in analysis.

Means followed by different letters in the same column are significantly different (LSD at $P \leq 0.05$).

species and the *G. mellonella* control. The largest number of IJs per g insect (1068 IJs) was recorded in *G. mellonella* (Fig. 1). Among the fly species, the two muscid flies were the poorest species for the reproduction of *S. feltiae*. No significant differences in the reproduction of the nematode occurred between the two calliphorid species and between the two muscid species (Fig. 1).

DISCUSSION

In our tests, *S. feltiae* showed great variation in its pathogenicity towards second and third instar larvae of the four tested fly species. Second instar larvae of *L. sericata* were the most susceptible to *S. feltiae* compared with other fly species. However, *S. feltiae* showed the highest virulence toward third instar larvae of *M. domestica*. This is in accord with Taylor *et al.* (1998) who found that, out of 40 EPNs species, two strains of *S. fel-*

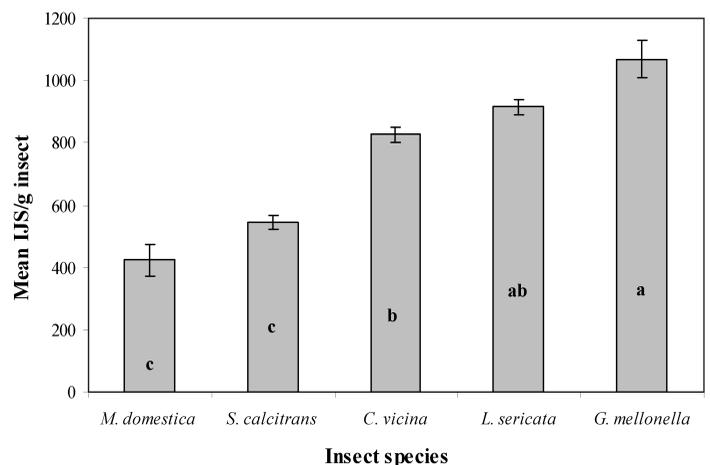


Fig. 1. Mean number of infective juveniles (IJs) of *S. feltiae* produced per g by each insect species under laboratory conditions. Bars with different letters indicate significant difference (LSD at $P \leq 0.05$).

Table IV. The virulence of *Steinernema feltiae* to second and third instar larvae and pupae of four fly species based on the mortality rates 72 h after treatment.

Host insect	2 nd instar larvae		3 rd instar larvae		Pupae in soil		Pupae in manure	
	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
<i>L. sericata</i>	47.01	193.8	634.2	1208	2148	4118	7117	15157
<i>C. vicina</i>	200.50	540.0	320.5	618.6	8983	17268	21662	43699
<i>M. domestica</i>	474.50	995.2	75.1	292	11340	25117	3789	9038
<i>S. calcitrans</i>	712.30	1412.0	147.7	338.8	2437	10907	8543	19200

tiae were the most virulent to *M. domestica* third instar larvae. The results of the pathogenicity of *S. feltiae* to *L. sericata* agree with those of Molyneux *et al.* (1983), who found that EPNs are potential bioagents for controlling blowfly larvae in Australia.

The virulence of *S. feltiae* to second and third instar maggots was very different in the four fly species tested. Inconsistent results on the susceptibility of insect larvae to EPNs has been reported previously. Toledo *et al.* (2005) found that younger larvae of the same instar of *Anastrepha ludens* Loew were more susceptible than older larvae to infection with *Heterorhabditis bacteriophora* Poinar, probably because the period of exposure, from inoculation to pupation, was longer in younger larvae than in older larvae. However, Mahmoud and Osman (2006) found that third instar larvae of *Bactrocera zonata* Saunders were more susceptible to *S. feltiae* than second instar ones. Variations in *S. feltiae* infectivity to the four tested fly species may be attributable to the differences in attractiveness of fly maggots to the IJs (Peters and Ehlers, 1994; Abdel-Razik, 2006), size of the natural orifices, *i.e.* spiracles, anus pore and mouth openings, which are used by *S. feltiae* in penetration (Mahmoud and Osman, 2006), or suitability of maggots to nematode reproduction (Toth *et al.*, 2005).

In this study, pupae of the tested fly species appeared to be less susceptible to nematode infection than maggots. In earlier studies, pathogenicity in insect pupae was also less pronounced compared to insect larvae. For example, pupae of *Spodoptera littoralis* Boisd. (Kaya and Hara, 1980), *M. domestica* (Renn *et al.*, 1985), *B. zonata* (Mahmoud and Osman, 2006) and *Delia antiqua* Meigen (Mahmoud and Pomazkov, 2004) were totally refractory to penetration by EPNs. However, the results with *S. calcitrans* in our study disagree with those of Poinar and Boxler (1984), who found that *Neoplectana bibionis* Boviën and *N. carpocapsae* Weiser are capable of infecting and developing inside pupae and adults of *S. calcitrans* and have potential for practical control of this pest. The low rate of infectivity of fly pupae may be attributable to the toughness of the puparium, and the limited ability of EPNs to penetrate through pupal spiracles (Beavers and Calkins, 1984; Toledo *et al.*, 2005). Therefore, it would be of significant importance to apply the EPNs in the soil before pupation of fly larvae.

In spite of the higher application rates of *S. feltiae*

used in the manure bioassay, mortality rates of fly pupae were higher in soil than in manure and this finding agrees with Shapiro *et al.* (1996), who stated that manure reduced nematode pathogenicity when added to soil as a fertilizer. Belton *et al.* (1987) also found that EPNs (*Heterorhabditis heliothidis* Poinar and *Steinernema* spp.) survived only a few days in moist manure and, therefore, had little potential to control *M. domestica*. Moreover, *H. heliothidis* was more effective in killing fly maggots in chicken manure than *Steinernema* spp. (Belton *et al.*, 1987). Georgis *et al.* (1987) demonstrated that poultry manure was toxic to infective nematode larvae. In contrast, our results differed from those reported by Taylor *et al.* (1998), who stated that the pathogenicity of *S. feltiae* to *M. domestica* in manure was as high as in a filter paper bioassay. Also, Bednarek and Gaugler (1997) reported that populations of *S. feltiae* increased when manure was added to the soil.

Steinernema feltiae reproduced successfully within all the fly species. However, the reproduction into *L. sericata* disagrees with the findings of Toth *et al.* (2005), who found that two strains of *S. feltiae* (Vija Norway and IS6) failed to reproduce in *L. sericata* maggots. The different results in the two studies may be explained by differences in nematode strains.

In conclusion, *S. feltiae* showed high virulence toward second and third instar larvae of *M. domestica*, *S. calcitrans*, *C. vicina* and *L. sericata*; however, its virulence toward fly pupae was less pronounced, particularly in manure. Laboratory bioassays of virulence of EPNs are important for developing a biological control programme for a particular insect pest (Ricci *et al.*, 1996). However, the higher infectivity of EPNs in the laboratory may not be consistent with what may occur under field conditions (Grewal and Georgis, 1998) and, therefore, virulence studies of *S. feltiae* should be conducted in cattle a feedlot environment before drawing any ultimate conclusion.

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