THE INFECTIVITY OF THE ENTOMOPATHOGENIC NEMATODE, STEINERNEMA GLASERI AGAINST THE MORINGA HAIRY CATERPILLAR, EUPTEROTE MOLLIFERA

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Summary. The entomopathogenic nematode, Steinernema glaseri was tested against the Moringa hairy caterpillar, Eupterote mollifera in laboratory investigations. It was highly effective against third, fourth and fifth instars of E. mollifera with LC₅₀ and LT₅₀ in the range of 16.5-24.1 infective juveniles (IJ) per larva and 50.3-69.7 h, respectively. The mortality rate decreased with increase in age of the larvae. Penetration of IJ was less in E. mollifera larvae (18-56%) than in Galleria mellonella larvae (45-85%) but penetration of both species differed significantly with the number of IJ applied and the age of the larvae. The yield of IJ per larva was significantly less (0.11-0.21 × 10⁵ per g of larva) in E. mollifera than in G. mellonella (2.75-3.87 × 10⁵ per g of larva). Spray application of IJ of S. glaseri at a concentration of 1000 IJ per 10 ml was found to be effective, with a mortality of 83.3% of fourth instar larvae of E. mollifera.

Entomopathogenic nematodes (EPN) belonging to the family Steinernematidae have been recognized as effective insect control agents (Poinar, 1990). Their unique association with symbiotic bacteria of the genus Xenorhabdus render them more lethal to insects than any other nematode group (Kaya and Gaugler, 1993). They are highly effective against soil-inhabiting insects and insects with aggregation behaviour (Mráček and Bécvár, 2000). Steinernema glaseri (Steiner, 1929) Wouts, Mráček, Gerlin et Bedding, 1982 was found to infect a large number of insect species including coleopteran grubs (Wouts, 1991) and lepidopteran larvae (Burnell and Stock, 2000).

We investigated the potential of S. glaseri against the moringa hairy caterpillar, Eupterote mollifera Walker, an oligophagous pest feeding on the foliage and soft bark of commercially cultivated moringa, Moringa oleifera Lam. in India (Sivagami and David, 1968). The pest tends to aggregate on the bark of the trees in the night and feeds on the foliage during daytime (Ayyar, 1940). It has assumed great significance because of the widespread cultivation of annual moringa and amenity forestry plantations of moringa trees (Ramachandran et al., 1980; Nautiyal and Venkataraman, 1987; Kantharajah and Dodd, 1991). Few of the reports on the management of this pest are related to cultural practices (Ayyar, 1940). Raj et al. (1994) reported the effect of the insecticide malathion on food consumption in different instars of E. mollifera. Moringa is put to multivarious uses, including a natural coagulant from seeds for low-cost water purification and soap industry (Ramachandran et al., 1980; Nautiyal and Venkataraman, 1987), tender shoots, buds, flowers and fruits of moringa as vegetable and fodder (Ramachandran et al., 1980; Jahn et al., 1986), and medicine (Caceres et al., 1991). Hence, the chemical control of E. mollifera is restricted as it may lead to accumulation of toxic residues (Ramachandran et al., 1980; Odee, 1998).

This paper describes the results of experiments on (i) age-related susceptibility of E. mollifera to S. glaseri, (ii) the invasiveness and multiplication of IJ per cadaver of E. mollifera compared with the greater wax moth Galleria mellonella Linnaeus, and (iii) the effect of site-directed application of the nematode on moringa.

MATERIALS AND METHODS

Insect culture

Eupterote mollifera. The insects were collected at the egg stage from moringa plants grown in the orchard of the Horticultural Research Farm, Tamil Nadu Agricultural University, Coimbatore during the north east monsoon season of 2002. The eggs were maintained in 500 ml disposable plastic containers for larval emergence. The hatched larvae were held in groups of 50 to 75 on moringa leaves kept in well-aerated 10-litre plastic containers covered with cotton fabric. The leaves were maintained turgid by placing their petioles in a conical flask filled with water and plugged with non-absorbent cotton. On alternate days the water was replaced with fresh water. The larvae were maintained at 25 ± 2 °C throughout and constantly examined for association of pathogenic organisms (Poinar and Thomas, 1978) before they were used in experiments. Only larvae of uniform age and stage were used.
Galleria melonella. A culture of the wax moth, routinely maintained at the Department of Agricultural Entomology, Tamil Nadu Agricultural University was used for propagation of S. glaseri. The stock came from a population infesting honeycombs of Apis cerana indica Linnaeus in Coimbatore. The larvae were reared on a semi-synthetic diet at 25 ± 5 °C (Hussaini, 2003). Galleria mellonella was used as a susceptible check for comparison with E. mollifera in invasiveness and in vivo production experiments.

Nematode culture

Infective juveniles (IJ) of S. glaseri, obtained from the Sugarcane Breeding Institute, Coimbatore, were mass-produced in vivo using G. mellonella larvae. Steinernema glaseri, being a cruiser forager (Koppenhöfer et al., 1996) with superior gut penetration ability (Wang and Gaugler, 1998), was used in this study as the target pest was a hairy caterpillar feeding on the foliage and aggregating in the bark of the tree (Ayyar, 1940). Five fourth instar larvae of G. mellonella were inoculated with one ml of water containing 100 IJ in a Petri dish (9 cm diameter) containing a wet filter paper (Whatman No.1) and maintained at 25 ± 0.5 °C. The infected larvae developed a dark color 36 h after infection. The IJ were harvested by a white trap method between 96 and 120 h after infection (Woodring and Kaya, 1988). They were washed with 0.1% formaldehyde and stored at 8.5 ± 1.5 °C under aerated conditions until further use (Hussaini et al., 1999).

Infectivity test

Second, third, fourth, fifth and sixth instar larvae of E. mollifera were exposed to a concentration of 25 IJ per larva. Polypots (one-ounce polypropylene containers of basal diameter 40 mm and 40 mm depth) were lined all over the internal surface with sterile filter paper (Whatman 1) and 1 ml of distilled water was added into the polypots and covered with filter paper strips moistened with sterile water. Four replicates per treatment were established for each instar. Finally, the polypots were covered with tightly secured muslin fabric for better aeration. To prevent the filter paper from drying out, sterile distilled water was added when required. The larvae were incubated at 25 ± 0.5 °C. The mortality of the larvae was observed at 12 h intervals up to 96 h after inoculation.

The penetration of S. glaseri was determined in third, fourth and fifth instars of both E. mollifera and G. mellonella. The concentrations of IJ used were 10, 20, 30, 40 and 50 per larva. Again, for each species of insect, each instar and each concentration of nematodes, a polypot bioassay as described above was done, with 10 larvae per replicate and four replicates of each treatment. The larvae were dissected and the populations of nematodes counted at 72 h after inoculation for E. mollifera and at 48 h after inoculation for G. mellonella, as the rate of growth and reproduction was faster in G. mellonella (Hussaini et al., 1999).

Concentration and time response

A polypot bioassay was carried out as described earlier to study the concentration and time response of third, fourth and fifth instar E. mollifera larvae to S. glaseri. Concentrations of 25, 20, 15, 10 and 5 IJ per larva were used along with an untreated control. Thirty E. mollifera larvae were used per dose of S. glaseri in each of the three replicates. The mortality of the larvae was observed at 12 h intervals up to 96 h after inoculation.

A further polypot bioassay was carried out to determine the multiplication of the nematode in fourth instar E. mollifera and G. mellonella larvae. The doses tested were 25, 50, 100, 150 and 200 IJ per larva. Thirty E. mollifera larvae were used per dose of S. glaseri in each of the three replicates. The mortality of the larvae was observed at 12 h intervals up to 96 h after inoculation and the dead larvae were transferred to ‘white-traps’ to collect emerging IJ.

Bark application of S. glaseri

The effects of S. glaseri were examined in field conditions simulated in the laboratory. Pieces of bark (25 cm × 1.5 cm) separated from moringa trees were moistened with distilled water and sprayed with 2000, 1000, 500, 250 or 125 IJ of S. glaseri in 10 ml of water mixed with 0.1% Tween 20. The control received only water with 0.1% Tween 20. Each treatment was replicated three times with ten insect larvae in each replicate. After treatment, the bark pieces were placed individually inside plastic buckets (30 cm × 20.5 cm) and the ten fourth instar larvae of E. mollifera were added. The buckets were closed with muslin fabric. The larvae were allowed to feed on moringa leaves 24 h after starting the experiment and the mortality of the larvae was observed at 24 h intervals.

Statistical analysis

The data (as percentages) were transformed to arcsine values and checked for normality. Analysis of variance was performed as a Completely Randomized Design (CRD) using the statistical software IRRISTAT version 3/93, Biometrics unit, IRRI and means were separated using Duncan’s Multiple Range Test (DMRT) (Gomez and Gomez, 1984). Probit analyses were performed by SPSS package version 7.5.

RESULTS AND DISCUSSION

The mortality of E. mollifera to S. glaseri at a concentration of 25 IJ per larva decreased significantly with the increase in age of the larvae (F = 15.05, df = 4, P = 0.01) (Fig. 1). The susceptibility of the second and third instars was significantly different from that of the fourth and fifth instars. The final instar had a significantly low-
er mortality rate (55%) than all other instars but the fifth. A similar decrease in susceptibility with age of larvae of *Pseudaletia unipuncta* Haworth (Noctuidae: Lepidoptera) to a strain of *Steinernema carpocapsae* Weiser was found by Medeiros *et al.* (2000). However, there are no earlier reports on the susceptibility of *E. mollifera* to *S. glaseri*. This decrease in mortality might be attributed to the presence of physical barriers, in the form of large numbers of tufts of hairs, which hinder the penetration of IJ into the later instars of *E. mollifera*. A similar decrease in susceptibility to *Steinernema* sp. with increased age of the larvae was observed in groundnut red hairy caterpillar *Amsacta* sp. (Arctiidae: Noctuidae) (Muralibaskaran *et al.*, 1996). However, the inverse was true in the case of *Spodoptera exigua* Hübner (Noctuidae: Lepidoptera) when tested with *Steinernema feltiae* Filippiev (Kaya, 1985). Karunakaran *et al.* (1999) and Rosa *et al.* (2002) found that the pathogenicity of entomopathogenic nematodes varied with the species of insect and nematode and the stage of the insect host and that no generalization can be made.

The penetration of different doses of *S. glaseri* IJ into different instars of *G. melonella* and *E. mollifera* differed significantly with both the stage of the insect (*F* = 57.38, *df* = 2,29, *P* = 0.01) and dose of the nematode inoculum (*F* = 18.28, *df* = 4,29, *P* = 0.01) (Fig. 2 and 3). The penetration of the nematode into *E. mollifera* increased up to 20-30 IJ per larva and the fourth instar larval stage and decreased at higher doses and later instars of the larva (Fig. 2). In *G. mellonella*, penetration increased with the dose of nematode inoculum up to 50 IJ per larva, for fourth and fifth instar larvae; however, for the third instar larvae maximum penetration was observed at 30 IJ per larva and penetration decreased at higher concentrations (Fig. 3). A variety of reasons could explain the differences, including size, immune response and host behaviour. The portals of entry for nematodes may be smaller in the younger instars (Gaugler and Malloy, 1981; Jackson and Brooks, 1995) and smaller instars may be less attractive in terms of host cues such as CO₂ or kairomones (Kaya, 1985). However, older larvae may also become less susceptible if their immune system becomes stronger (Watanabae, 1987) and reduced feeding might reduce nematode entry through these barriers.

### Table I. Dose and time mortality response of different instars of *Eupterote mollifera* to *Steinernema glaseri*.

<table>
<thead>
<tr>
<th>Instar</th>
<th>Response* (95% fiducial limits)</th>
<th>Slope ± S.E.</th>
<th>χ² (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Third</td>
<td>LC₅₀ 16.5 (12.3 – 26.1)</td>
<td>1.63 ± 0.44</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td>LT₅₀ 50.3 (45.3 – 56.5)</td>
<td>4.99 ± 0.78</td>
<td>2.89</td>
</tr>
<tr>
<td>Fourth</td>
<td>LC₅₀ 22.1 (16.5 – 24.7)</td>
<td>1.71 ± 0.55</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>LT₅₀ 62.1 (55.6 – 73.1)</td>
<td>4.96 ± 0.93</td>
<td>1.50</td>
</tr>
<tr>
<td>Fifth</td>
<td>LC₅₀ 24.1 (17.9 – 49.0)</td>
<td>1.78 ± 0.48</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>LT₅₀ 69.7 (63.9 – 81.3)</td>
<td>7.84 ± 1.71</td>
<td>0.71</td>
</tr>
</tbody>
</table>

*LC₅₀ - IJ per larva LT₅₀ - hours.
All lines were of significantly good fit (*P* = 0.05); χ² were not significant and no heterogeneity was used in the calculation of confidence limits.

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**Fig. 1.** Bioassay of *Steinernema glaseri* infective juveniles to assess susceptibility of different larval instars of *Eupterote mollifera*. Twenty five IJ per larva were used in the bioassay, which was conducted at 25 ± 0.5 °C. Different letters indicate significantly different % mortality at *P* = 0.05. Bars are means ± SE.
the mouth, which is the main portal of entry for nematodes into a host (Shapiro et al., 1999). The penetration was also significantly higher in *G. mellonella* (45-85%) than in *E. mollifera* (18-56%) \((F = 190.4, df = 4.29, P = 0.01)\) (Fig. 2 and 3). Morris et al. (1990) found that the numbers of nematodes invading *G. mellonella* were higher than in other lepidopteran insects. It is obvious that *G. mellonella*, which is the ideal laboratory host and so is greatly preferred (Kaya and Gaugler, 1993), attracted IJ strongly, resulting in high penetration in this experiment. Hui and Webster (2000) reported that cues from the larval cuticle influence the host finding ability of the nematode. Host defence may also have played a bigger role in reducing penetration of *S. glaseri* into *E. mollifera* than *G. mellonella*. Host defences to nematodes are influenced by cues from both the host (Hui and Webster, 2000) and the parasite cuticle (Brivio et al., 2002). Behavioural responses to host and host environmental cues are critical steps in the infection process of nematode species such as *S. glaseri* for finding, recognizing, and penetrating insects (Wang and Gaugler, 1998).

The dose mortality responses of *E. mollifera* to *S. glaseri* indicated that *E. mollifera* was highly susceptible to *S. glaseri* irrespective of the age groups tested. The LC50 and LT50 were in the range of 16.5-24.1 IJ per larva and 50.3-69.7 h, respectively (Table I). Although the

**Fig. 2.** Penetration of *S. glaseri* into different larval instars of *E. mollifera*. A common letter in lower case indicates no significant difference in penetration between different instars of insects within a given dose of inoculum by DMRT \((P = 0.05)\). A common capital letter indicates no significant difference in penetration rates over different doses of inoculum within given instars of insects by DMRT \((P = 0.05)\). Bars are means ± SE.

**Fig. 3.** Penetration of *S. glaseri* into different larval instars of *Galleria mellonella*. A common letter in lower case indicates no significant difference in penetration between different instars of insects within a given dose of inoculum by DMRT \((P = 0.05)\). A common capital letter indicates no significant difference in penetration rates over different doses of inoculum within given instars of insects by DMRT \((P = 0.05)\). \* indicates significant and N indicates no significant differences between the two species of insects at given doses of inoculum and stages of insect by DMRT \((P = 0.05)\). Bars are means ± SE.
LC₉₀ and LT₉₀ increased slightly with increase in larval age, they did not differ significantly as is evident from the overlapping 95% fiducial limits. Entomopathogenic nematode multiplication in fourth instar E. mollenlera was significantly lower (0.11 - 0.21 × 10⁴ IJ per g of larva) than G. mollenlera (2.75 - 3.87 × 10⁵ IJ per g of larva) (F = 3516.9, df = 1.9, P = 0.01) (Table II). Josephrajkumar and Sivakumar (1998) also found decreased multiplication of Steinernema sp. in the hairy caterpillar, Utetheisa pulchella Linnaeus (Arctiidae: Noctuidae). The dose of IJ used for inoculation significantly influenced the multiplication of IJ in G. mollenlera, but did not influence the multiplication of IJ in E. mollenlera (F = 11.85, df = 4,9, P = 0.01). Koppenhöler and Kaja (1995) reported that reproduction of S. glaseri in G. mollenlera increased up to 58 IJ per larva, above which it decreased until at 184 IJ per larva no progeny emerged from the larvae.

In our studies, we found that the multiplication of S. glaseri could be slow as the time taken by IJ to emerge from E. mollenlera was longer than from G. mollenlera. This might be attributed to the lower nutritional status of E. mollenlera larvae compared to that of G. mollenlera larvae and to host plant influence, as G. mollenlera was reared on a rich nutritional diet. Hussaini et al. (1999) determined the nutritional requirement of IJ of Steinernema sp. and identified G. mollenlera as the most suitable host with a yield of 5.14 × 10⁵ IJ per g of larva; the larvae of G. mollenlera contain more protein and lipid than those of Corcyra cephalonica Stainton (Pyraulidae: Lepidoptera), Agrotis ipsilon Hufnagel (Noctuidae: Lepidoptera), Helicoverpa armigera Hübner (Noctuidae: Lepidoptera) and Spodoptera litura Fabricius (Noctuidae: Lepidoptera). Similar reports on the variability of host quality have been documented by several other authors (Mamiya, 1989; Barbercheck, 1993; Epsky and Capinera, 1993).

The experiment on the application of the nematode to bark indicated that a spray dose of 1000 IJ was optimum for the control of the pest under the conditions used, with 83.3% mortality of the host insect; at higher dosage levels mortality was not significantly greater (F = 387.1, df = 4,24, P = 0.01) (Table III).

**Table II.** Multiplication of S. glaseri IJ in larvae of E. mollenlera and G. mollenlera.

<table>
<thead>
<tr>
<th>Dose of IJ used</th>
<th>IJ per g of larva (x 10⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. mollenfera*</td>
</tr>
<tr>
<td>25</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>50</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>100</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>150</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>200</td>
<td>0.11 ± 0.03</td>
</tr>
</tbody>
</table>

* Means are not significantly different by DMRT (P > 0.05).
** Means followed by the same letter do not differ significantly by DMRT (P = 0.05).

Steinernema glaseri is a cruiser and has the greatest ability among the steinernematids to find its host (Wouts, 1991). Mráček and Bečvár (2000) observed, during a survey, that insect aggregation influenced nematode activity. The congregation behaviour of E. mollenlera larvae at night on the bark of the trees could be exploited to target the pest using entomopathogenic nematodes. Such a biological approach is preferable to the use of insecticides for pest management in moringa as insecticides can leave undesirable residues. Detailed field studies would yield valuable information on the effectiveness of entomopathogenic nematodes against E. mollenlera as an alternative to insecticides for the eco-friendly management of E. mollenlera.

**ACKNOWLEDGEMENT**

The authors are grateful to the Tamil Nadu Agricultural University for providing the facilities and granting permission to carry out the ad hoc experiments. The supply of S. glaseri by Dr. S. Easwaramoorthy, Head, Division of Crop Protection, Sugarcane Breeding Institute, Coimbatore, India is gratefully acknowledged.

**Table III.** Efficacy of S. glaseri sprays against fourth instar E. mollenlera larvae.

<table>
<thead>
<tr>
<th>IJ used/10 ml</th>
<th>72</th>
<th>96</th>
<th>120</th>
<th>144</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>16.7 ± 1.7 aD</td>
<td>41.7 ± 4.4 aC</td>
<td>66.7 ± 1.7 aB</td>
<td>83.3 ± 3.3 aA</td>
<td>83.3 ± 3.3 aA</td>
</tr>
<tr>
<td>1000</td>
<td>8.3 ± 1.7 bE</td>
<td>26.7 ± 3.3 bD</td>
<td>50.0 ± 2.9 bC</td>
<td>66.7 ± 3.3 bB</td>
<td>83.3 ± 1.7 aA</td>
</tr>
<tr>
<td>500</td>
<td>0.0 ± 0.0 bE</td>
<td>8.3 ± 1.7 cD</td>
<td>16.7 ± 1.7 cC</td>
<td>33.3 ± 3.3 cB</td>
<td>58.3 ± 1.7 bA</td>
</tr>
<tr>
<td>250</td>
<td>0.0 ± 0.0 bE</td>
<td>8.3 ± 1.7 cD</td>
<td>25.0 ± 2.9 cB</td>
<td>41.7 ± 4.4 cA</td>
<td>41.7 ± 4.4 cA</td>
</tr>
<tr>
<td>125</td>
<td>0.0 ± 0.0 bE</td>
<td>0.0 ± 0.0 dB</td>
<td>0.0 ± 0.0 dB</td>
<td>8.3 ± 3.3 dA</td>
<td>8.3 ± 1.7 dA</td>
</tr>
</tbody>
</table>

* In a column, means followed by the same letter in lower case do not differ significantly according to DMRT (P = 0.05) at different doses of nematodes. In a row, means followed by the same capital letter do not differ significantly according to DMRT (P = 0.05) over different times.
LITERATURE CITED


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