[1]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 LC_{50} and LT_{50} 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 × 10^5 per g of larva) in *E. mollifera* than in *G. mellonella* (2.75-3.87 × 10^5 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 Bečvár, 2000). *Steinernema glaseri* (Steiner, 1929) Wouts, Mráček, Gerdin *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 (Jahn, 1984), edible oil from seeds for use in cosmetic 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.

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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 colour 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 No. 1) and 1 ml of distilled water was added to the filter paper along with the nematode juveniles. Ten larvae of *E. mollifera* per replicate were transferred 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 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 by 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-

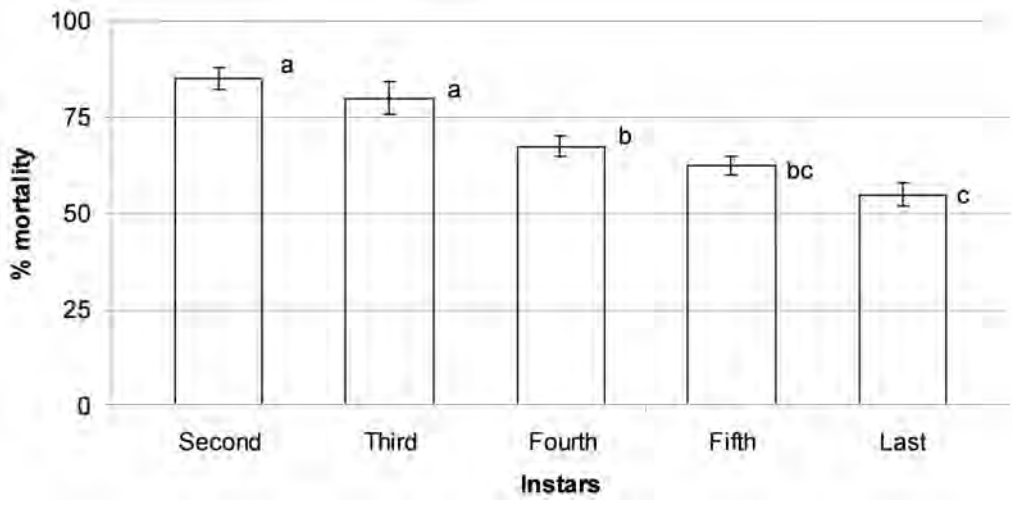

Fig. 1. Bioassay of *Steinernema glaseri* infective juveniles to assess susceptibility of different larval instars of *Eupterote mollifera.* Twenty five IJ per larva were used in the bioassay, which was conducted at 25 ± 0.5 ºC. Different letters indicate significantly different % mortality at $P = 0.05$. Bars are means ± SE.

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* Filipjev (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 CO_2 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

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

Instar		Response* (95% fiducial limits)	Slope ± S.E.	$\chi^{2\,*(n-2)}$
Third	LC_{50}	16.5 (12.3 – 26.1)	1.63 ± 0.44	1.31
	LT_{50}	50.3 (45.3 – 56.5)	4.99 ± 0.78	2.89
Fourth	LC_{50}	22.1 (16.5 – 42.7)	1.71 ± 0.55	1.13
	LT_{50}	62.1 (55.6 – 73.1)	4.96 ± 0.93	1.50
Fifth	LC_{50}	24.1 (17.9 – 49.0)	1.78 ± 0.48	0.60
	LT_{50}	69.7 (63.9 – 81.3)	7.84 ± 1.71	0.71

* LC_{50} - IJ per larva LT_{50} - hours.
All lines were of significantly good fit ($P = 0.05$); χ^2 were not significant and no heterogeneity was used in the calculation of confidence limits.

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 LC_{50} and LT_{50} were in the range of 16.5-24.1 IJ per larva and 50.3-69.7 h, respectively (Table I). Although the

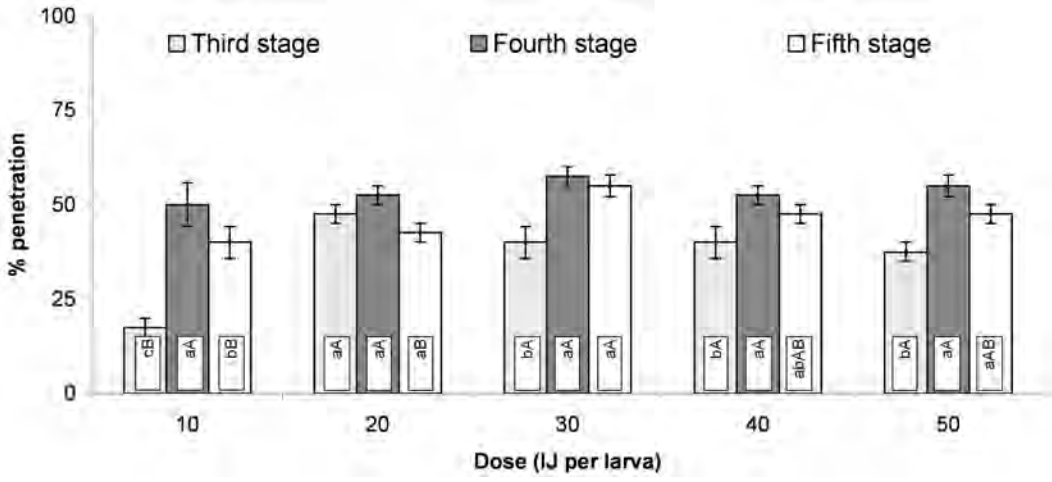

Fig. 2. Penetration of *S. glaseri* into different larval instars of *E. mollifera.* A common letter in lower case indicates no significant difference in penetration between different instars of insects within a given dose of inoculum by DMRT (P = 0.05). A common capital letter indicates no significant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

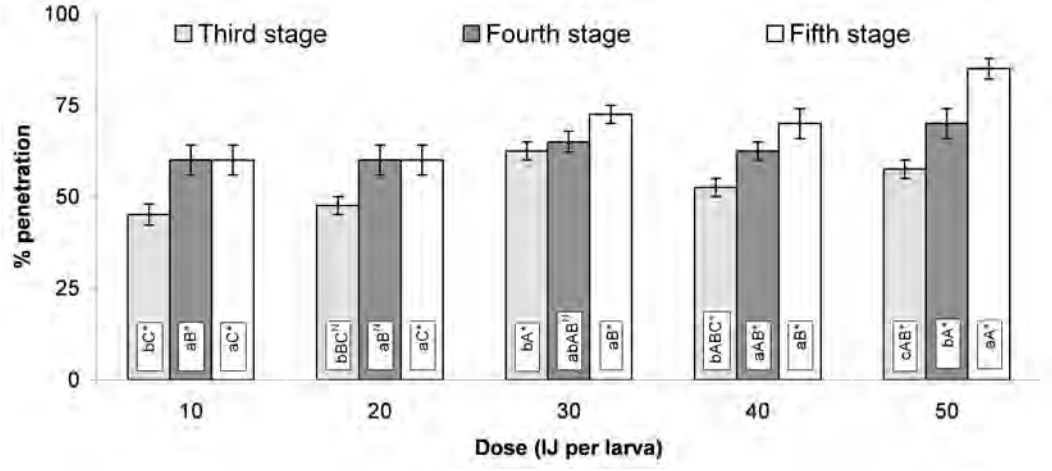

Fig. 3. Penetration of *S. glaseri* into different larval instars of *Galleria mellonella.* A common letter in lower case indicates no significant difference in penetration between different instars of insects within a given dose of inoculum by DMRT (P = 0.05). A common capital letter indicates no significant 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.

LC_{50} and LT_{50} 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. mollifera* was significantly lower (0.11 - 0.21 × 10^5 IJ per g of larva) than *G. mellonella* (2.75 - 3.87 × 10^5 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, *Utethesia pulchella* Linnaeus (Arctiidae: Noctuidae). The dose of IJ used for inoculation significantly influenced the multiplication of IJ in *G. mellonella,* but did not influence the multiplication of IJ in *E. mollifera* (F = 11.85, df = 4,9, P = 0.01). Koppenhöfer and Kaya (1995) reported that reproduction of *S. glaseri* in *G. mellonella* 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. mollifera* was longer than from *G. mellonella.* This might be attributed to the lower nutritional status of *E. mollifera* larvae compared to that of *G. mellonella* larvae and to host plant influence, as *G. mellonella* was reared on a rich nutritional diet. Hussaini *et al.* (1999) determined the nutritional requirement of IJ of *Steinernema* sp. and identified *G. mellonella* as the most suitable host with a yield of 5.14 × 10^5 IJ per g of larva; the larvae of *G. mellonella* 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. mollifera* and *G. mellonella*.

Dose of IJ used	IJ per g of larva (x 10^5)	
	*E. mollifera**	*G. mellonella***
25	0.19 ± 0.04	3.12 ± 0.14 b
50	0.21 ± 0.01	3.24 ± 0.07 b
100	0.11 ± 0.02	3.87 ± 0.15 a
150	0.15 ± 0.05	3.22 ± 0.04 b
200	0.11 ± 0.03	2.75 ± 0.11 c

* 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. mollifera* 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. mollifera* as an alternative to insecticides for the ecofriendly management of *E. mollifera*.

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

IJ used/10 ml	Mean per cent mortality at different hours after inoculation*				
	72	96	120	144	168
2000	16.7 ± 1.7 aD	41.7 ± 4.4 aC	66.7 ± 1.7 aB	83.3 ± 3.3 aA	83.3 ± 3.3 aA
1000	8.3 ± 1.7 bE	26.7 ± 3.3 bD	50.0 ± 2.9 bC	66.7 ± 3.3 bB	83.3 ± 1.7 aA
500	0.0 ± 0.0 cE	8.3 ± 1.7 cD	16.7 ± 1.7 cC	33.3 ± 3.3 cB	58.3 ± 1.7 bA
250	0.0 ± 0.0 cD	8.3 ± 3.3 cC	25.0 ± 2.9 cB	41.7 ± 4.4 cA	41.7 ± 4.4 cA
125	0.0 ± 0.0 cB	0.0 ± 0.0 dB	0.0 ± 0.0 dB	8.3 ± 3.3 dA	8.3 ± 1.7 dA

* 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.

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