MASS PRODUCTION OF ENTOMOPATHOGENIC NEMATODES USING SILKWORM (*BOMBYX MORI* L.*)* FOR MANAGEMENT OF KEY AGRICULTURAL PESTS

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Abstract

Entomopathogenic nematodes are biological control agents of arthropod pests that can be multiplied by *in vivo* and *in vitro* methods. They are effective when well marched with host arthropods but the major drawback to their wide use is their availability on demand. The *in vivo* method is appropriate for a cottage industry and the *in vitro* method for massive production. The objective of the study was to determine the suitability of the silkworm (*Bombyx mori*) as an alternative host to the greater wax moth (*Galleria mellonella*) for multiplication of entomopathogenic nematodes. This study demonstrated that a dose of 100 infective juveniles per the fifth instar silkworm (*Bombyx mori*) can yield over 60,000 *Heterorhabditis indica* infective juveniles, 40,000 *Steinernema karii* juveniles and 15,000 *Steinernema yirgalemense* juveniles at 20-25^{0}C and at a relative humidity of 60% in seven days. The results compared well with yields of 50,000 *H. indica*, 45,000 *S. karii* and 80,000 of *S. yirgalemense* from *Galleria mellonella* (the greater wax moth) which is the conventionally used insect host for *in vivo* multiplication of entomopathogenic nematodes. The results indicate that for optimum yields of entomopathogenic nematodes, there is need to match the host with the nematode species.

Key Words: Entomopathogenic nematodes, *Bombyx mori*, *Galleria mellonella*

Introduction

Entomopathogenic nematodes will only be widely used as pest control products when they become available on demand by the different clients (commercial growers and small scale farmers). Small scale farmers will benefit from *in vivo* production of nematodes using cheap materials and ideally those from their farms while large scale commercial farms' nematode needs can be met by the capital investment mass propagation methods using fermentation chambers (Ehlers *et al.,* 1998). The latter have been developed fairly well (Ehlers and Shapiro, 2005; Shapiro and McCoy, 2000). The start up costs for *in vitro* methods is beyond the scope of small-scale farmers. The hosts used in *vivo* methods must be susceptible, have high multiplication potential, not prone to become crop pests and reared easily using cheap materials. *In vivo* nematode production is labour intensive and produces good quality nematodes (Shapiro *et al.,* 2000).

Diets used to raise nematode hosts have effects on juvenile yields with antibiotics like nipagin lowering host susceptibility by 50-250% (Kermarrec and Mauleon, 1989; Nunchanart, 2002). *Galleria mellonella* is the conventional host for *in vivo* multiplication of entomopathogenic nematodes. *Galleria* occurs naturally in bee hives and is reared using artificial diets made of cereals, wax, yeast and glycerol. These dietary components are purchased from markets and are therefore an additional expense (Costa *et al.,* 2007). The silkworm is a Lepidopteran that is related to *Galleria* but that feeds on mulberry leaves and twigs (Goldsmith *et al.,* 2004). The mulberry tree has been domesticated for over four centuries in the orient and over three decades in Kenya for silk production (MOA&RD, 2003). Preliminary tests showed that the silk worm is highly susceptible to entomopathogenic nematodes and a potential host for multiplication of entomopathogenic nematodes. The objective of this study was to determine the relative ease of obtaining *Heterorhabditis indica, Steinernema karii* and *Steinernema yirgalemense* from the silkworm larval instars compared to the third *Galleria* larval instar at two infective dose levels per larva. The most efficient and cost effective host would be promoted for use by farmers.

Materials and Methods

Three hundred Petri dishes were lined with Whatman filter paper and the nematodes S*teinernema karii*, *Heterorhabditis indica* and *Steinernema yirgalemense* applied at the rate of 100 ij per dish (9 x 3.5) in fifty dishes for each nematode species and at 200ij/dish to another batch of fifty for each nematode species. The nematodes were applied in 1ml distilled water and given 30 minutes to distribute on the filter. The fifty Petri dishes treated with 100ij of *S. karii* per dish were subdivided into five groups of ten. The treatments (third instar *G. mellonella* and the 5th, 4th, 3rd and 2nd silk worm larval instars) were applied in ten replicates first to Petri dishes treated with 100 ij of *S. karii* and then to the 200 ij of *S. karii* dose. The procedure was repeated for *H. indica* and S. *yirgalemense* and the experiment laid out in a completely randomised design. The treatments were left on laboratory benches at room temperature (18-25°C) and 60% relative humidity for three days. The

cadavers from each treatment were placed in own White traps (Woodring & Kaya, 1988) for extraction of emerging entomopathogenic nematodes. Nematodes were harvested for seven days and cleaned by sedimentation and decantation. Nematodes from each treatment were counted under a binocular microscope.
The mortality data was subjected to Chi-square analysis while nematode yield data was analysed for variance. Significantly different treatment means were separated using the Student Neuman Keus (SNK).

Results

Galleria and silkworm mortality occurred between 24 and 72 hours in all treatments. Infected silkworm larvae were floppy and retained their cream colour while *Galleria* infected with *S. karii* and *S. yirgalemense* maintained the pale colour and those infected with *H. indica* the brick red colour characteristic of heterorhabditid infected *Galleria* (Woodring and Kaya, 1988). Younger larval instars died faster than older ones in all treatments. The lower dose (100ij) of *Steinernema karii* and *S. yirgalemense* was faster acting on larvae of all ages than the higher dose (200ij) but the rate of larval mortality in *H. indica* treatments was higher for the 200ij/larva dose for the 4th and 5th silk worm instars and *Galleria* (Table 1). The silk worm treatments were harvested thrice but it took more than five harvest times for *Galleria* cadavers to be depleted.

Table 1: Mean percent insect host mortality twenty four hours after nematode application

Insect host	S. karii		H. indica		S. yirgalemense	
	100ij/larva	200ij/larva	100ij/larva	200ij/larva	100ij/larva	200ij/larva
Galleria mellonella	0 b	0a	10b	100a	100a	0b
Silk worm stage 5	0 b	0 a	90a	80a	10b	0b
Silkworm Stage 4	20 b	0a	0b	90a	10b	0b
Silkworm stage 3	100 a	0a	100a	100a	100a	100a
Silkworm stage 2	100 a	0a	100a	100a	100a	100a
LSD	20		52		20	

Means in the same column sharing a superscript letter are not significantly different at P<0.05

Steinernema yirgalemense did not reproduce juveniles in the second silkworm larval instar while only ten to twenty percent of the replicates of *S. karii* and *H. indica* treatments reproduced in this larval instar for both the 100 and 200 ij per larva doses. All the silkworm larval instars reproduced *H. indica* nematode juveniles. The second instar, however, reproduced poorly at both the lower and upper dose levels (Table 2).

Table 2: Mean percent replicates reproducing entomopathogenic nematodes

Nematode	Insect instar	Percent productive replicates	
		100ij/larva	200ij/larva
Steinernema yirgalemense	3rd instar Galleria larva	90 a	60a
	Silkworm stage 2	0c	0c
	Silkworm stage 3	70ab	10c
	Silkworm stage 4	80a	80a
	Silkworm stage 5	50b	70a
Steinernema karii	Galleria stage 3	60b	60b
	Silk worm stage 2	20c	10c
	Silkworm stage 3	80ab	80ab
	Silkworm stage 4	90a	70b
	Silkworm stage 5	90a	100a
Heterorhabditis indica	Galleria stage 3	100a	100a
	Silkworm stage 2	20b	10b
	Silkworm Stage 3	100a	100a
	Silkworm stage 4	100a	100a
	Silkworm stage 5	100a	100a
LSD		24	

Means (per nematode treatment) in the same columns that share a superscript letter are not significantly

different at P<0.05

Steinernema yirgalemense was the highest yielding nematode in *G. mellonella* while *H. indica* yielded the highest number of infective juveniles in the 5th silkworm larval instar. *Steinernema karii* yielded moderately in the 4th and 5th silkworm instars and the 3rd *Galleria* instar. The mean yield of *S. karii* in the three larval instars (4th and 5th silkworm and 3rd *Galleria* instars) were not significantly different among themselves at P<0.05 (Table 3)

Table 3: Mean number of nematode juveniles reproduced per insect host

Insect host	S. yirgalemense	S. karii	H. indica
Galleria mellonella	60,680 a	43,780a	43,470a
Bombyx mori 2nd instar	3b	60b	99cb
Bombyx mori 3rd instar	583b	7170b	6620b
Bombyx mori 4th instar	4430b	35,620a	10,920b
Bombyx mori 5th instar	8945b	38,970a	63,170 a
LSD	31,850		

Means in the same columns sharing a superscript letter are not significantly different at P< 0.05

The nematode yields were higher at the 100ij/larva dose than the 200ij/larva rate with the exception of *S. yirgalemense* in the 5th silkworm larval instar which yielded higher numbers of infective juveniles at the 200ij dose per larva treatment (Table 4).

Table 4: Mean number of nematode juveniles reproduced per dose of applied nematodes

Nematode	Insect host	Dose(ij/larva	Yields (infective juveniles)	Yields/ Juvenile applied
Steinernema yirgalemense	Galleria mellonella	100	80000 a	800
		200	41000ab	205
	Silkworm stage 2	100	10b	0
		200	0b	0
	Silkworm stage 3	100	1100b	11
		200	20b	0
	Silkworm stage 4	100	2600b	26
		200	60b	0
	Silkworm stage 5	100	3200b	32
		200	15000b	75
Steinernema karii	Galleria mellonella	100	46000ab	460
		200	42000ab	210
	Silkworm stage 2	100	100b	1
		200	0b	0
	Silkworm stage 3	100	4400b	44
		200	10000ab	50
	Silkworm stage 4	100	53000a	530
		200	18000ab	90
	Silkworm stage 5	100	40000ab	400
		200	38000ab	190
Heterorhabditis indica	Galleria mellonella	100	51000ab	510
		200	41000ab	205
	Silkworm stage 2	100	100c	1
		200	90c	1
	Silkworm stage 3	100	5200c	52
		200	8100bc	41
	Silkworm stage 4	100	12000b	120
		200	9000bc	45
	Silkworm stage 5	100	62000a	620
		200	63,000a	315
LSD		45,700		

Means (per nematode treatment) in the same column sharing a superscript letter are not significantly

different at P< 0.05Discussion

The fifth silkworm larval instar was comparable to *Galleria* in suitability for multiplication of entompathogenic nematodes juveniles. *Galleria* was the most suited host for reproducing *Steinernema yirgalemense* at both the lower and upper dose of infective juveniles per larva and the 5th silkworm larval instar the most suited for *H. indica*. *Steinernema karii* however reproduced moderately in *Galleria*, the fourth and fifth silkworm instar. *Galleria* and silk worm are Lepidopterans which share the silk producing gene (Goldsmith *et al.,* 2004). The good performance of the silkworm could probably be attributed to both its high levels of amino acids and large body weight (mean body weight of 5th silkworm larval instar was 1.6g compared to *Galleria* 0.2g) (Zang *et al.,* 1990: Flanders *et al.,* 1996). The 5th silkworm instar was fatty. Diets rich in lipids increased juvenile yields in both *in vivo* and *in vitro* reproduction (Moeen *et al*., 1998; Gil *et al.,* 2002). Nematodes with small juveniles were also more productive than large nematodes (Zervos, 1991). This may explain the higher yields of *H. indica* whose juveniles are small in size in this study but *S. yirgalemense* is a large nematode and yet the highest yielding nematode species in *Galleria*. Incubation temperature during reproduction is important (Moeen *et al,* 1998). Earlier on, while testing different media for nematode storage, *S. karii* and *H. indica* survived better and were more infective at 15^0C while *S. yirgalemense* stored better at 25^0C. The laboratory temperatures during the study ranged between 21 and 25^0C which could be ideal for the *S. yirgalemense* and *Galleria* match but not for the silkworm *S. Yirgalemense* match.

The 5th larval instar takes 12 days to pupate which is enough time for farmers to make decisions on whether to use the larva for producing nematodes or cocoons (pupae) for silk production depending on demand. These results suggest that for optimum nematode yields, the nematodes should be screened for effectiveness on important pests and selected nematodes matched with appropriate hosts for propagation. The establishment of the silkworm as an alternative host for entomopathogenic nematodes reproduction is an important finding because the silkworm is easily raised by farmers in most farming districts of Kenya (MOA&LD, 2003). The raising of the silkworm requires some quarantine measures which farmers have mastered over the years. The activity is sustainable as it fits with farmer practice and nematode propagation is an easy task that farmers can take up. This means that the silkworm can be dual purpose for the farmer (for nematode multiplication or for silk production depending on the demand of the moment). The insect host to be selected for nematode multiplication will be determined by the cost and nematode species to be reproduced.

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