EVALUATION OF ENTAMOPATHOGENIC NEMATODES AGAINST *AETHERASTIS CIRCULATA*

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A total of 345 soil samples from rubber growing areas were collected from ten districts of Kerala to find out the natural occurrence of entomopathogenic nematodes (EPN). Bioassay of samples was conducted by soil baiting technique using fifth instar larvae of greater wax moth, *Galleria mellonella*, under the laboratory condition. Seventy three samples (21%) yielded EPN, among these 61 samples (18%) collected from Thiruvananthapuram, Kottayam, Thrissur, Palakkad, Kozhikode and Malappuram regions yielded both *Heterorhabditis* and *Steinernema* spp. Twelve soil samples (3%) collected from Kollam, Pathanamthitta, Ernakulam and Idukki regions showed the presence of *Heterorhabditis* sp. alone. Laboratory and field evaluation of EPN against bark feeding caterpillar, *Aetherastis circulata* infesting rubber plants was conducted. The results of the laboratory study showed 84 per cent mortality of second instar larvae of *A. circulata* within 24 h followed by 88 and 92 per cent mortality of third and fourth instar larvae, respectively when they were inoculated with EPN @ 320 ijls/5 larvae. Hundred per cent mortality was recorded at 48 h of exposure. The multiplication rate was found to be reduced at lower as well as higher inoculum levels. No significant effect was noticed upon the field application of EPN against *A. circulata*.

Keywords : *Aetherastis circulata*, Biocontrol, Entomopathogenic nematodes

Entomopathogenic nematodes (EPN) belonging to the genera, *Steinernema* and *Heterorhabditis* are recognized as potential biocontrol agents for a range of insect pests of agricultural importance (Poinar, 1979). The important attributes making these nematodes ideal for biocontrol are their broad host range, high virulence, safety to non-target organisms, ability to search for hosts, high efficiency in favourable habitats, high reproductive potential, ease of mass production and compatibility with control strategies (Kaya and Gaugler, 1993). Hence, numerous surveys were conducted world wide and these nematodes were recovered from many habitats. The objectives of the present study were to investigate the occurrence of EPN in rubber growing soils, collect locally adopted isolates and evaluate their bio-control efficiency against the bark feeding caterpillar, *Aetherastis circulata*, under laboratory and field conditions.

A total of 345 soil samples were collected from ten districts of Kerala, *viz.* Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam, Ernakulam, Thrissur, Palakkad, Kozhikode, Malappuram and Idukki to find out the natural occurrence of EPN in rubber growing soils. The samples were collected

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randomly at a depth of 0 to 15 cm from an area of about 1m,2 pooled and transported in polythene bags to laboratory. Bioassay of the samples was conducted by baiting technique using fifth instar larvae of greater wax moth, *Galleria mellonella* (Bedding and Akhurst, 1975). Five to ten larvae were placed at the bottom of a plastic container (500 mL capacity) filled with sampled soil and incubated at room temperature for six to ten days. Mortality of the larvae was observed at 24 h interval for ten days. The genus identification was carried out based on the colour of the cadavers. The galleria larvae infested with *Steinernema* spp. became flaccid and their colour changed from white to yellow brown, while in case of *Heterorhabditis*, the colour changed from white to brownish red or brick red. The number of samples showing the presence of EPN and the percentage of occurrence of *Heterorhabditis* and *Steinernema* spp. were also recorded. The dead larvae were washed with 4 per cent sodium hypochlorite, twice with distilled water and placed on white trap in BOD incubator at 30 ^{0}C for the collection of infective juveniles (White, 1927). The emergence of nematodes from the cadavers started after 5 to 6 days and 9 to10 days for *Steinernema* spp. and *Heterorhabditis* spp. respectively. The harvesting of infective juveniles was made thrice a week until production ceased.

The infective juveniles (ijls) were tested for their pathogenicity against bark feeding caterpillar, *Aetherastis circulata* by conducting laboratory and field experiments. Second, third and fourth instar larvae of *A. circulata* from the field was collected and inoculated with different concentrations of EPN such as 10, 20, 40, 80, 160 and 320 ijls per 5 larvae with 4 replications. The mortality were recorded at 24, 48 and 72 h of exposure. Progeny productions of EPN (multiplication rate) at different inoculum levels were also evaluated.

A field study was conducted to evaluate the comparative effectiveness of EPN @ 2 lakh per tree along with insecticides imidacloprid 0.005 per cent and fenvalarate 0.02 per cent. A combination of insecticides with a stem remedy formulation (SRF, a product developed by Kerala Agricultural University) was also sprayed on the stem up

Table 1. **Occurrence of entomopathogenic nematodes in rubber growing soils of Kerala**

District	No. of samples	No. of samples positive for EPN		
		Heterorhabditis spp.	*Steinernema* spp.	Total
Thiruvananthapuram	40	6 (15%)	2 (5%)	8 (20%)
Kollam	25	3 (12%)	-	3 (12%)
Pathanamthitta	45	4 (9%)	-	4 (9%)
Kottayam	35	7 (20%)	2 (6%)	9 (26%)
Ernakulam	35	2 (6%)	-	2 (6%)
Thrissur	35	10 (29%)	2 (6%)	12 (35%)
Palakkad	40	10 (25%)	3 (8%)	13 (33%)
Kozhikode	30	9 (30%)	1 (3%)	10 (33%)
Malappuram	30	7 (23%)	2 (7%)	9 (30%)
Idukki	30	3 (10%)	-	3 (10%)
Total	345	61 (18%)	12 (3%)	73 (21%)

Table 2. **Per cent mortality of *A. circulata* larvae after exposure to infective juveniles of EPN (Average of four replications)**

No. of ijls/	24 h			48 h			72 h		
5 larvae	2nd instar larvae	3rd instar larvae	4th instar larvae	2nd instar larvae	3rd instar larvae	4th instar larvae	2nd instar larvae	3rd instar larvae	4th instar larvae
10	0	0	20	32	44	76	76	88	92
20	12	8	32	52	60	80	80	80	88
40	20	28	44	76	64	84	92	92	84
80	24	36	64	72	76	84	100	100	100
160	40	52	68	76	84	92	100	100	100
320	84	88	92	92	100	100	100	100	100
Control	0	0	0	0	0	0	0	0	0

to a height of 5M. The experiment was conducted in CRD in clone RRII 105 with six treatments and four replications (one tree per plot). The pre- and post-treatment numbers of caterpillars up to one metre height were recorded to evaluate the effect of treatments.

The survey indicated the presence of EPN in rubber growing soils (Table 1). Out of 345 soil samples tested, 73 samples (21%) yielded EPN, among these 61 samples (18%) collected from ten districts in Kerala yielded both *Heterorhabditis* and *Steinernema* spp. Twelve samples (3%) collected from Kollam, Pathanamthitta, Ernakulam and Idukki regions showed the presence of *Heterorhabditis* sp. alone. The reason for the low percentage of occurrence of EPN may be due to the non- availability of enough soil fauna for the recycling of EPN in rubber plantations (Josephkumar and Sivakumar, 1997).

The results of the laboratory study on the effect of EPN @ 320 ijls/5 larvae of bark feeding caterpillars showed 84 per cent mortality of second instar larvae within 24 h followed by 8 and 92 per cent mortality of third and fourth instar larvae, respectively. Hundred per cent mortality of third and fourth instar larvae was recorded at 48 h of exposure (Table 2). When *A. circulata* larvae was inoculated with 40ijls, the rate of multiplication was 201200 followed by 107460 at 20 and 83808 at 80 ijls/5 larvae, respectively. The multiplication rate was found to be reduced at lower as well as higher inoculum levels (Table 3). A dose that is too low results in low host mortality and too high dose results in high level of non-infection due to competition with secondary invaders. Razak and Sivakumar (1989) studied the influence of inoculum levels in multiplication of *Steinernema feltiae* (DD- 136 strain) on *Corcyra cephalonica* and observed that the nematode multiplication was reduced at the lowest and highest level of inoculum. Similar study conducted by Karunakar *et al.* (1993) also showed that the

Table 3. **Effect of inoculum levels on the multiplication of EPN in *A. circulate***

Inoculum levels of ijls/5 larva	Production of ijls/5 larva
10	43350
20	107460
40	201200
80	83808
160	31800
320	27547

Table 4. **Effect of EPN and insecticides on *A. circulata* in rubber**

Treatment	Mean per cent reduction of caterpillar	
	After one week	After three weeks
Imidacloprid 0.005% +SRF	28.99	40.84
Imidacloprid 0.005%	32.70	40.35
Fenvalerate 0.02%+ SRF	41.35	54.28
Fenvalerate 0.02%	45.57	53.43
EPN 2lakhs ijls /tree	19.31	26.25
Control	14.63	23.31
CD (P = 0.05)	*4.16*	*8.39*

dosage of 20 ijls of *H. indicus* per fifth instar larvae of sugarcane internode borer, *Chilosacchariphagus indicus* (Kapur) yielded significantly highest multiplication rate of 210283.3 ijl/larva followed by 199472.5 @ 10 ijls/larva.

The field study showed 54 per cent control of bark feeding caterpillars by the application of fenvalarate 0.02 per cent after three weeks followed by imidacloprid 0.005 per cent (40.35%) (Table 4). No additional effect was recorded by the combination of insecticides with SRF. Similarly, no significant effect was noticed on the field application of EPN against bark feeding caterpillar, *A. circulata*. It showed only 19.31 and 26.25 per cent control of bark feeding caterpillars after one and three weeks, respectively. Though EPN showed very good mortality of the larvae of *A. circulata* in the laboratory, it was not effective in the field condition. Hence, EPN is not advisable for the control of bark feeding caterpillars in rubber plantations.

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