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Distribution of entomopathogenic nematodes in coconut gardens of Kerala State and pathogenicity of *Steinernema feltiae* to red weevil, *Rhynchophorus ferrugineus*

V.K. Sosamma

Division of Crop Protection, Central Plantation Crops Research Institute, Regional Station, Kayangulam, Krishnapuram P.O., Kerala, India - 690533.

Abstract

Steinernema feltiae and *Steinernema* spp. were found at five and *Heterorhabdtiis indica* at 128 sites out of 480 samples collected from coconut gardens in 10 districts of Kerala. Two hundred and fourteen (45%) were positive for *Rhabditis* sp. and 16(3%) for *Aphelenchus* sp. Analysis of soil samples revealed the highest occurrence of EPN in sandy loam soil followed by laterite and alluvial soil types. Soil samples collected from termite mounts did not yield any EPN. Forty per cent of soil samples yielded an earthworm *Fridericia khallikottosus* alongwith entomophilic nematodes. This indicates that EPN is not harmful to other non-target/beneficial organisms. Ninety per cent of the frass collected from red weevil infested coconut palms yielded *Teratorhabditis palmarum*, which was only entomophilic and not pathogenic to red weevil. In bioefficiency tests of *S. feltiae*, on red weevil grubs, mortality occurred in 3-4 days. When the last instar larvae of Red weevil inoculated with 1000 nematodes, the adults emerged, were of reduced size compared to healthy ones. Soil application of *S. feltiae* against red weevil adults in the net house in the field resulted in 55-60% mortality of weevils, while in control it was only 10-12%.

Key words: Coconut, entomopathogentic nematodes, *Steinernema feltiae*, *Heterorhabditis indica*, red weevil

Introduction

Entomopathogenic nematodes (EPN) belonging to families Heterorhabditidae and Steinernematidae have considerable potential as biological control agents of insects. Attributes making these nematodes ideal as biological control agents are their broad host range, high virulence, safety to non-target organisms. ability to search for hosts, high efficacy in favorable habitats, high reproductive potential, ease of mass production, application and compatibility with other control strategies (Kaya and Gaugler, 1993).

Red weevil, *Rhynchophorus ferrugineus* Oliv. is a hidden voracious damaging pest, fatal to palms particularly during the early periods of its growth. Cryptic behaviour of all developmental stages of the insect can make the control very difficult. However, EPN remains the best possible means for controlling this pest. The present study includes survey for presence of EPN in soil and the pathogenic effect of *Steinernema feltiae* on red weevil of coconut.

Materials and Methods

Soil samples were collected from ten districts viz. Thiruvananthapuram, Kollam, Kottayam, Alappuzha, Thrissur, Palakkad, Pathanamthitta, Kozhikode, Wynad and Kasaragod in Kerala State to find out the natural occurrence of entomopathogenic nematodes. A total of 480 soil samples were collected from rhizosphere of coconut and from areas near cow dung pits. Each sample consisted of three random sub samples collected at a depth of 15 cm from an area of one square meter. They were taken in polyethylene bags, labeled and brought to the laboratory.

Greater wax moth, *Galleria melonella* L. (Galleridae: Lepidoptera) was used as a bait to isolate entomophilic nematodes. Last instar larvae of *Galleria* (3-5 numbers) were placed at the bottom of 300 ml plastic

jars containing the soil lightly packed into it and moistened a little. The containers were kept at 25-28°C for 3-5 days after which the dead larvae or the larvae showing characteristic colour change (reddish brown for *Heterorhabditis* sp. and yellow, orange, brown or black for *Steinernema* sp.) were taken out (Bedding and Akhurst, 1975). They were then washed first with distilled water and then in 0.1 % formalin and incubated in modified White traps (White, 1927). The infective juveniles were collected and used for identification and multiplication. The last instar larvae of *Galleria* were used for multiplying EPN. The nematodes were stored at 25°C in aerated tissue culture flasks filled with 30 ml of 75 per cent Ringer's solution. Only infective juveniles that had a maximum age of four weeks after emergence from *Galleria* larvae were used for the trials.

Rhynchophorus ferrugineus grubs were collected from red weevil infested coconut palms. The grubs were maintained on fresh cut petioles of coconut leaves in plastic containers. Pathogenicity of S2 isolate (*S. feltiae*) to red weevil grubs (25 numbers) was studied in the laboratory using inoculum levels of 500, 1000, 2000 and 4000 infective juveniles in petri plates. The dead insects (cadavers) were kept on White trap and infective juveniles emerged were counted.

Soil application of *S. feltiae* isolate against red weevil adults was tried in net houses in the field. Four coconut seedlings in one net house were used as control while infective juvenile suspension (1 lakh) was poured into the basins of another four potted seedlings in a separate net house. Twenty adult red weevils were released into the cages the next day. After two days the dead weevils obtained, if any, were collected and kept in White trap for nematode extraction. There were four replications.

Results and Discussion

Out of 480 samples collected from ten districts in Kerala State, 363 samples yielded entomophilic nematodes. Of these, 214 (44.6%) were positive for *Rhabditis* sp., 128 (26.7%) for *Heterorhabditis indica*, 16 (3.3%) for *Aphelenchus* sp. and 5 (1%) for *Steinernema* spp. (Table 1). Analysis of soil samples revealed the highest occurrence of nematodes in sandy loam soil followed by laterite and alluvial types (Table 2). Soil samples collected from termite mounts did not yield any nematodes.

Forty per cent of soil samples yielded an earthworm *Fridericia khallikottosus* along with entomophilic nematodes. This indicates that EPN may not be harmful to other non-target/beneficial organisms like earth worms. Ninety per cent of the frass collected from red palm weevil infested coconut palms yielded a nematode *Teratorhabditis palmarum*, which was only entomophilc and not pathogenic to the red weevils.

S. feltiae isolate tested for their pathogenicity on red weevil was found to be a virulent isolate which was a first report. Males, females along with juveniles were found in large numbers from cadavers of red weevil grubs indicating their pathogenic effect on red weevil. The nematode caused 100 percent mortality in sevan days at an inoculum level of 4000 juveniles/plate. (Table 3). When the last instar grubs of red weevil got infested they pupated earlier and could develop to adults of under size. To check whether there is any difference in the number of nematodes after six days, an analysis using

Table 1. Occurrence of entomophilic nematodes in soils of Kerala.

District	No.of samples collected	No. of samples positive for EPN				
		Rhabditis sp.	*H. indica*	*Aphelenchus* sp.	*Steinernema* sp	Total
Alappuzha	217	126 (58%)	56 (26%)	6 (3%)	2 (1%)	190 (88%)
Kasaragod	10	-	-	-	2 (20%)	2 (20%)
Kollam	20	-	2 (10%)	-	-	2 (10%)
Kottayam	20	8 (40%)	4 (20%)	-	-	12 (60%)
Kozhikode	43	18 (42%)	18 (42%)	3 (7%)	-	39 (91%)
Palakkad	67	20 (30%)	15 (22%)	-	-	35 (52%)
Pathanamthitta	26	12 (46%)	9 (35%)	5 (19%)	-	26 (100%)
Thiruvananthapuram	21	4 (19%)	7 (33%)	-	-	11 (52%)
Thrissur	31	16 (52%)	14 (45%)	-	-	30 (97%)
Waynad	25	10 (40%)	3 (12%)	2 (8%)	1 (4%)	16 (64%)
Total	**480**	**214 (45%)**	**128 (27%)**	**16 (3%)**	**5 (1%)**	**363 (76%)**

(Figures in parentheses are per cent value)

Table 2. Effect of soil type on distribution of EPN in Kerala.

Soil type	No. of samples collected	No. of samples positive	
		H. indica	*Steinernema sp.*
Laterite	138	44 (32)	-
Alluvial	39	10 (26)	-
Sandy loam	255	69 (27)	5 (2)
Clay	36	5 (14)	-

(Figures in parentheses are per cent)

Table 3. Infectivity of *Steinernema feltiae* (S_2) to grubs of red weevil.

Dosage (Juveniles/plate)	Mortality (%)	No. of days	No. of nematodes after six days
500	50	9	35875 (189.3)
1000	70	7	140555 (374.8)
2000	70	7	284966 (533.2)
4000	100	7	24967 (158.0)
Control			0
CD(P=0.05)			25.28

(Figures in the parentheses are the square root values

completely randomizes design was done. There were is five dosages viz, 500, 1000, 2000, 4000 juveniles/plate and control. The analysis showed a significant difference among the treatments. Since the observed values are the count of nematodes after applying different dosages of the treatment, a square root transformation of the original values is used for the analysis. The highest number of nematodes was obtained in the third dosage (2000) while there was no count in the control. The 3rd dosage was significantly different from all other treatments.

Isolates of entomopathogenic nematodes were tested against red weevil in earlier studies too (Sosamma and Resmi, 2001; Sosamma, 2003; Banu *et al.*,1998 and 2002). *H. indica* and *S. abbasi* were isolated from date palm and found to be virulent (Abbas *et al.*, 2000 and 2001).

Soil application of *S. feltiae* against red weevil in net house in the field gave 55-60% mortality, while in control it was only 10-12%. Acceptable level of control was not obtained in soil application of EPN as the grubs are hidden in the crown. Red weevil being a hidden enemy, the EPN can be applied to the leaf axils of coconut palms around red weevil infested palms in the field, so that the nearby palms could be protected. Various modes of application of EPN against red weevil have been tried. When infective juveniles were introduced into the stem of red weevil infested palms by making holes, the results were not encouraging. This may due to the deleterious effect of frass on the nematodes. In most cases foliage feeding lepidopteran larvae are highly susceptible to infection by EPN in petri dishes but are rarely effective in the field (Kaya and Gaugler, 1993; Nguyen, 1988 and 2005). Soil is the natural habitat of *Stenernema* and *Heterorhabditis* and hence the ability of the infective juveniles of EPN to disperse actively through soil and locate their host is the key element for success of biocontrol.

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