ETIOLOGY - C. NEMATODES

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The coconut root (wilt) is a debilitating disease and root rotting takes place much before the appearance of any visual symptom like flaccidity, yellowing and necrosis. Root degeneration to the extent of 90 per cent has been reported in root (wilt) affected palms (Butler, 1908; Menon and Nair, 1949; Radha and Menon 1954; Menon and Pandalai, 1958). Increasing evidence on soil transmissible nature of the disease and the suspected involvement of virus in the sixties led to the thinking of involvement of plant parasitic nematodes as probable vectors of the disease (Shanta, Pillai and Lal, 1972; Mathen *et al.*, 1976). Weischer (1967) examined a total of 60 soil samples covering six soil types in the diseased tract and four samples from three soil types from healthy tract and reported the occurrence of plant parasitic nematodes belonging to the genera *Criconema, Criconemoides, Dolichodorus, Helicotylenchus, Hemicriconemoides, Hemicycliophora, Hoplolaimus, Longidorus, Meloidogyne, Paratylenchus, Pratylenchus, Radopholus, Rotylenchulus, Tylenchorhynchus* and *Xiphinema* from the rhizosphere of coconut. Weischer (1967) concluded that the presence of species of *Xiphinema* or *Longidorus* in all soil types could be of importance if viruses were involved in the disease. While *Xiphinema* was present in both the diseased and healthy areas, *Longidorus* was found only in the diseased zone or very near the border between these two areas (Weischer, 1967). Later Khan *et al.* (1971) reported *Dolichodorus pulvinus, Macroposthonia oachirai, Discocriconemella recensi, Longidorus saginus* and *Paralongidorus flexus* from sandy loam soil around the rhizosphere of coconut at Kayangulam. The vector role of *L. saginus* and *P. flexus* in the disease need to be studied in the light of reported soil transmissible nature of the disease (Radha and Menon, 1954; Shanta *et al.*, 1972; Weischer, 1967).

Organised research in plant nematology was initiated at CPCRI in 1972. In addition to the nematodes reported by earlier workers (Weischer, 1967; Mathen, 1969; Mathen, Kurian and Lal, 1970; Khan *et al.*, 1971) twentynine genera of plant parasitic nematodes were recorded from soil (Koshy, Sosamma and Sundararaju, 1979). Besides *Radopholus similis, Pratylenchus zeae* and *Tylenchorhynchus coffeae* have also been recovered from roots occasionally. *L. saginus, Hoplolaimus seinhorsti* and *D. pulvinus* have been encountered from various depths within the root zone of coconut. They have also been found to multiply on roots of coconut seedlings on inoculation.

Initial investigations showed very high populations of *R. similis* from roots of root (wilt) affected as well as healthy

palms in disease tracts (Koshy, Sosamma and Nair, 1975; Koshy, Sundararaju and Sosamma, 1978). Conclusive evidences against the viral etiology in the seventies and observations of root rotting coupled with the notoriety of the burrowing nematode in spreading decline of citrus and pepper yellows prompted concentrated investigations on the role of *R. similis* in the etiology of root (wilt) disease.

R. similis infestation produces small elongated orange coloured lesions on tender creamy white roots. Consequent to nematode parasitisation and multiplication, these lesions enlarge and coalesce to cause extensive rotting of roots. On merging of lesions, cracks develop on the epidermis of the semi-hard orange coloured main roots (Fig. 1). Lesions and rotting are confined to the tender portion of roots. Lesions are not conspicuous on the secondary and tertiary roots as they are narrow and rot quickly on infestation (Fig. 2). Tender roots of coconut seedlings on heavy infestation become spongy in texture.

Burrowing nematodes do not enter hardened or suberised epidermis of coconut roots; but they penetrate the absorbing region behind the root cap covered by very delicate epidermis by lysis of cells. Such entry points or holes are of 1-2 cells in diameter and surrounded by sclerenchymatous cells to a depth of 10 to 15 cells. The cavities that form in the outer cortex are always surrounded by deeply stained and heavily suberised cells of irregular shape, whereas those formed in the inner cortex do not have any such deformed darkly stained border cells. Maximum number of nematodes and cavities are seen in the outer cortex. Nematodes have not been observed in the stelar region or in closely packed 4-6 layers of cells outside the strongly suberised endodermis even in heavily infested roots. The endodermis and the 4-6 layers of cells around it appear to serve as an effective barrier against the invasion of the stele. In the early stage of infection, roots have cavities of independent origin separated by several cells. Consequent to nematode multiplication and lysis of cytoplasm and cell walls, adjacent cavities merged with each other. Multiple cavities and their coalescence destroy the cortex to a great extent. The stelar tube remains intact even in heavily infested roots in transverse and longitudinal sections. Eggs and all stages of nematodes with different orientations are seen in cavities in longitudinal sections (Koshy and Sosamma, 1987).

In an extensive survey carried out comprising 965 samples each of soil and root from Kerala (836), Karnataka (13) and Tamil Nadu (116) during 1973-1982 the widespread occurrence of the burrowing nematode, *R. similis* on coconut was noticed. From the soil samples collected from the root zone of coconut, 39 genera of plant parasitic nematodes were recorded including five new species viz. *Chronogaster spinicorpus, Ecphyadophora teres, Ecphyadophoroides leptocephalus, E. macrocephalus* and *Epicharinema keralense* (Sosamma, 1984; Maggenti *et al.*, 1983; Raski, Koshy and Sosamma, 1982).

The methodology followed for the survey is as follows: Soil and root samples for detection of *R. similis* were collected at a distance of one meter away from the bole of the palm from a depth of 50-100 cm which is the active root zone. Longitudinally split root bits of 2-3 cm were

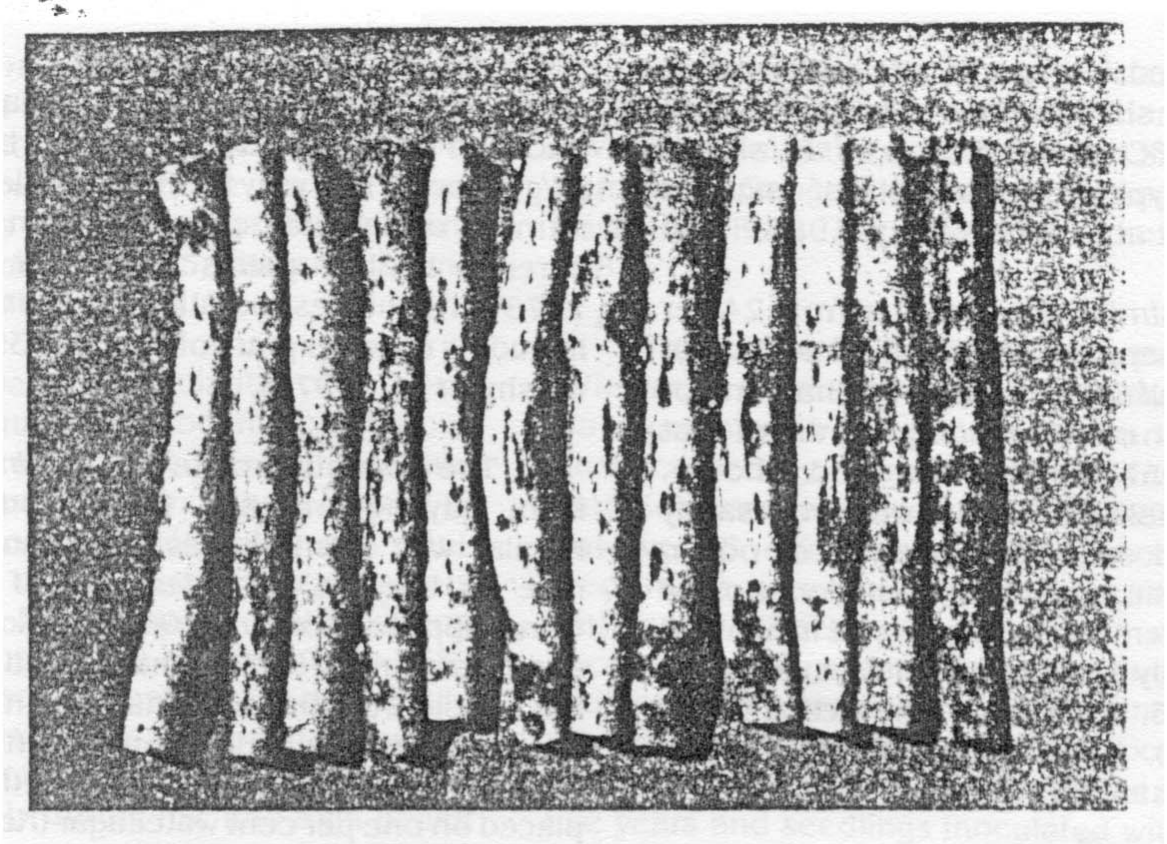

Fig. 1 Tender, white main roots of coconut with various intensities of lesions and rotting on infestation by *R. similis*.

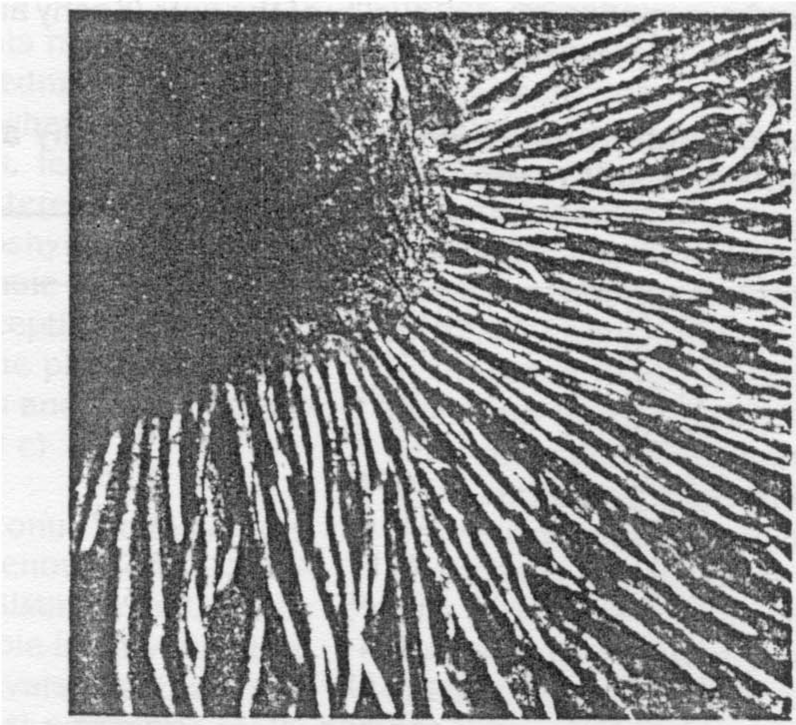

Fig. 2 Tender roots at the base of the palm showing lesions and rotting on infestation by the burrowing nematode.

submerged in water contained in petri dishes or shallow pans at a temperature of 20-25°C for extraction of *R. similis* from polyphenol rich roots of coconut (Koshy *et al.*, 1975; Koshy, 1986)

R.similis was recorded from 24 per cent of samples (230 out of 965). Of these, half the number (115) had one or more than one *R. similis* per gram of root weight (57 yielding ten and above). Among these 93 samples were from sandy loam soil. A further analysis of occurrence of the nematode population showed that the percentage occurrence in healthy, apparently healthy and diseased palms was 12.6, 39.3 and 18.8 respectively (Table 1), which again varied between soils (Sosamma, 1984).

The percentage occurrence of *R. similis* was 12.6 in healthy areas compared to 29 in root (wilt) disease prevalent areas. The samples from apparently healthy palms yielded more nematodes (75/g of root) when compared to diseased palms (34/g of root) in the diseased tract and from palms in the healthy tract (5/g of root). This may be due to the large number of samples collected from apparently healthy palms (Koshy *et al.*, 1978). The highest population recorded including eggs per gram of root was 3941 (Koshy *et al.*, 1975).

The coconut isolate of *R. similis* from Kayangulam and Kasaragod in Kerala, was identified as the 'Banana race' as they do not infest any of the *Citrus* spp. and *Poncirus trifoliata* (Koshy and Sosamma, 1977). The population has a haploid chromosome number (n=4). The *R. similis* population from coconut is easily cultured axenically on carrot discs placed on one per cent water agar (Koshy and Sosamma, 1980). It can also be cultured within the mesocarp of tender coconut without affecting the size or quality of the nuts (Koshy and Sosamma, 1982 a).

Table 1. Occurrence of *Radopholus similis* in roots of healthy and diseased palms in different soil types

Soil types	Healthy	Apparently healthy	Diseased	Total
Sandly loam	5/47[a]	109/213	49/184	163/444
	(10.6)	(51.2)	(26.0)	(26.6)
Laterite	11/84	9/62	9/89	29/235
	(13.0)	(14.5)	(10.1)	(12.3)
Riverine alluvium	4.38	4.28	4.50	12.116
	(10.5)	(14.3)	(8.0)	(10.3)
Clayey	1/31	5/16	5/32	11/79
	(3.2)	(31.0)	(15.6)	(13.9)
Red loam	15/85	0/4	0/2	15/91
	(17.6)	(0.0)	(0.0)	(16.5)
Total	36/285	127/323	67/357	230/965
	(12.6)	(39.3)	(18.8)	(23.8)

Figures in parentheses are the percentages;
a = Number of samples yielding *R. similis* / Total number of samples

R. similis has a wide host range and among the 115 plant species tested at CPCRI Regional Station, Kayangulam, 48 are hosts which include several crops and weeds in coconut gardens (Koshy and Sosamma, 1975; Sosamma and Koshy, 1977 and 1981).

Studies on population of the burrowing nematode in coconut plantations in Kerala, show that infested coconut roots yield maximum number of *R. similis* during October to November and minimum or nil during March to July. Factors favourable for nematode multiplication are soil temperature between 23 to 25°C and moist soils coupled with availability of tender fleshy roots. Nematode population in roots of individual palms varies considerably during low and high peaks depending upon the age, variety and disease index of the palms involved (Koshy and Sosamma, 1978 a).

Coconut sprouts raised by sowing partially husked seednuts (Koshy and Sosamma, 1978 b) when inoculated on roots with *R. similis*, lesions could be detected under a stereoscopic microscope after 24 h (Koshy and Sosamma, 1982 b). The plumule of germinating seed coconut is susceptible to *R. similis* and on infestation the plumule exhibits characteristic lesions and rotting (Koshy and Sosamma, 1978 c)

Forty four coconut cultivars (29 exotic and 15 indigenous) and 15 hybrids screened for resistance to *R. similis* were found susceptible in varying intensities. In the cultivars Kenthali and Klappawangi, the least nematode multiplication and lesion indices were recorded. Similar reaction was noticed in hybrids such as Java Giant x Kulasekharam Yellow Dwarf, Kulasekharam Yellow Dwarf x Java Giant, Java Tall x Malayan Yellow Dwarf and San Ramon x Gangabondam (Sosamma, Koshy and Rao, 1980, 1986; Sosamma, 1984).

Three fungi viz. *Cylindrocarpon effusum*, *C. lucidum* and *Cylindrocladium clavatum* have been isolated from lesions caused by *R. similis* on coconut root (Sosamma and Koshy, 1978; 1983). However, *C. effusum* has been associated more often with lesions. The pathogenic effects of *R. similis* on coconut seedlings were studied through two pot culture experiments using sandy loam soil. In the first experiment the pots were kept in the open field for a period of five years and seedlings inoculated with namatodes extracted from roots of naturally infested coconut palms. In the second experiment the pots were kept under greenhouse conditions for one year and the seedlings were inoculated using axenic nematode population. In both the experiments reduction in growth of plants, intensity of root lesions and root rotting were directly proportional to the initial inoculum levels used. The initial inoculum levels ranged from 100 to 62,500 nematodes per seedling kept in the open field. At the highest initial inoculum level, 48, 21, 76 and 79 per cent reduction over control in height, girth, shoot and root weight respectively was recorded at the end of 5 years. 100 nematodes per seedling or one nematode in 576 cm^3 or 800 g sandy loam soil over a period of five years caused significant reduction in various growth parameters. In the greenhouse an initial inoculum density of 100,000 nematodes caused 40, 55, 20, 62, 71, 57 and 51 per cent reduction over control with regard to

height, shoot weight, number of leaves, leaf area, number of lateral roots, volume and root weight, respectively, over a period of one year. No appreciable damage occurred in seedlings inoculated with the fungus, *C. effusum* alone. The multiplication rate of nematode was reduced in seedlings inoculated simultaneously with the fungus and nematode. The pathogenic threshold level of *R. similis* is 1000 nematodes per seedling or one nematode per 10 cm^3 of sandy loam under greenhouse conditions (Koshy and Sosamma, 1987).

Though these experiments have clearly established the pathogenic potential of the nematode on coconut, the role of the nematode in the etiology of the root (wilt) disease could not be established as the plants got pot bound. To fecilitate normal growth of the plant to flower, yield and exhibit the disease under natural conditions a detailed pathogenicity trial was initiated in October 1982 in 1.8 M x 1.8 M x 1.2 M field tanks (microplots) filled with sandy loam soil fumigated with methyl bromide. The seedlings were inoculated with fungus and different levels of nematode inoculum viz. 100 to one million per seedling.

The absence of production of typical root (wilt) disease symptoms even after eight years on plants inoculated with one million nematodes clearly shows that *R. similis* is not involved as an incitant in the etiology of root (wilt) disease. However, the pathogenic potential of the nematode has been established from this experiment. The infestation of the nematodes causes heavy root rotting, stunting, delay in flowering and reduction in yield (Fig. 3). The root (wilt) affected palms may decline at a faster rate on

Fig. 3 Showing the seedling infested with 10000 *R. similis* in the front and the uninfested at the back.

infestation by *R. similis*, which is widespread on coconut as well as on intercrops like banana, black pepper etc.

Thirty per cent increase in yield and 5 to 10 per cent decrease in disease indices of palms affected with root (wilt) disease has been recorded by the application of *Hydnocarpus* oil cake @ 4 kg per palm as well as with phorate @ 10 g a.i./palm in June-July and October-November (Koshy, 1986). Maximum increase in yield is obtained with application of phenamiphos @ 10 g a.i./palm. The control palms, on the contrary recorded 10 per cent increase in disease indices and 2-5 per cent reduction in yield.

Use of nematicides for the contro

of plant parasitic nematodes on coconut may pose problems of residual toxicity in their products as well as those of the intercrops. Therefore, control of nematodes by application of nematicides alone is not advisable specially in view of the adoption of high density multispecies cropping systems in the infested areas. Hence, an integrated nematode management schedule using organic amendments, minimising quantity of nematicide application, use of nematophagous fungi and bacteria and cultivation of tolerant cultivars of coconut and other crops and colonization with effective mycorrhizae for increasing tolerance to drought and nematodes is to be developed.

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