PRELIMINARY STUDIES ON *RADOPHOLUS SIMILIS* (COBB, 1893) THORNE, 1949 INFESTING COCONUT AND ARECANUT PALMS IN SOUTH INDIA

BY

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Radopholus similis infestation on coconut and arecanut is reported from various districts of Kerala, Karnataka and Tamil Nadu. The semi-hard orange-coloured portion of the main roots of coconut is found to harbour maximum number of nematodes. Peeling and slicing of roots during processing resulted in maximum nematode recovery. Optimum temperature range for extraction of *R. similis* from coconut roots is 4.0°C to 14.0°C. *R. similis* produces small, elongate reddish brown cortical lesions on roots of coconut and arecanut which later coalesce and cause extensive root rotting. Populations from coconut, arecanut and banana did not differ in their pathogenicity. The coconut cultivars West Coast Tall, Dwarf Orange, Dwarf Green, Gangabondam, Tall x Dwarf, Dwarf x Tall and Tall x Gangabondam are all susceptible to the burrowing nematode.

The burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne, 1949 has a wide geographical distribution and is notorious for causing root rot of banana in almost all banana growing regions of the world, spreading decline of citrus in Florida and pepper yellows in Indonesia. Van Weerdt *et al.* (1959) recorded *R. similis* from soil samples around roots of coconut from Florida and Jamaica. Its occurrence in Kerala in banana roots causing severe yellowing of leaves and rotting of roots has been reported by Nair *et al.* (1966) and in coconut roots by Weischer (1967) and Mathen *et al.* (1970). Kumar *et al.* (1971) recorded this nematode from root zones of arecanut, cardamom, banana, ginger and pepper. Setty (1974, personal communication) reported the occurrence of this nematode in banana roots in Bangalore in Karnataka and Muthukrishnan (1975, personal communication) intimated having found it in the same host in parts of Coimbatore, Madurai, Tirunelveli, Salem, Dharmapuri and North Arcot Districts of Tamil Nadu. The investigations reported herein were initiated to determine the possible role of *R. similis* in causing severe root rot in coconut (*Cocos nucifera* L.) and arecanut (*Areca catechu* L.) in South India.

MATERIALS AND METHODS

Sampling and nematode extraction : Root samples of coconut and arecanut were collected from various districts of Kerala, Karnataka and Tamil Nadu. Sampling was confined to the main feeder root zone of the palms at a distance of 50-100 cm away from the bole of the palms and at a depth of 10-50 cm. Samples of 50 g each, washed and cut into 2.5 cm bits after peeling off the epidermis, were sliced into 8 longitudinal pieces and left in 15 cm petri plates containing 150 ml of tap water for 72 hours in the laboratory (25-27°c). The nematodes were extracted after passing the suspension through 60 and 350 mesh sieves. The above method was followed in all experiments, unless otherwise stated.

Recovery of nematodes from different parts of the roots : Main roots were collected from a young palm (7-10 years old) known to have a very high nematode infestation, and cut and grouped into three types of root bits : (i) reddish brown coloured hard (ii) orange coloured semi-hard and (iii) creamy white soft tips. The root samples were processed in triplicate for extraction as described earlier and emergence recorded.

Nematode recovery by different methods of processing : The semi-hard orange coloured root bits harbouring maximum number of nematodes were processed by four different methods. Fifty grams each of root samples peeled entire, unpeeled entire, peeled and sliced, and unpeeled and sliced were kept in triplicate for extraction of *R. similis* as described earlier and counts recorded.

Effect of temperature on extraction : Roots were collected from a heavily infested cononut palm. The semi-hard orange coloured roots were processed for nematode extraction as mentioned earlier. The roots were kept moist by sprinkling water while cutting and slicing. There were seven treatments (seven temperature ranges between 2.5°-31.0°C, see Table I) replicated three times, each replicate consisting of 50 g root pieces soaked in 150 ml water in a petri plate at each temperature range. The nematode suspension was collected at 24 hr intervals and water renewed in the petri dish for further recovery. At the end of five days, the samples were brought to the temperature range giving maximum extraction (4°-14°C) and recovery continued for three more days in the same manner. Further recovery of nematodes and renewal of water supply were done only at 72 hr intervals till the 14th day when emergence became negligible.

Symptom development : Seedlings of coconut (one year old West Coast Tall) and arecanut (local) used for this purpose were made nematode-free by giving a dip treatment in 1000 ppm DBCP aqueous solution for 15 minutes (unpublished). Three such treated seedlings were planted individually in large cylindrical cement tubs, 85 cm dia x 75 cm depth containing 550 kg sterilised soil

and inoculated with *R. similis* at the rate of one nematode/2 g soil. An uninoculated seedling served as control. The inoculation was done by incorporating the required quantity of infested banana roots cut into small pieces in four instalments at intervals of 45 days. The growth characters such as girth at collar, height and number of leaves of each plant were recorded. Eighteen months after inoculation root samples were drawn from these seedlings and the nematodes were extracted and counts recorded.

In the case of arecanut two plants growing in the same tub were inoculated with *R. similis* population isolated from arecanut roots from Palode in Trivandrum district. The number of plants studied was limited due to the large size of pots involved.

Nematode population in relation to root symptoms : Coconut roots of more or less uniform thickness showing various intensities of lesions and rotting, collected from an infested palm were cut into five cm long pieces and arranged into six categories in the order of severity of lesions, rotting and intensity of natural root colour development due to ageing. (Fig. 1). The categories ranged from white root tips showing very thin, elongate, faint orange coloured cortical lesions, separate from one another with the tip one inch without any lesions to those showing severe rotting and discolouration. The intermediate ones were creamy white to orange in colour showing reddish to dark brown lesions coalescing with one another, with rotting of various intensities.

Root pieces of known weight sliced thin longitudinally were rinsed in 25 ml sterile water in a 10 cm petri plate. After drawing out this water for recovery of any nematodes that might have come out, the root pieces were left in fresh sterile water at 10°C for extraction. Every 24 hours the water was drawn out to record the population and replaced with fresh sterile water. On the fifth day when the extraction became negligible, all the root bits in every petri plate were stained in 1% boiling acid fuchsin-lactophenol for five minutes, blended and the population recorded. The total population extracted from each root piece was classified separately as males, females, larvae and eggs/g root weight. In all these the whole suspension was observed without passing through sieves so that the eggs were not lost. No other nematode was extracted from these roots.

Cross inoculation trials : *R. similis* is found to infest coconut, arecanut and banana in this area. To find out differences if any among them, populations extracted from each of these were inoculated separately on to the other two hosts raised in sterilised soil.

Varietal screening : To find out resistance, if any, of different cultivars of coconut to the burrowing nematode, a small scale field survey was conducted.

Root samples of the varieties West Coast Tall, Dwarf Orange, Dwarf Green, Gangabondam, Tall x Dwarf, Dwarf x Tall and Tall x Gangabondam were drawn from young and adult palms grown on CPCRI farms at Kasaragod and Kayangulam. In addition, one year-old seedlings of these varieties raised in sterilised soil were inoculated with *R. similis* collected from coconut roots.

RESULTS AND DISCUSSION

Occurrence and distribution of the nematode : *R. similis* was recovered from coconut roots collected from various places in Trivandrum, Quilon, Alleppey, Kottayam, Ernakulam, Trichur and Cannanore districts of Kerala, and Kanyakumari and Tirunelveli districts of Tamil Nadu.

A preliminary survey of the arecanut gardens showed their presence as below :

Districts		*Total No. of samples examined*	*No. of samples which yielded* R. similis	*Percentage of samples which yielded* R. similis	
Trivandrum	Kerala	75	42	56	rainfed
Quilon	Kerala	71	19	28	rainfed
S. Kanara	Karnataka	47	33	72	irrigated
Chikmagalur	Karnataka	23	17	74	irrigated

It was found that the continuous availability of soil moisture in the irrigated arecanut gardens favoured the survival and multiplication of *R. similis.*

Recovery of nematodes from different parts of the root : The semi-hard orange coloured portion of roots was found to harbour maximum number of nematodes followed by the hard brown coloured portion and the least in the soft creamy white portion (4589, 936 and 36 nematodes/20 g root weight respectively, being mean of three replicates).

In the reddish brown portion the hard nature of the roots, the higher tannin content and the setting-in of rotting apparently make the conditions unfavourable for the nematode. The populations present in the soft white tip portion (10 cm long) are obviously those which have just penetrated since coconut roots are known to grow at the rate of 50 mm a day, and the nematodes which have entered the soft portion may be expected to complete two or three generations by the time the roots become orange in colour.

Nematode recovery by different methods of processing : Maximum extraction of nematodes from the semi-hard orange coloured root bits was effected by the peeled and sliced method (4589/20 g, av. of 3 replicates) while the unpeeled entire, peeled entire, and unpeeled and sliced methods yielded 51, 491 and 1049 nematodes respectively. The peeling and slicing obviously enable the nematodes to come out easily, the thick and hard nature of the epidermis and the cortex in coconut roots otherwise acting as natural barriers against their recovery.

Effect of temperature on extraction : The data given in Table I represent the average emergence for three replicates for a period of 5 days. The maximum extraction is effected in T6 at a temperature range of 4.0°C—14.0°C which is significantly higher than all the other treatments. This is followed by T5 and T7 without any significant difference between the latter. On the whole temperature ranges at T6, T5 and T7 are more favourable and gave significantly higher extraction than other treatments. There was no significant improvement in the rate of emergence in T1, T2, T3 and T4 even when the samples were brought to the most favourable temperature range after five days. In all cases, more than 80% of the total emergence took place during the first 24 hours. Nematodes extracted at lower temperatures were found to be more active than those extracted at higher temperatures.

TABLE I

Effect of temperature on extraction of R. similis *from coconut roots*

Sl. No.	Temp. range	Nemotode emergence (mean of 3 replicates)
T^1	27.0-31.0°C	928 (2.9896)
T^2	22.0-27.0°C	1050 (3.0395)
T^3	25.5-30.0°C	1089 (3.0395)
T^4	21.0-26.0°C	1118 (3.0395)
T^5	10.0-20.0°C	1662 (3.2678)
T^6	4.0-14.0°C	2695 (3.4612)
T^7	2.5-12.0°C	1531 (3.2254)

(Figures in parentheses are the log transformed values)

C. D. at 1% between treatments = 0.0867

The highest nematode population is recorded during September to November, but the recovery becomes negligible or nil in summer months even from palms known to be heavily infested (unpublished).

Symptom development : On inoculation the healthy coconut seedlings grown in sterilised soil exhibited lesions after 35 to 45 days. Initially the lesions produced are small reddish brown and elongate on the young white and orange coloured roots, those on the older brown roots being seen clearly only when the hypodermis (functional epidermis) is peeled off. These lesions later coalesce with one another causing extensive root rotting. The early attack on the new main roots leads to failure of lateral root production. While the uninoculated seedlings produce plenty of lateral roots interspersed with one another giving a mat-like appearance, the inoculated seedlings lose their vigour, present a retarded growth and produce very few lateral roots. Table II gives the percentage of growth increase in each of the three seedlings over the initial readings. Root samples drawn 18 months after inoculation yielded 80 nematodes per gram weight.

TABLE II

Percentage of growth increase in coconut seedlings with and without R. similis *18 months after inoculation*

	Control	Inoculation with *R. similis* (Three replicates)		
		1	2	3
Collar girth	180	75	113	158
Height	215	126	100	160
No. of leaves	143	125	89	129

In arecanut the inoculated plants showed general yellowing and poor growth. Apart from elongate, brown cortical lesions throughout the root system, blackening of the root tip was invariably observed (Fig. 2). Such symptoms have been reported in citrus also (Suit & DuCharme, 1953).

It may be seen from Table III that in root pieces (Nos. 1 and 2) showing the initial symptoms, the male population is almost nil as against the female population of 20 and 31 respectively. This indicates that males do not infect roots and most of the larvae penetrated in the initial stages become females. This is in agreement with DuCharme & Price (1966) who reported that males of

R. similis are not capable of penetrating citrus roots. In the other root pieces (Nos. 3 to 6) the male-female ratios vary from 1 : 3 to 1 : 5 with a mean of 1 : 4. Maximum population of 3941/g was recorded in root No. 3 where the lesions had started coalescing with one another out of which as much as 61.6% are eggs. With the usual sieving method this egg population is lost and not accounted for. It may also be seen that with the increase in degree of rotting in root Nos. 4 to 6 there is a corresponding reduction in population which may be due to nematode migration from roots into the soil as a result of population density, food shortage and putrefaction by secondary invaders as suggested by DuCharme (1968) for *R. similis* in citrus.

TABLE III

R. similis *population/g in root pieces showing different degrees of damage* (cf. Fig. 1)

Root Nos.	Females	Males	Larvae	Eggs	Total Population
1	20 (88.9)	1 (2.2)	1 (2.2)	2 (6.7)	24
2	31 (58.2)	0 (0)	14 (25.4)	9 (16.4)	54
3	564 (14.4)	114 (2.9)	836 (21.2)	2427 (61.6)	3941
4	311 (29.5)	74 (7.1)	272 (25.8)	397 (37.6)	1054
5	135 (29.0)	39 (8.5)	114 (24.6)	176 (37.9)	464
6	43 (18.0)	8 (3.6)	7 (3.9)	178 (74.5)	236

*Figures in parentheses are the percentages of the total population.

Cross-inoculation trials : Irrespective of the sources of nematode inoculum, roots of coconut, arecanut and banana (Fig. 3) developed typical symptoms, indicating that the populations from banana, coconut and arecanut are the same and has no specificity to these hosts.

Varietal screening : Roots of West Coast Tall, Dwarf Orange, Dwarf Green, Gangabondam, Tall x Dwarf, Dwarf x Tall, Tall x Gangabondam yielded *R. similis* in the field survey and on inoculation, developed typical root lesions which yielded the nematode on extraction. However, their comparative susceptibility could not be ascertained due to inadequate number of samples drawn and

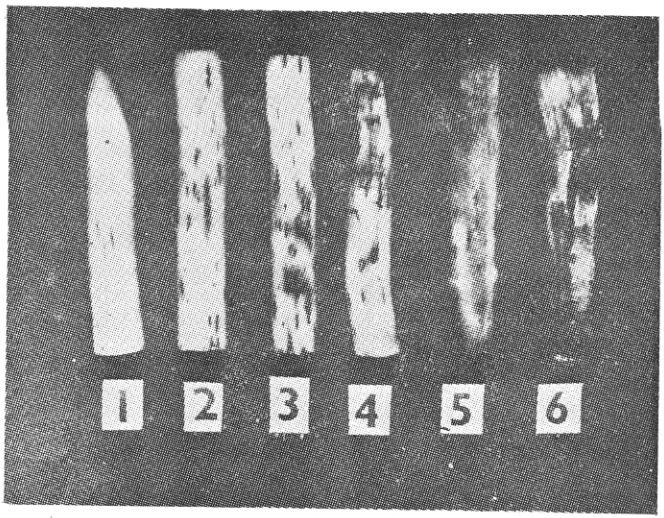

Fig. 1. Progressive development of symptoms of *R. similis* infestation on coconut roots. (1) Root tip showing very thin elongate faint orange coloured cortical lesions separate from one another ; roots showing, (2) lesions in the initial stages of coalescing with one another, (3) reddish to dark brown lesions, most of them coalescing with one another, (4) coalescing of lesions and initial stages of rotting, (5) lesions, rotting and discolouration, and (6) severe rotting, discolouration and cracking of epidermis.

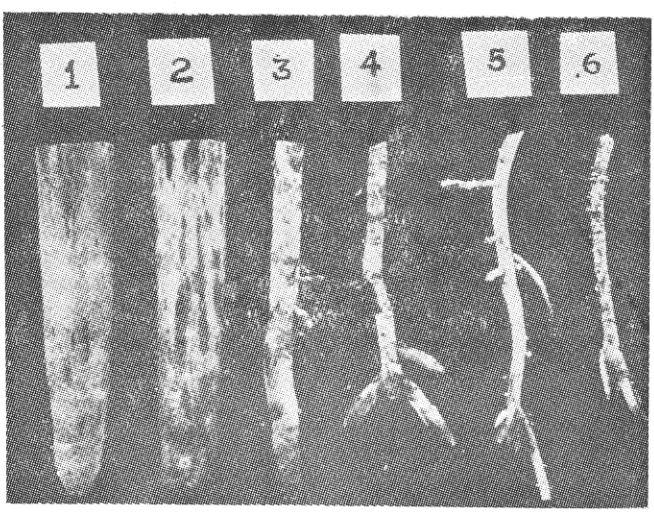

Fig. 2. Symptoms of *R. similis* infestation on arecanut roots (1) Main root showing orange coloured lesions separate from one another at the tip with coalescing of lesions farther away, (2) main root showing coalescing of lesions and rotting and (3-6) secondary and tertiary roots showing blackening of their tips.

non-uniformity in their growing conditions. In the field survey a maximum population of 1000 nematodes/g of root (mean of 50 g) was recorded from West Coast Tall.

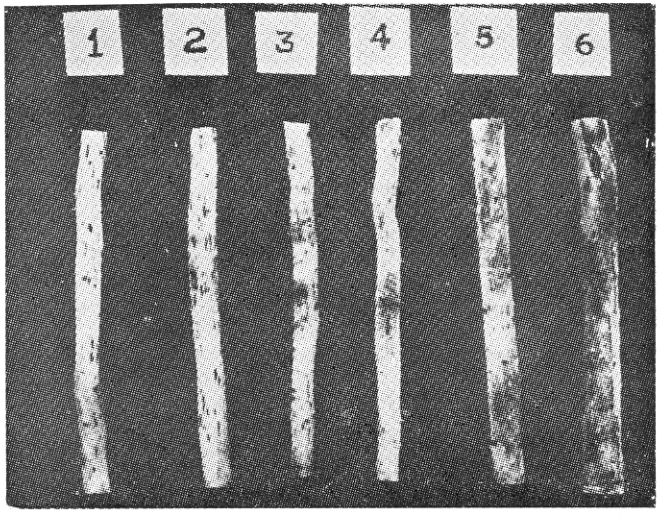

Fig. 3. Symptoms of *R. similis* infestation on banana roots showing (1) small reddish brown elongate lesions separate from one another, (2) lesions coalescing with one another, (3) lesions coalescing with one another with initiation of rotting, (4) lesions and rotting, (5) extensive rotting, middle region with lesions in the initial stages, and (6) rotting of the entire root.

It may be stated in conclusion that although the above investigations are of a preliminary or exploratory nature, the widespread occurrence of the burrowing nematode in the coconut and arecanut plantations in South India has been clearly established. Extraction of *R. similis* from coconut roots presents several problems and the standardization of techniques will go a long way in facilitating further studies on this nematode. The recovery of high populations of the nematode in all developmental stages from infested roots and the results of inoculation experiments are indicative of the potentialities of this nematode to cause severe damage to the root system of coconut. Further studies are in progress.

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