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PATHOGENICITY OF *RADOPHOLUS SIMILIS* ON COCONUT (*COCOS NUCIFERA* L.) SEEDLINGS UNDER GREENHOUSE AND FIELD CONDITIONS

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**Abstract :** The pathogenic effects of *Radopholus similis* on coconut seedlings was established through two experiments, the first with a duration of 5 years, using nematode inoculum extracted from roots of naturally infested coconut palms under field conditions and the second using axenic nematode population, reared on carrot discs under greenhouse conditions over a period of one year. In both cases, reduced growth of plants and intensity of root lesions and rotting were directly proportional to the increase in initial inoculum used. An initial inoculum density of 62, 500 nematodes per seedling caused 48, 21, 76 and 79 per cent reduction over control in height, girth, shoot and root weight respectively, over a period of 5 years, in sandy loam soil in pots under field conditions. However, the threshold inoculum density causing significant reduction of various growth parameters was 100 nematodes per seedling or one nematode in 576 cm<sup>3</sup> or 800 g sandy loam soil over a period of five years. 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 was noticed in plants inoculated with the fungus, *Cylindrocarpon effusum* alone. Plants inoculated simultaneously with the fungus and nematode recorded reduced rate of multiplication of the nematode. This study established the potential of the burrowing nematode as a pathogen of coconut. The pathogenic threshold level of *R. similis* was 1000 nematodes per plant or one nematode per 10 cm<sup>3</sup> of sandy loam soil under greenhouse conditions.

**Key words :** *Radopholus similis*, coconut, pathogenicity

Koshy *et al.* (1978) have reported widespread occurrence of *Radopholus similis* in South India on coconut, arecanut, banana and black pepper and the population infesting coconut has been reported as the 'banana race' (Koshy & Sosamma, 1977). Maximum population of the nematode is known to occur during September to November and minimum during March to June (Koshy & Sosamma 1978a). The pathogenic effect of the burrowing nematode on coconut seed-

lings, informations on which are lacking, was therefore studied by conducting two experiments, the first under field conditions using field inoculum and the second one in the greenhouse using axenic population.

MATERIALS AND METHODS

**Field experiment :** Twentyeight cement tubs (75 × 80 cm) were inserted, 60 cm deep into the soil, after opening pits at

1.5 × 1.5 m distance in the field at CPCRI, Kayangulam leaving the top 10 cm of the tub above the soil surface. Each pot was filled with 80 Kg (57,600 cm<sup>3</sup>) steam sterilised sandy loam soil. One seednut, cv. West Coast Tall, was sown in each tub in September, 1974. *Pueraria phaseoloides* was grown as cover crop in the interspace to avoid heating up of soil around the cement tubs and to prevent soil splashing during the rainy season. *R. similis* infected coconut roots were collected from palms of the farm and active nematode inoculum was extracted (Koshy *et al.*, 1975). There were seven treatments (uninoculated check, associated micro-organisms, *R. similis* @ 100, 500, 2,500, 12,500 and 62,500) with 4 replications. The plants were inoculated in October, 1975 at 5 leaf stage. Just before inoculations, the feeder roots of the seedlings were exposed carefully by removing the top layer of the soil. The inoculum (females and larvae) was poured uniformly all over the exposed roots and covered immediately with the top soil. After 5 years, soil samples were collected from three places, from each tub, using G. I. pipes (5 cm OD) and mixed thoroughly. An aliquot of 250 cm<sup>3</sup> soil was drawn and analysed by Cobb's sieving and sifting method followed by the modified Baermann's funnel method.

Each plant was uprooted from tubs with a strong jet of water. uprooted seedlings were again washed thoroughly to remove the adhering soil particles. Plant growth characters such as girth at collar, height of the plant, number of leaves, lamina length, maximum leaflet, shoot weight and root weight were recorded. As there was no clear cut differentiating line between the stem base and the bole portion where roots emerge, this portion was weighed along with the shoot. The maximum width of the leaf was recorded by measuring the length of the longest leaflet in the middle portion of the leaf. Counts of leaflet number and measurement of lamina

length were done on the youngest fully opened leaf. It was difficult to index uniformly the lesions and rotting visually because of the presence of large number of lateral roots and their heavy coilings. Hence, it was decided to assess only the root population by drawing well represented samples. The root system was cut into small pieces after taking root weight and three aliquots of 10 g samples were drawn for population assessment. The roots were stained in boiling acid fuchsin-lactophenol for three minutes, cleared and churned for 40 seconds in a Waring blender. The total root population was computed with the average per gram population.

*Greenhouse experiment*: Coconut seedlings cv. West Coast Tall were raised from partially husked open pollinated nuts in methyl bromide fumigated sandy loam soil (14 Kg or 10080 cm<sup>3</sup> dry soil) contained in earthen pots (35 cm) (Koshy & Sosamma, 1978 b). At two-leaf-stage, 70 seedlings having uniform growth were selected. Nematode population was axenically multiplied on carrot discs placed on one per cent water agar (Koshy and Sosamma, 1980). The fungus, *Cylindrocarpum effusum*, was multiplied on potato dextrose agar (PDA) medium. There were seven treatments (T<sub>1</sub>—uninoculated control, T<sub>2</sub>—*C. effusum*, T<sub>3</sub>—*C. effusum* + *R. Similis*, 10,000 T<sub>4</sub>—*R. similis* 100, T<sub>5</sub>—*R. similis* 1000, T<sub>6</sub>—*R. similis* 10,000 and T<sub>7</sub>—*R. similis* 100,000) each replicated 10 times. The fungal inoculations were done through 100 ml aqueous suspension having 100,000 spores/0.5 ml. Combined inoculations of fungus and nematode were done by adding the 100 ml fungus suspension into the 100 ml nematode suspension and pouring the resultant mixture on to the exposed root system. In the uninoculated control sterile water was poured. All plants were kept in the greenhouse (27 to 34° with even sunlight and watered daily to sustain vigorous growth. Water was brought to boiling and cooled for irriga-

tion in greenhouse.

After 12 months, the plants were carefully depotted and thoroughly washed to remove the adhering soil particles. Plant growth characters were recorded. Visual observation of lesions and rotting of roots were also recorded on individual main roots and indexed (1-5 scale) as follows: 1—no lesions, 2—very thin elongate faint orange coloured lesions separated from one another, 3—reddish to dark brown lesions in the initial stage of coalescing with the adjoining one, 4—coalescing lesions with initial stages of rotting, 5—severe rotting, discolouration and cracking of epidermis.

Nematode population in soil, roots and in leaf bases at collar region (3 cm length) were assessed also. Population of burrowing nematodes in 10 g apple (haustorium) was extracted by keeping the shredded material immersed in water at  $15 \pm 1^\circ\text{C}$  in a BOD incubator and counts made.

Root volume was measured by placing all the roots of the test plant into a glass measuring cylinder containing known volume of water and the displaced

quantity of water was recorded as volume of the roots. Leaf area of individual leaf was calculated by using the formula  $L \times B \times 0.61 + 27.30$  in sq. cm (Satheesan *et al.*, 1983).

One plant, from each treatment was kept in 6 l water, in a polyethylene bucket with air tight covering to prevent evaporation for recording water absorption. These plants were kept in a dark room at  $25 - 26^\circ\text{C}$ . The reduction in volume of water was recorded after 48h.

Tender, creamy white roots showing orange to reddish brown lesions were cut into 1 cm bits and fixed in FAA. Further they were processed in tertiary butyl alcohol series and embedded in paraffin. Longitudinal and transverse sections were cut at  $10 \mu\text{m}$  thickness and stained in safranin and fast green following the method of Johansen (1940).

## RESULTS AND DISCUSSION

*Field experiment* (Tables 1 and 2; Fig. 1)

Generally a progressive reduction in growth characters was recorded with an increasing inoculum levels. An initial

TABLE 1. *Effect of different inoculum levels of Radopholus similis on growth parameters of coconut seedlings in pots under field conditions*  
(Mean of four seedlings)

Initial inoculum level	Height (cm)	Girth (cm)	Shoot weight (g)	Root weight (g)
Check	287.0 <sup>a</sup>	47.75	8600 <sup>a</sup>	5235 <sup>a</sup>
Associated	254.5 <sup>a</sup>	43.00	4600 <sup>b</sup>	3158 <sup>b</sup>
Check	(11)	(10)	(46.5)	(39.7)
100	186.8 <sup>b</sup>	41.25	3013 <sup>b</sup>	1818 <sup>c</sup>
	(35)	(14)	(65.0)	(65.3)
500	184.5 <sup>b</sup>	35.75	2713 <sup>b</sup>	1950 <sup>c</sup>
	(36)	(25)	(68.5)	(62.8)
2,500	166.5 <sup>b</sup>	38.00	2775 <sup>b</sup>	1725 <sup>c</sup>
	(42)	(20)	(67.7)	(67.1)
12,500	163.5 <sup>b</sup>	37.75	2338 <sup>b</sup>	1419 <sup>c</sup>
	(42)	(21)	(72.8)	(72.9)
62,500	149.8 <sup>b</sup>	37.50	2100 <sup>b</sup>	1098 <sup>c</sup>
	(48)	(22)	(75.6)	(79.0)
C.D. at 1%	69.48	N.S.	2637	2289
C.D. at 5%	51.05		2672	1681

Means with notations by the same letter do not differ at 5% level.  
Figures in parentheses are percentage reductions over control.

TABLE 2. *Effect of different inoculum levels of Radopholus similis on leaf characters of coconut seedlings in pots under field conditions*

(Mean of four seedlings)

Initial inoculum level	No. of leaves	Per cent reduction over control	No. of leaflets per leaf	Per cent reduction over control	Leaflet length (cm)	Per cent reduction over control	Lamina length (cm)	Per cent reduction over control
Check	29.40 (5.4226) <sup>a</sup>	—	103.20 (10.1590) <sup>a</sup>	—	67.00 <sup>a</sup>	—	187.5 <sup>a</sup>	—
Assoc. Check	28.24 (5.3141) <sup>a</sup>	4.0	96.38 (9.8173) <sup>a</sup>	6.6	55.75 <sup>ab</sup>	16.8	152.5 <sup>ab</sup>	18.7
100	27.46 (5.2404) <sup>ab</sup>	6.6	94.65 (9.7287) <sup>ab</sup>	8.3	42.25 <sup>be</sup>	37.0	138.25 <sup>b</sup>	26.3
500	24.73 (4.9733) <sup>bc</sup>	15.9	88.37 (9.4004) <sup>abc</sup>	14.4	42.75 <sup>bc</sup>	36.2	111.00 <sup>c</sup>	40.8
2,500	24.00 (4.8990) <sup>c</sup>	18.4	74.52 (8.6327) <sup>c</sup>	27.8	40.50 <sup>c</sup>	39.9	97.75 <sup>c</sup>	47.9
12,500	24.98 (4.9980) <sup>bc</sup>	15.0	77.66 (8.8128) <sup>bc</sup>	24.8	39.50 <sup>c</sup>	41.1	97.00 <sup>c</sup>	48.3
62,500	23.99 (4.8984) <sup>c</sup>	18.4	77.20 (8.7865) <sup>c</sup>	25.2	37.50 <sup>c</sup>	40.0	97.75 <sup>c</sup>	47.9
C.D. at 1%	0.38		1.3130		20.83		50.24	
C.D. at 5%	0.28		0.9647		15.31		36.91	

Mean with notations by the same letter do not differ at 5% level.

Figures in parentheses are square root transformed values.

inoculum level of 62,500 nematode caused 48, 22, 76, 18, 25, 40, 48 and 79 per cent reduction in respect of height, girth at collar region, shoot weight, number of leaves, number of leaflets/leaf, leaflet length, lamina length and root weight over control plants. The effect of parasitization of the nematode was more pronounced on the root system, especially on the number and mass of feeder roots. The normal matting of lateral roots, seen in check seedlings, was absent in inoculated plants.

The population build up of nematodes in soil and roots are summarised in Table 3. It was noticed that more nematodes were present in roots than in soil. The threshold inoculum density required for causing significant reduction

of various growth parameters was 100 nematodes per seedling or one nematode in 800 g soil under field conditions over a period of five years.

*Greenhouse experiment* (Table 4 and 5; Fig. 2)

The inoculated plants were stunted with reduced vigour compared to control plants. (Table 4). The root system of all inoculated plants showed lesions and root rotting, reduction in number of lateral roots in various intensities (Table 5). The pathogenic effect of the nematode inoculum was maximum on the number and weight of lateral roots than the other studied growth parameters.

Inoculations with fungus alone caused minimum reduction in root weight but

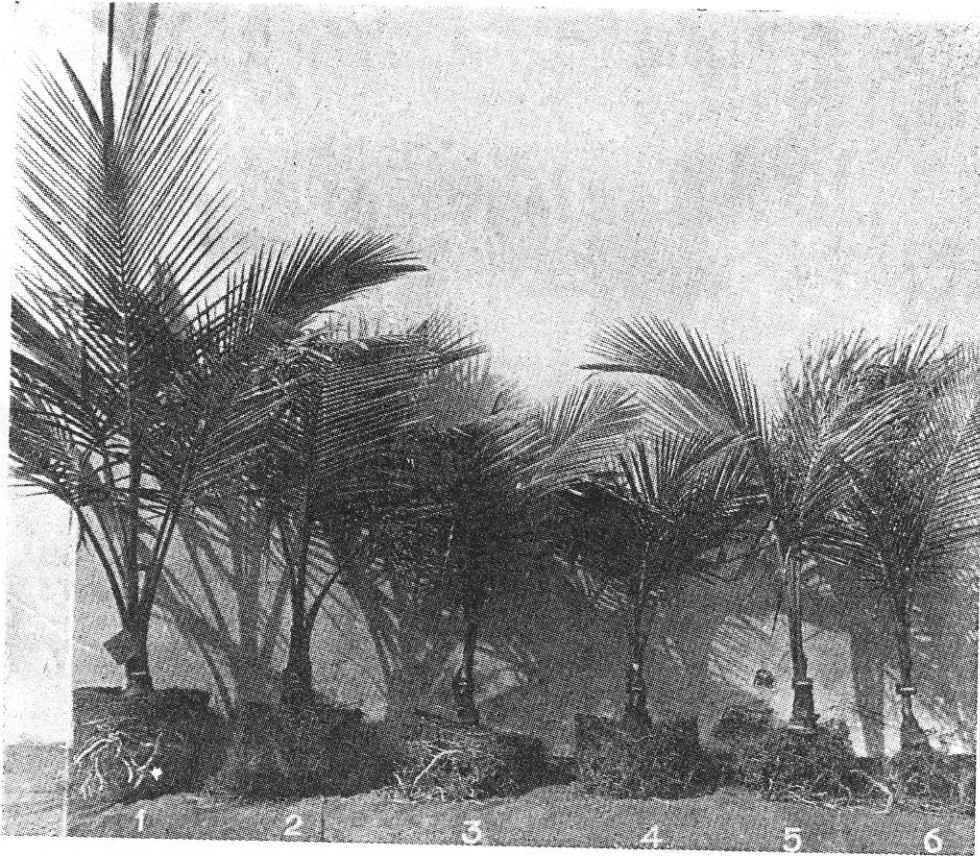


FIG. 1. Effect of different inoculum levels of *Radopholus similis* on growth of coconut seedlings in pots under field conditions. 1. Control 2. *R. similis* @ 100 3. 500 4. 2,500 5. 12,500 and 6. 62,500

the combined inoculations of the fungus and nematode resulted in high reduction in the total root weight. However, it was still at par with the control. The inoculations affected the total volume of the roots and the differences between treatments were significant at one per cent level. The maximum reduction in root volume was recorded in plants that received an initial inoculum level of 100,000 followed by 10,000 and 1000 nematodes. The water absorption rate was more with seedlings having higher root volume than otherwise. Uninoculated seedling with a root volume of 185 cm<sup>3</sup> absorbed 151 ml

water. Plants belonging to other treatments with root volumes in the range 12 to 80 cm<sup>3</sup> absorbed only 70 to 120 ml water.

The fungus *C. effusum*, alone did not cause any damage to the root system. All levels of nematode inocula caused root lesions and rotting and the indices varied from 4 to 4.5. An inoculum level of 10,000 nematodes combined with the fungus produced a root lesion index of 2.8 against 4.5 recorded at the same level when inoculated singly. Thus, the simultaneous inoculation of fungus and nematode adversely affected reproduction of nematodes on coconut seedlings.

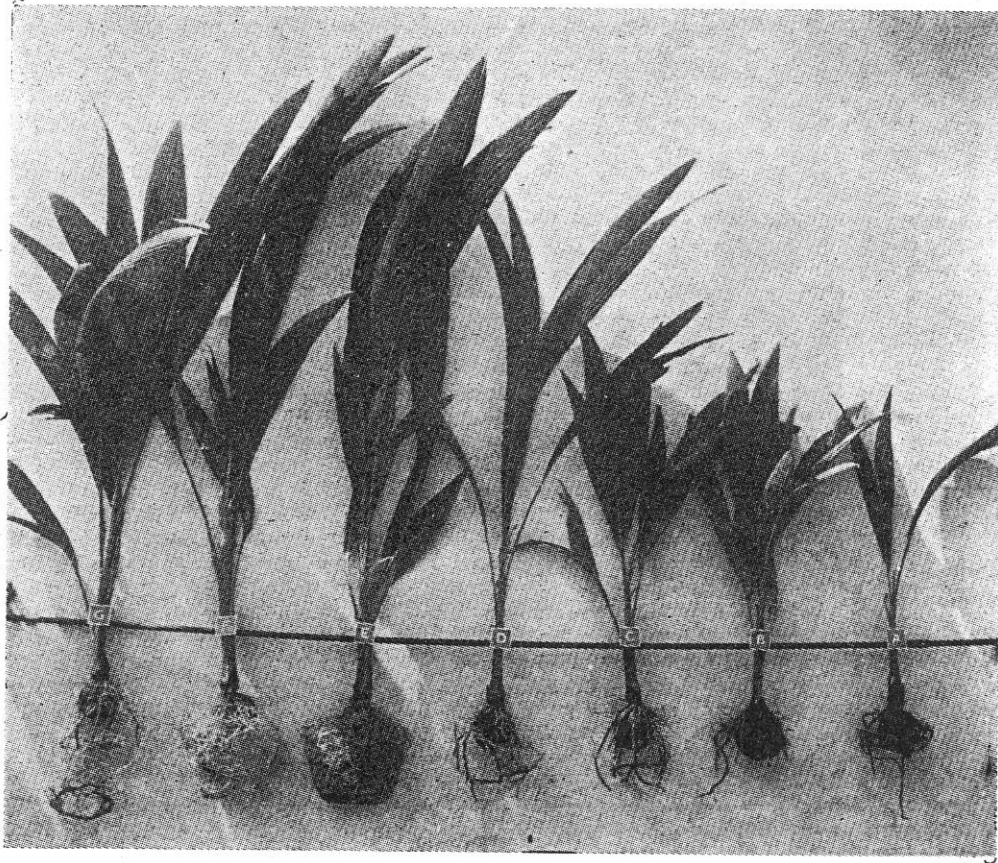


FIG. 2. Effect of *C. effusum*, *R. similis* + *C. effusum* and four initial inoculum levels of *R. similis* on growth of coconut seedlings in pots under greenhouse conditions. A.—*R. similis* @ 100,000 B.—10,000 C.—1,000 and D.—100 E.—Control F.—*C. effusum* G.—*R. similis* + *C. effusum*

The population build up of the nematode in various treatments, separately in soil and in roots, along with their total is given in Table 6. Burrowing nematode being an endoparasite, more of its populations could be recovered from roots than from soil. Minimum population build up was recorded with combined inoculation of nematode and fungus. A maximum population of 297,666 was recorded with an initial inoculum level of 10,000 nematodes, followed by 100, 100,000 and 1000 in that order. The rate of multiplication was maximum

(2204 times) with 100 nematodes and minimum (2 times) with 100,000 nematodes.

Irrespective of the treatments, in all experimental seedlings, the strands of vascular tissues running through the soft eye, that connect apple with the young seedlings, were intact and the seednuts remained attached to the seedlings. The haustoria (apples) in control seedlings were bigger in size with the corresponding decrease in thickness of the kernel compared to that of plants inoculated

TABLE 3. *Effect of different inoculum levels of R. similis on nematode multiplication on coconut seedlings in pots under field conditions*  
(Mean of five seedlings)

Initial inoculum levels	Nematode population		
	Soil	Root	Total
Check	0	0	0
Assoc. check	0	0	0
100	9605 (3.9825)	42555 (4.6290) <sup>bc</sup>	53481 (4.7282) <sup>b</sup>
500	4823 (3.6833)	48136 (4.6825) <sup>b</sup>	53469 (4.7281) <sup>b</sup>
2,500	14004 (4.1462)	25358 (4.4041) <sup>c</sup>	40668 (4.6092) <sup>b</sup>
12,500	8849 (3.9469)	30348 (4.4821) <sup>bc</sup>	40112 (4.6033) <sup>b</sup>
62,500	13170 (4.1196)	126052 (5.1005) <sup>a</sup>	144178 (5.1589) <sup>a</sup>
C.D. at 1%		0.3502	0.3215
C.D. at 5%	NS	0.2532	0.2325

Means with notations by the same letter do not differ at 5% level.  
Figures in parentheses are log transformed values.

TABLE 4. *Effect of C. effusum R. similis + C. effusum and four initial inoculum levels of R. similis on shoot growth of coconut seedlings in pots under greenhouse conditions*  
(Mean of ten replications)

Treatment	Height (cm)	Girth (cm)	Shoot weight (g)	No. of leaves	Petiole length (cm)	Lamina length (cm)	Lamina breadth (cm)	Leaf area (sq. cm)
T <sub>1</sub>	176.40 <sup>a</sup>	15.15 <sup>a</sup>	526.40 <sup>a</sup>	8.90 <sup>a</sup>	56.50 <sup>a</sup>	75.30 <sup>a</sup>	21.80 <sup>a</sup>	6719.40 <sup>a</sup>
T <sub>2</sub>	166.30 <sup>a</sup> (5.7)	14.20 <sup>abc</sup> (6.3)	498.50 <sup>ab</sup> (5.3)	8.90 <sup>a</sup> (0.0)	57.80 <sup>a</sup> (+2.3)	74.70 <sup>a</sup> (1.5)	21.20 <sup>ab</sup> (2.8)	5889.90 <sup>ab</sup> (12.4)
T <sub>3</sub>	156.70 <sup>a</sup> (11.2)	13.45 <sup>bc</sup> (11.2)	471.10 <sup>ab</sup> (10.5)	8.50 <sup>a</sup> (3.3)	41.55 <sup>b</sup> (26.5)	65.80 <sup>ab</sup> (13.2)	17.50 <sup>ab</sup> (19.7)	6090.40 <sup>ab</sup> (9.4)
T <sub>4</sub>	157.70 <sup>a</sup> (10.6)	15.05 <sup>ab</sup> (10.7)	379.60 <sup>bc</sup> (27.9)	7.60 <sup>b</sup> (14.6)	54.20 <sup>a</sup> (7.6)	73.40 <sup>a</sup> (3.2)	21.20 <sup>abc</sup> (2.8)	4660.70 <sup>b</sup> (30.6)
T <sub>5</sub>	105.00 <sup>b</sup> (40.5)	13.00 <sup>c</sup> (14.2)	210.60 <sup>d</sup> (60.0)	7.40 <sup>b</sup> (18.0)	37.90 <sup>b</sup> (32.9)	60.40 <sup>b</sup> (20.3)	17.90 <sup>bc</sup> (17.9)	2830.10 <sup>c</sup> (57.9)
T <sub>6</sub>	115.20 <sup>b</sup> (34.7)	13.23 <sup>c</sup> (12.7)	289.40 <sup>d</sup> (45.0)	7.50 <sup>b</sup> (17.0)	38.30 <sup>b</sup> (32.2)	57.60 <sup>b</sup> (24.0)	18.20 <sup>c</sup> (17.0)	2809.10 <sup>c</sup> (58.2)
T <sub>7</sub>	106.20 <sup>b</sup> (39.8)	12.44 <sup>d</sup> (17.9)	236.00 <sup>cd</sup> (55.2)	7.10 <sup>b</sup> (20.2)	35.70 <sup>b</sup> (36.8)	55.80 <sup>b</sup> (26.4)	17.30 <sup>c</sup> (20.7)	2559.30 <sup>c</sup> (61.9)
C.D. at 5%	42.66	2.47	171.1	1.15	11.8	16.45	4.7	2154.0
C.D. at 5%	32.07	2.9	128.6	0.86	8.8	12.38	3.5	1619.9

Means with notations by the same letter do not differ at 5% level.  
Figures in parentheses are percentage reductions over control.

TABLE 5. *Effect of C. effusum, R. similis + C. effusum and four initial inoculum levels of R. similis on root growth of coconut seedlings in pots under greenhouse conditions*  
(Mean of ten replications)

Treatment	Main roots			Lateral roots		Total root weight (g)	Root volume (cm <sup>3</sup> )	Root lesion index
	No.	Length (cm)	Weight (g)	No.	Weight (g)			
T1	11.30	520.60 <sup>a</sup>	95.10 <sup>a</sup>	553.60 <sup>a</sup>	28.40 <sup>a</sup>	123.50 <sup>a</sup>	142.10 <sup>a</sup>	1.0 <sup>c</sup>
T2	10.80 (4.4)	609.90 <sup>a</sup> (+17.2)	76.60 <sup>ab</sup> (19.5)	524.20 <sup>a</sup> (3.3)	24.30 <sup>a</sup> (14.4)	101.20 <sup>ab</sup> (18.1)	128.10 <sup>ab</sup> (9.9)	1.0 <sup>c</sup>
T3	11.30 (0.0)	529.00 <sup>a</sup> (+1.6)	70.50 <sup>bc</sup> (25.9)	420.40 <sup>ab</sup> (25.9)	19.20 <sup>ab</sup> (32.4)	90.70 <sup>bc</sup> (26.6)	100.90 <sup>bc</sup> (29.0)	2.8 <sup>b</sup>
T4	11.40 (+0.9)	323.40 <sup>b</sup> (37.8)	58.80 <sup>bc</sup> (38.2)	325.00 <sup>bc</sup> (41.3)	12.10 <sup>b</sup> (57.4)	70.90 <sup>c</sup> (42.6)	105.90 <sup>ab</sup> (25.8)	4.0 <sup>a</sup>
T5	10.00 (11.5)	212.20 <sup>b</sup> (59.2)	53.80 <sup>bc</sup> (43.4)	185.40 <sup>c</sup> (66.5)	10.70 <sup>b</sup> (62.3)	64.70 <sup>d</sup> (47.6)	66.20 <sup>c</sup> (53.4)	4.2 <sup>a</sup>
T6	10.20 (9.7)	258.30 <sup>b</sup> (50.4)	62.20 <sup>bc</sup> (34.6)	174.90 <sup>c</sup> (68.4)	10.60 <sup>b</sup> (62.7)	71.80 <sup>cd</sup> (41.1)	66.20 <sup>c</sup> (53.4)	4.5 <sup>a</sup>
T7	10.10 (10.6)	218.70 <sup>b</sup> (58.1)	49.80 <sup>c</sup> (47.6)	161.30 <sup>c</sup> (70.7)	10.20 <sup>b</sup> (64.1)	60.10 <sup>d</sup> (51.3)	61.10 <sup>c</sup> (57.0)	4.1 <sup>a</sup>
C.D. at 1%	—	185.3	30.1	253.7	11.9	37.1	50.14	0.9
C.D. at 5%	NS	139.3	22.6	190.7	8.9	27.2	37.7	0.7

Means with notations by the same letter do not differ at 5% level.  
Figures in parentheses are percentage reductions over control.

with nematodes. However, apples of control seedlings weighed less due to their utilisation for the vigorous growth of the seedlings. In nematode inoculated seedlings, the haustoria were small in size and retained mere of kernel because of the reduced conversion of the kernel by the haustoria due to the nonvigorous and reduced growth of seedlings.

In addition, the plants, inoculated with the fungus and fungus plus nematode, put out small leaves subsequent to inoculations, but soon after they started putting out normal leaves. Though *C. effusum* did not cause much root damage, it could be reisolated from the roots which exhibited some rotting (T2 and T3). Burrowing nematodes could be recovered from the haustoria and leaf bases at the collar region of all the inoculated plants irrespective of the level of inoculum used.

Burrowing nematodes do not enter or penetrate the coconut roots that have developed hardened or suberised epidermis but they are found to penetrate the absorbing region behind the root cap covered by very delicate epidermis by lysis of cells. Such entry points or holes were of 1-2 cells in diameter and surrounded by sclerenchymatous cells to a depth of 10 to 15 cells. The cavities that formed in the outer cortex were always surrounded by deeply stained and heavily suberised cells of irregular shape, whereas those formed in the inner cortex did not have any such deformed darkly stained border cells. Transverse sections of large number of nematodes could be seen in intra as well as intercellular positions in and around the cavities (Fig. 3). Nematodes were not observed in the stelar region as well as in closely packed 4-6

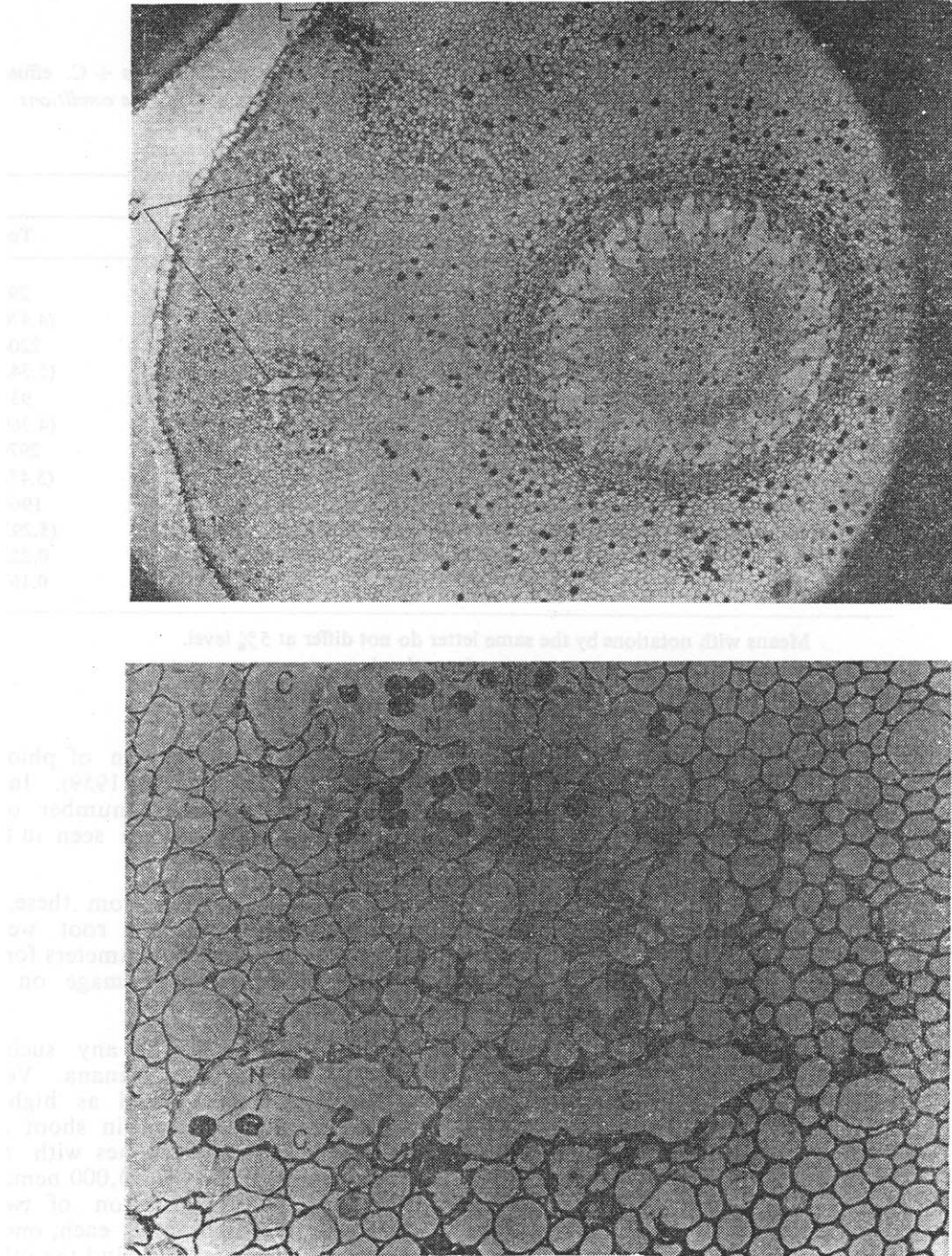


Fig. 3 & 4. T.S. of a coconut root infested by burrowing nematodes showing lesions(L), cavities(C) and cross sections of nematodes (N) in them.

TABLE 6. *Effect of four initial inoculum levels of R. similis and R. similis + C. effusum on nematode multiplication on coconut seedlings in pots under greenhouse conditions*

(Mean of ten replications)

Treatment	Nematode population		
	Root	Soil	Total
T3	17502 (4.2431) <sup>c</sup>	7773 (3.8906) <sup>bc</sup>	29510 (4.4700) <sup>d</sup>
T4	213831 (5.3301) <sup>a</sup>	6031 (3.7804) <sup>c</sup>	220495 (5.3439) <sup>ab</sup>
T5	82533 (4.9166) <sup>b</sup>	9488 (3.9772) <sup>bc</sup>	93128 (4.9691) <sup>c</sup>
T6	167683 (5.2245) <sup>a</sup>	120651 (5.0815) <sup>a</sup>	297666 (5.4737) <sup>a</sup>
T7	184548 (5.26661) <sup>a</sup>	10923 (4.0384) <sup>b</sup>	196462 (5.2933) <sup>b</sup>
C.D. at 1%	0.2761	0.2832	0.2234
C.D. at 5%	0.2068	0.2121	0.1673

Means with notations by the same letter do not differ at 5% level.

Figures in parentheses are log transformed values.

Control (T1) and Fungus (T2) are not included for analysis.

layers of cells outside the endodermis even in heavily infested roots (Fig. 4). In the early stage of infection, roots showed cavities of independent origin separated by several cells. Consequent to nematode multiplication and lysis of cytoplasm and cell walls, adjacent cavities merged each other. Multiple cavities and their coalescence destroyed the cortex to a great extent. Stellar tube was found intact even in heavily infested roots in transverse and longitudinal sections. Eggs and all stages of nematodes with different orientations were seen in cavities in longitudinal sections (Fig. 5).

The strongly suberised endodermis and closely packed 4-5 layers of parenchymatous cells around the endodermis appear to serve as an effective barrier against the invasion of the stele as in the case of banana (Blake, 1966) but in citrus the nematode enters the central cylinder or stele through the passage cells in the endodermis and accumulate in large

numbers in the region of phloem-cambium ring (Ducharme, 1959). In the case of coconut maximum number of nematodes and cavities are seen in the outer cortex.

It was apparent, from these, experiments, that shoot and root weights are the two important parameters for assessing the nematode damage on coconut seedlings.

There are hardly any such studies either with citrus or banana. Venkitesan & Setty (1977) reported as high as 80 to 90 per cent reduction in shoot and root growth of pepper vines with an initial inoculum level of 10,000 nematodes in 55 days. In a comparison of two citrus groves of four hectares each, one planted with healthy seedlings and the other with burrowing nematode infested seedlings, the healthy grove produced 1,322 boxes of citrus/ha and the infested grove only 62 boxes at the end of nine years

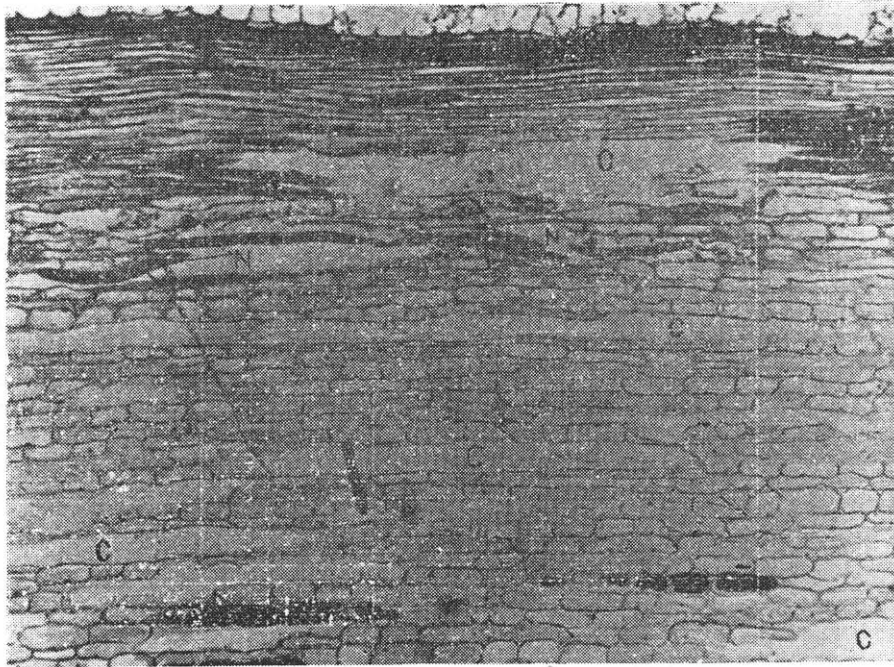


Fig. 5 L. S. of a coconut root infested by burrowing nematode showing nematodes (N) and their cavities (c).

(O'Bannon, 1977). Blake (1972) reported yield increases of 30 to 60 per cent in banana following the control of *R. similis* over a range of conditions.

The results of the greenhouse experiment indicated that the fungus is a secondary pathogen which required the presence of nematode for its successful entry into coconut roots. Further, it was again established that the nematode is pathogenic on coconuts.

The pathogenicity of the burrowing nematode on coconut has been well established by these two experiments. In the field experiment an initial inoculum of 100 nematodes/seedling or one nematode in 800 g sandy loam soil over a period of five years was the threshold inoculum density causing significant reduction of growth parameters but in the greenhouse experiment

a higher initial inoculum of 1000 nematodes/seedling or one nematode in 14 g sandy loam soil was the threshold level. The reasons for the requirement of higher threshold inoculum level could be that the seedlings in the first year of its growth derives considerable quantity of nutrition from the kernel through its haustorium (apple) as such damage to root system by nematodes at lower inoculum levels may not affect the growth significantly, secondly the shorter duration experiment (one year) compared to the long period (five years) and thirdly in the greenhouse experiment seedlings were under shade whereas in the field experiment seedlings were exposed to sunlight all through five years, though plants in both experiments were watered regularly.

Eventhough it would have been ideal to have nematode and fungus combination with every level of nematode inocu-

lum, this could not be done to the difficulties involved in raising large number of seedlings in sterile soil. Hence, only one level of nematode in combination with the fungus was studied.

It is seen from Table 6 and 7 that though the level of *R. similis* in the combined inocula was 10,000 the total multiplication of the nematode, root lesion index and the per cent reductions in plant growth parameters studied were significantly different, not only with the same inoculum level of 10,000 nematodes, but were also significantly less than an initial inoculum density of 100 nematodes. This situation is totally different from the normal nematode-fungus complex disease syndromes. At the same time, it is very interesting to note that the situation is similar with many other *Cylindrocarpon* nematode complex diseases. Pinochet and Stover (1980) reported higher damage index on banana when inoculated with *R. similis* and *Cylindrocarpon* (4.6) than with *R. similis* alone (3.6). They also recorded 89 per cent reduction in *R. similis* population with combined inocula (60) compared to *R. similis* alone (537) over a period of 64 days. It is interesting to note that in the present studies also 90 per cent reduction in population was recorded with combined inocula compared to *R. similis* alone (Table 8) after a period of 12 months. In addition, a corresponding decrease in lesion index was recorded in combined inocula (2.8) compared to *R. similis* alone (4.5).

Reduction in nematode population could be the direct effect of fungus on nematodes or could be the result of rapid colonization of the root tissue by the fungus thereby reducing the feeding sites for *R. similis*. However, further, studies are necessary to support this hypothesis. A similar role of *Cylindrocarpon* in root rot of bulbs (Slootweg, 1956) and Strawberry (Wilhelm, 1959) had been described. *C. destructans* was reported as a minor egg pathogen of *Heterodera*

*avenae* (Tribe, 1969; Kerry and Crump, 1977 and Crump and Kerry, 1983). De Maeseneer (1964) isolated *C. radicicola* and *Fusarium oxysporum* from nematode infested conifer seedlings but concluded that parasitic nematodes were the primary pathogens and expressed doubt that a nematode-fungus complex was involved.

The recovery of *R. similis* from haustorium and leaf bases at the collar region in large numbers further corroborates the damage these nematode can cause to deep sown germinating seednuts in heavily infested areas, especially when banana is grown as a shade crop in coconut nursery.

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