

HOST PREFERENCE OF THE BURROWING NEMATODE, *RADOPHOLUS SIMILIS* POPULATIONS FROM INDIA*

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Abstract : Host specificity study of 28 populations of *Radopholus similis* was done. Designated 17 host differentials to identify races of *R. similis*. The host differentials are coconut (Cv. Laccadive Micro), arecanut (Var. Mangala), banana (Cv. Njalipoovan and Var. Robusta), black pepper (Var. Panniyur-1), nutmeg (Cv. Local), clove (Cv. Local), cardamom (Cv. Vazhukka), tea (Var. Ca-150), coffee (Cv. San Ramon), avocado (Cv. Furte), citrus (Cv. Musambi), pineapple (Cvs. Mauritius and Kew), sweet potato (Var. H-268) and sugarcane (Vars. Co. 449 and Co. 997). Ten races of *R. similis* in south India were identified among the 28 populations on the basis of their multiplication on the 17 identified host differentials. Citrus, clove, pineapple and sugarcane var. Co. 997 were immune to all the 28 populations. Race I multiplied on all the 11 susceptible differentials. Nutmeg was immune to race II and nutmeg and tea were immune to race III. Race IV did not multiply on avocado and nutmeg. Black pepper was immune to race V. Race VI did not multiply on banana Cv. Njalipoovan but it multiplied on tissue cultured banana Var. Robusta and nutmeg. In addition to banana Cv. Njalipoovan, race VII did not multiply on sugarcane Var. Co. 449, avocado, coffee and tea. Race VIII did not multiply on 4 differentials viz. black pepper, sugarcane, tea and nutmeg. Arecanut, tea and nutmeg were immune to race IX. In addition to arecanut and nutmeg, race X did not multiply on black pepper.

Key words : *Radopholus similis*, host specificity, races, differentials

Burrowing nematode, a potential pathogen of many plantation crops like coconut, arecanut, banana, black pepper etc., occurring in several states of South India has been identified as banana race of *Radopholus similis* (Koshy & Sosamma, 1977). The coconut isolate of this nematode parasitizes as many as 48 plant species indicating polyphagy of Indian populations. Subsequent detailed biometrical studies of Indian populations collected mainly from South India, revealed their identity as conspecific populations of *R. similis* (Koshy *et al.*, 1991). Since, as a prerequisite for successful management of any nematode, it is relevant to have precise information on racial picture of parasite,

this study was undertaken with 28 populations of *R. similis* collected from different areas of India to realize this objective.

MATERIALS AND METHODS

Soil and root samples were collected from coconut, arecanut, banana, black pepper, betel vine and from anthurium, from different areas of south India during October-November. Infested banana roots of B₄₁ (Jabalpur) Madhya Pradesh were obtained from Nematology Laboratory of JNKVV, Jabalpur. Samples were also collected from palms/plants/vines showing unthrifty growth, yellowing etc. Lesioned areas of roots were teased and nematodes (females & larvae) were hand picked. The

populations thus collected, were multiplied on respective host plants for a few generations initially and later cultured axenically on carrot discs for three generations.

Host specificity studies were carried out with host differentials as detailed under (Table 1). The host differentials were raised in sandy loam soil fumigated with MBr. For getting nematode/fungal free banana plants, suckers (CV. Njalipoovan) were pared, dipped in fungicide, dithane M.45 (0.3 per cent) and dried in shade. These were planted in sterile soil treated with phorate @ 0.3g a.i./kg of soil contained in plastic/aluminium trays (35 cm x 15 cm). After 45 days, the seedlings were carefully transferred to fresh fumigated soil contained in plastic trays after washing and cutting of all the roots. After three months, these plants were taken out and the roots present were cut off and planted in fresh fumigated soil in 20 cm earthen pots to make sure that roots put out were free from residues of phorate as well as free from nematodes. The other plants were raised from seeds/cuttings/crowns and grown in 20 cm earthen pots. the sugarcane and pineapple plants were kept outside the greenhouse for getting direct sunlight.

Two hundred active specimens consisting of females and juveniles collected from the carrot discs were used as inoculum and had five replications. The plants were watered daily with cold boiled water.

After three months, each plant was deputed carefully into an aluminium tray, soil mixed thoroughly and an aliquot sample of 200 cc was drawn. Soil samples were analysed by Cobb's sieving and sifting method (Cobb, 1918). The whole root system of each replication (plant) was

collected, washed thoroughly to remove the adhering soil particles, weighed and cut into 1 cm bits and sliced. Root bits were mixed thoroughly and three aliquots of one gram each were taken. These samples were stained in boiling acid fuchsin lactophenol for one minute (Franklin & Goodey, 1949), blended in waring blender and the suspension was made up to 200 ml. Three aliquots of 10 ml each were drawn and nematodes present in each aliquot were counted and population in 200 ml was assessed. Average of three aliquots was then multiplied with the total root weight. The sum of the nematodes present in soil and root gave the total multiplication on inoculation with 200 nematodes during three months.

In the case of coconut, arecanut, banana and black pepper inoculation trials were repeated five times, wherever there was no population build up. In banana, tissue cultured seedlings of var. Robusta were used after the population failed to build up on Cv. Njalipoovan.

RESULTS & DISCUSSION

The differential behaviour of 28 populations of *R. similis* with regard to multiplication is given in (Table 2).

Six coconut populations from Kerala used for cross inoculation study, multiplied well on coconut and banana. But on the other differentials these exhibited differential behaviour. Nutmeg, cardamom, tea and coffee were not good hosts of any one of the coconut populations. Out of the six populations, three (C_{17} , C_{34} and C_{37}) multiplied well on sweet potato and four populations (C_{17} , C_{24} , C_{34} and C_{37}) on sugarcane Var. Co. 449. But C_6 (Kasaragod) was unable to infest sugarcane Var. Co. 449.

TABLE 1 : Host differentials used for identification of races

Sl. No.	Common Name	Botanical Name	Variety/Cultivar	Family
1	Arecanut	<i>Areca catechu</i> Linn.	Mangala	Arecaeae
2	Avocado	<i>Persea americana</i> Mill	Furte	Lauraceae
3	Banana	<i>Musa paradisiaca</i> Linn.	Njalipoovan and Robusta	Musaceae
4	Black pepper	<i>Piper nigrum</i> Linn.	Panniyur-1	Piperaceae
5	Cardamom	<i>Elettaria cardamomum</i> (L.) Maton	Vazhukka	Zingiberaceae
6	Clove	<i>Eugenia caryophyllata</i> Willd.	Local	Myrtaceae
7	Coconut	<i>Cocos nucifera</i> Linn.	Laccadive Micro	Arecaeae
8	Coffee	<i>Coffea arabica</i> Linn.	San Ramon	Rubiaceae
9	Nutmeg	<i>Myristica fragrans</i> Houtt.	Local	Myristicaceae
10	Pineapple	<i>Ananas comosus</i> (L.) Merr.	Mauritius and Kew	Bromeliaceae
11	Sugarcane	<i>Saccharum officinarum</i> Linn.	Co. 449 and Co. 997	Graminae
12	Sweet orange	<i>Citrus sinensis</i> (L.) Osbeck	Musambi	Rutaceae
13	Sweet potato	<i>Ipomoea batatas</i> (L.) Lam.	H-268	Convolvulaceae
14	Tea	<i>Camellia sinensis</i> Linn.	Ca. 150	Ternstroemiaceae

TABLE 2 : Identification of *R. similis* races on the basis of their multiplication on host differentials

Place/State	Popu- lation code	Race	Coconut (L. Micro)	Areca- nut (Man- gala)	Banana (Njaln)	(Robu- sta)	Black Pepper (Panni- yur 1)	Sweet Potato (H.268)
Palode (Kerala)	*A21		4667	7654	2100	-	415	180
Koppa (Kerala)	*B39		6786	491	6297	-	1561	167
Kayangulam (Kerala)	*C17		24021	465	462	-	114	240
Valiyathura (Kerala)	C37		74349	476	406	-	179	304
Vittal (Karnataka)	A6		6455	6326	614	-	293	210
Chethali (Karnataka)	B29	1	17689	563	4569	-	46	102
Sringeri (Karnataka)	BV1		28576	718	1192	-	1018	140
Kazhakootam (Kerala)	C34		75751	790	732	-	1349	374
Anchal (Kerala)	B16		21785	40	12145	-	236	1680
Jabalpur (M.P.)	B41		7957	97	7336	-	814	223
Kumarakam (Kerala)	B32	2	659	49	1026	-	577	145
Koppa (Kerala)	*P20		234	102	93	-	741	74
Koothally (Kerala)	P9		9134	372	1810	-	789	131
Vallikunnam (Kerala)	C11		20501	77	467	-	76	20
Vytilla (Kerala)	C24		16082	58	205	-	311	104
Panniyur (Kerala)	P37	3	133	60	236	-	277	112
Neriamangalam (Kerala)	P52		153	6783	37	-	413	230
Thaliparambu (Kerala)	A24		3436	4277	4116	-	1342	240
Koppa (Karnataka)	A44		260	7498	129	-	77	20
Mananthawady (Kerala)	A26	4	144	1967	297	-	1693	86
Kasaragod (Kerala)	B21		3443	77	11088	-	0	44
Sringeri (Karnataka)	B38	5	342	16	8397	-	0	120
Emakulam (Kerala)	B8		801	179	5248	-	0	89
Palode (Kerala)	A40	6	220	24575	0	584	88	372
Perla (Kerala)	P67	7	18817	4089	0	940	2207	106
Kasaragod (Kerala)	C6	8	17472	391	920	-	0	83
Vithura (Kerala)	*An	9	372	0	5068	-	121	58
Palode (Kerala)	Bv	10	3719	0	2254	-	0	496

*A - Arecanut, *B - Banana, C - Coconut, *Bv - Betel vine, An - Anthurium, P - Black pepper

Sugarcane (Co. 449)	(Co. 997)	Avocado (Furte)	Carda- mom (Vazhu- kka)	Coffee (San- Ramon)	Tea (Ca- 150)	Nutmeg (Local)	Clove (Local)	Pineapple (Mauritius)	Citrus (Musambi)	(Kew)
106	0	621	141	68	16	220	0	0	0	0
177	0	160	109	95	70	1030	0	0	0	0
982	0	184	20	12	12	22	0	0	0	0
308	0	533	47	20	90	12	0	0	0	0
98	0	549	95	10	61	6	0	0	0	0
314	0	173	72	13	10	7	0	0	0	0
630	0	642	255	45	10	36	0	0	0	0
636	0	1760	28	52	6	24	0	0	0	0
102	0	2764	377	140	40	0	0	0	0	0
85	0	365	144	13	13	0	0	0	0	0
201	0	372	154	146	22	0	0	0	0	0
120	0	362	17	218	19	0	0	0	0	0
86	0	488	130	28	135	0	0	0	0	0
124	0	663	17	8	0	0	0	0	0	0
236	0	89	10	6	0	0	0	0	0	0
713	0	1232	21	19	0	0	0	0	0	0
98	0	274	23	10	0	0	0	0	0	0
260	0	736	29	10	0	0	0	0	0	0
41	0	0	132	17	127	0	0	0	0	0
1008	0	0	300	52	10	0	0	0	0	0
213	0	23	60	101	10	12	0	0	0	0
51	0	762	168	502	122	8	0	0	0	0
266	0	130	5	10	21	6	0	0	0	0
90	0	194	10	80	60	0	0	0	0	0
0	0	0	127	0	0	0	0	0	0	0
0	0	57	60	79	0	0	0	0	0	0
62303	0	20	1388	135	0	0	0	0	0	0
461	0	1101	9	105	6	0	0	0	0	0

Arecanut was reported as a host from India by Kumar *et al.*, 1971 and later by Koshy *et al.* (1975; 1976). Four populations from the State of Kerala and two from Karnataka were used for host specificity study. All six arecanut populations multiplied well on coconut and arecanut. In banana, A₄₀ from Palode did not multiply on the cultivar Njalipooan but it multiplied in tissue cultured cavendish variety 'Rousta'. Two populations (A₄₀ and A₄₄) multiplied poorly on black pepper. In nutmeg, population from Palode (A₂₁) multiplied well, but not others. Cardamom was found to be a good host for a single population (A₂₆). Except A₂₆ and A₄₄ all the others multiplied well on avocado. Coffee and tea were poor hosts of arecanut populations and two populations (A₂₄ and A₂₆) multiplied well on sugarcane Var. Co.449. Sugarcane, coffee, tea, avocado and sweet potato were reported as good hosts of *R. similis* in other countries.

Five populations from Kerala, three from Karnataka and one from Madhya Pradesh were used for host specificity study. All the nine populations were found to be infective on banana and coconut. Except B₂₉ and B₃₉, all other banana populations multiplied on arecanut. Black pepper was not a good host for most of the banana populations. Banana population from Koppa (B₃₉) multiplied on coffee, avocado, sweet potato and sugarcane. Tea was not a host for most of the banana populations. These results are in consistency with other reports made from several laboratories with regard to difference between "Banana Race" of *R. similis* in infecting plant species (Keetch, 1972; Milne & Keetch, 1976; Edwards & Wehunt, 1971).

All the five black pepper populations

multiplied well on black pepper and showed variations in their multiplication on other hosts.

R. similis has been reported to cause yellows/ slow wilt disease of betel vine in India (Koshy & Sosamma, 1975; Sundararaju & Suja, 1986; Eapen *et al.*, 1987). Betel vine and anthurium populations multiplied well on coconut and banana. Anthurium population (An) did not infest arecanut. Betel vine population (Bv) multiplied well on black pepper, while the population build up of An₁ was poor. Cardamom and sugarcane Var. Co. 449 were good hosts for both these populations. Nutmeg, coffee and sweet potato were poor hosts for anthurium and betel vine populations. Avocado was highly susceptible to betel vine population but not for anthurium population. The betel vine population (Bv) was almost similar to pepper populations (P₉ and P₂₀) except for their difference in multiplication of nutmeg.

The sugarcane variety Co. 997 was found immune to *R. similis* populations whereas the variety Co. 449 supported the multiplication of most of the populations except C₆ (Kasaragod) and P₆₇ (Perla). Sugarcane has been reported as a good host of burrowing nematode in Louisiana (Rands, 1929), Cuba, India, Java, the Philippines, Australia, Hawaii, Florida and Mauritius (Williams, 1969; Prasad, 1972) and poor host in Natal (Milne & Keetch, 1976).

Avocado, a known host of the *R. similis* population in Florida was found to be a good host of all populations except for two arecanut populations (A₂₆-Mananthawady, A₄₄-Koppa) and one black pepper population (P₆₇-Perla). In South Africa, in a host range test conducted

among infested banana var. Dwarf Cavendish recorded avocado as a 'poor host' (Milne & Keetch, 1976).

D'Souza *et al.*, (1970) reported cardamom as a host of *R. similis* from coffee tracts of south western India. In this study only 4 populations (A₂₆, B₁₆, Bv & An) multiplied well on cardamom.

Only two populations (B₃₈ & P₂₀) multiplied well on coffee and others did not. Tea variety Ca 150 was susceptible only to three populations viz. A₄₄ (Koppa), B₃₈ (Sringeri) and P₉ (Koothaly) and a poor host for all other populations.

Koshy & Sosamma (1975) reported nutmeg as a host of the coconut population of *R. similis* at Kayangulam in a host range study. The results obtained in the present study indicated that out of the 28 populations only two populations (A₂₁ & B₃₉) multiplied well on nutmeg.

Non-multiplication of all the 28 populations of *Citrus sinensis* clearly brings out the fact that none of these 28 populations belonged to the citrus race or to *R. citrophilus*. This is in agreement with the earlier studies conducted at the Central Plantation Crops Research Institute, Kayangulam, Kerala, India that the populations from coconut, arecanut and banana belong to the "banana race" as none of these populations infested *Citrus* spp. (Koshy & Sosamma, 1977). Though large number of publications have appeared on the host range of *R. similis* the race status of the populations or many a times the variety of the host used has not been given in most of the reports. The present study has brought out the importance of this showing the sugarcane var. Co. 997 as immune to all populations while var. Co.

449 as highly susceptible to most of the populations and immune to two populations.

In the inoculation trial different ornamental plants, especially different species of *Anthurium* were found to be good hosts of *R. similis*. They were found to be heavily infested with *R. similis* in various government and private nurseries in Kerala. These non-conventional hosts may help in the dissemination of this serious pest to uninfested areas through the network of large number of private and government ornamental nurseries in different parts of India.

Though the inoculum, duration, season of the experiment and variety and age of the host were the same, varied multiplication of nematode was noted on all the 17 differentials when different populations were used. This clearly brings out the differences in their reproductive potential on different hosts which is in agreement with the study conducted in Central America (Edwards & Wehunt, 1971); Natal (Milne & Keetch, 1976); Central Florida (O'Bannon & Ford, 1979); S. America (Tarte *et al.*, 1981) and Puerto Rico (Rivas & Roman, 1985).

Although the 28 populations varied in their host specificity, they still had many hosts in common. The results revealed definite differences in the multiplication of different populations on the same host (A₂₁ from Palode and B₂₁ from Kasaragod showed varied multiplication on black pepper) as well as multiplication of populations from the same crop from the same area (A₂₁ and A₄₀ from Palode showed differences in multiplication on nutmeg and banana).

When we look into the total population and the root weight of the host plant it is seen that coconut, arecanut, banana, sugarcane, sweet potato or avocado had more of susceptible succulent roots compared to black pepper, tea, coffee, cardamom etc. and hence multiplication of nematode population was more. A perusal of the data on per gram population (Table 3) showed that the populations collected from coconut, arecanut, banana and black pepper multiplied maximum on their respective hosts except in a few cases, while there were populations that multiplied poorly on the original host viz. A₂₄, B₁, B₃₂, B₄₁ and P₅₂. This led to the conclusion that differences in multiplication was not due to the availability of plenty of susceptible roots but might be because of host preferences.

The pooled data of the results of host specificity studies indicated the presence of 10 races in *R. similis* by using 17 host differentials. They are grouped according to their multiplication on 17 differentials (Table 4). The results indicated existence of 10 host specific populations/races of *R. similis* in India.

The difference in host specificity can be due to various factors. The isolates collected from roots of coconut, arecanut, banana and black pepper in the field could

possibly be a mixture of various populations or otherwise under the high density multispecies cropping system that exists in the west coast of south India. To avoid this initial differences, these populations were multiplied initially on the respective host plants for a few generations and later cultured axenically on carrot discs for three generations. All host range studies were conducted only with the populations from carrot discs to avoid the initial specificity, if any, because of their original host.

Irrespective of the populations involved, coconut appeared to be the best host for most of the populations followed by banana, arecanut, sugarcane, black pepper, sweet potato and avocado. Of these, black pepper was found to suffer maximum damage and exhibit yellowing, within three months with an initial inoculum of 200 nematodes, whereas in all other cases, though there was reduction in growth, the damage done by the nematode was not very apparent.

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TABLE 3 : Multiplication of different populations of *R. similis* on four different hosts (Per gram population) (Inoculum level : 200, replications : 5, duration : 3 months)

Crop	Population Code	Coconut	Arecanut	Banana	Black pepper
Coconut	C ₆	690	140	43	0
	C ₁₁	711	70	15	16
	C ₁₇	1805	51	52	24
	C ₂₄	433	4	15	47
	C ₃₇	2107	52	7	38
Arecanut	A ₆	195	588	192	85
	A ₂₁	72	1756	55	315
	A ₂₄	220	186	227	502
	A ₂₆	2	305	42	263
	A ₄₀	8	2753	18	24
	A ₄₄	4	1500	10	20
Banana	B ₁	260	0	188	0
	B ₈	25	13	407	0
	B ₁₆	436	8	1155	80
	B ₂₁	296	66	684	0
	B ₂₉	200	33	200	9
	B ₃₂	8	20	30	313
	B ₃₈	55	7	41	0
	B ₃₉	260	46	526	80
	B ₄₁	378	8	370	462
Black pepper	P ₉	349	851	49	773
	P ₂₀	166	13	9	580
	P ₃₇	7	36	117	130
	P ₅₂	9	621	5	205
	P ₆₇	1343	524	33	1453
Betel vine	Bv	94	47	90	198
Anthurium	An	53	0	203	26

TABLE 4 : Key for identification of races of the burrowing nematode, *Radophobus similis*

RACE	POPULATIONS	HOSTS	NON-HOSTS
I	A ₂₁ , B ₃₀ , C ₁₇ , C ₃₇ , A ₆ , B ₂₉ , C ₃₄ , BV ₁	Coconut, Arecanut, Banana, Black pepper, Sweet potato, Sugarcane (CO ₄₄₉), Avocado, Cardamom, Coffee, Tea, Nutmeg	Sugarcane (CO ₉₉₇), Sweet orange (Musambli), Pineapple (Mauritius), Pineapple (Kew), Clove (local)
II	B ₁₆ , B ₄₁ , B ₃₂ , P ₂₀ , P ₅	Coconut, Arecanut, Banana, Black pepper, Sweet potato, Sugarcane (CO ₄₄₉), Avocado, Cardamom, Coffee, Tea	Sugarcane (CO ₉₉₇), Sweet orange (Musambli), Pineapple (Mauritius), Pineapple (Kew), Clove (local), Nutmeg
III	C ₁₁ , C ₂₄ , P ₅₂ , A ₂₄	Coconut, Arecanut, Banana, Black pepper, Sweet potato, Sugarcane (CO ₄₄₉), Avocado, Cardamom, Coffee	Sugarcane (CO ₉₉₇), Sweet orange (Musambli), Pineapple (Mauritius), Pineapple (Kew), Clove (local), Tea, Nutmeg
IV	A ₄₄ , A ₂₆	Coconut, Arecanut, Banana, Black pepper, Sweet potato, Sugarcane (CO ₄₄₉), Cardamom, Coffee, Tea	Sugarcane (CO ₉₉₇), Avocado, Sweet orange (Musambli), Pineapple (Mauritius), Pineapple (Kew), Clove (local), Nutmeg
V	B ₉ , B ₂₁ , B ₃₈	Coconut, Arecanut, Banana, Sweet potato, Sugarcane (CO ₄₄₉), Avocado, Cardamom, Coffee, Tea, Nutmeg	Black pepper, Sugarcane (CO ₉₉₇), Sweet orange (Musambli), Pineapple (Mauritius), Pineapple (Kew), Clove (local)

RACE	POPULATIONS	HOSTS	NON-HOSTS
VI	A ₄₀	Coconut, Arecanut, Banana, (Robusta), Black pepper, Sweet potato, Sugarcane (CO ₄₄₉), Avocado, Cardamom, Coffee, Tea	Banana (Njalipoovan), Sugarcane (CO ₉₉₇), Sweet orange (Musambi), Pineapple (Mauritius), Pine-apple (Kew), Nutmeg, Clove (local)
VII	P ₆₇	Coconut, Arecanut, Banana (Robusta), Black pepper, Sweet potato, Cardamom	Banana (Njalipoovan), Sugarcane (CO ₄₄₉), Sugarcane (CO ₉₉₇), Avocado, Coffee, Tea, Nutmeg, Sweet orange (Musambi), Pine-apple (Mauritius), Pineapple (Kew), Clove (local)
VIII	C ₆	Coconut, Arecanut, Banana, Sweet potato, Avocado, Cardamom, Coffee	Black pepper, Sugarcane (CO ₄₄₉) Sugarcane (CO ₉₉₇), Tea, Nutmeg, Sweet orange (Musambi), Pineapple (Mauritius), Pineapple (Kew), Clove (local)
IX	An	Coconut, Banana, Black pepper, Sweet potato, Sugarcane (CO ₄₄₉) Avocado, Cardamom, Coffee	Arecanut, Sugarcane (CO ₉₉₇), Tea, Nutmeg, Sweet orange (Musambi), Pineapple (Mauritius) Pineapple (Kew), Clove (local)
X	B ₁	Coconut, Banana, Sweet potato, Cardamom, Coffee, Tea, Sugarca (CO ₄₄₉), Avocado	Arecanut, Black pepper, Sugarcane (CO ₉₉₇), Nutmeg, Sweet orange (Musambi), Pine apple (Mauritius), Pine apple (Kew), Clove (local)

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