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TECHNIQUES FOR SCREENING BLACK PEPPER GERMPLASM TO *RADOPHOLUS SIMILIS* AND *MELOIDOGYNE INCOGNITA**

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Abstract : Techniques for screening black pepper (*Piper nigrum* L.) germplasm to *Radopholus similis* and *Meloidogyne incognita* were standardised. An inoculum of 250 nematodes and recording root lesion index five months after inoculation or an inoculum of 150 nematodes and recording root lesion index six months after inoculation for *R. similis* and an inoculum of 1000 second stage juvenlies and recording root-knot index four months after inoculation for *M. incognita* were found optimum for screening black pepper germplasm for their reaction to these nematodes.

Key words : Screening technique, *Radopholus similis*, *Meloidogyne incognita*, **Black pepper,** *Piper nigrum*.

The burrowing nematode, *Radopholus similis* and the root-knot nematode, *Meloidogyne incognita* are the two important endoparasitic nematodes infesting black pepper vines (*Piper nigrum* L.) in Kerala, a major pepper growing state in India (Jacob & Kurian, 1979; Ramana & Mohandas, 1987) causing considerable damage to pepper cultivation. Application of nematicides reduces nematode population in black pepper (Mohandas & Ramana, 1987), the cost, difficulty in handling the chemicals and environmental pollution are limiting factors in their use. Hence, there is an urgent need to identify genotypes resistant/tolerant to these species of nematodes. No standard methods for screening black pepper germplasm to plant parasitic nematodes are available. The results of such studies are presented in this paper.

MATERIALS AND METHODS

Radopholus similis : Single node rooted cuttings of black pepper hybrid 'Panniyur-1' were planted singly in polythene bags (15×10 cm) containing 1.5 kg fumigated nursery mixture (forest soil 2 : sand 1 : cowdung 1). Three months after planting, one hundred plants of uniform growth with 4-6 leaves were selected and grouped into five sets consisting of twenty plants each. In all sets four plants each were inoculated with 10, 50, 150 and 250 nematodes per plant and the remaining four uninoculated plants in each set served as controls. Monoxenic culture of *R. similis* raised on carrot discs was used for inoculations. Plants in each set were removed from polythene bags at monthly intervals starting from second month after inoculation. Roots were washed free of soil particles and root lesion index was recorded on 1-5 scale (Ramana *et al.*, 1987). Root weight (wet) of individual plant was also recorded and one gram of root sample was drawn. Soil in each bag was mixed thoroughly and nematode population in 100 ml soil sample was estimated using modified Cobb's seiving and decanting technique. Nematode populations in root samples were estima-

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ted after staining the roots in acid fuchsin lactophenol and blending.

Meloidogyne incognita : Three node cuttings of 'Panniyur-1' were planted singly in polythene bags containing fumigated nursery mixture for rooting. Three months after planting, one hundred fifty plants of uniform growth having 4-5 leaves were selected and grouped into five sets, each consisting of 30 plants. Five plants each in all sets were inoculated with 100, 250, 500, 1000 and 2000 freshly hatched second stage juveniles of *M. incognita*. Five plants in each set were left uninoculated to serve as controls. Nematode populations in root samples were estimated as per the methods described for *R. similis*. Root-knot index was recorded on 0-5 scale (Taylor & Sasser, 1978).

RESULTS AND DISCUSSION

Radopholus similis : The data presented in Table I showed that the root lesion index (R.L.I) varied with initial inoculum levels at different intervals and it gradually increased with the increase in the period of exposure of the plants to nematodes. Highly susceptible reaction of the host plant (R.L.I 4 and above) was recorded in plants inoculated with higher inoculum levels (150 and 250 nematodes) six months after inoculation. Plants inoculated with the above inoculum levels also showed high nematode population (1173.05 and 1305.5 respectively) at three months after inoculation which reduced gradually in subsequent months. In plants inoculated with lower inoculum levels (10 and 50 nematodes) nematode population reached its peak at fourth month. A similar trend was observed with regard to nematode multiplication and population in one gram of roots in all the treatments. Nematode population did not show a corresponding increase with increase in the inoculum levels and the multiplication rate was maximum in the lowest inoculum. Similar results were observed in *Heterodera* spp. where greatest nematode population occurred at low/moderate inoculum levels and as the inoculum levels increased the rate of increase became less (Chitwood & Feldmesser, 1948; Jones, 1959; Peters, 1961).

Nematode population build up (final population (Pf.)/initial population (Pi) calculated on the basis of nematode population at a particular month (Pf) and the nematode population in the preceding month (Pi) was negative at fourth month in higher inoculum levels and at fifth month with lower inoculum levels. The results showed that there was no further increase in nematode population after reaching a certain level irrespective of the initial inoculum level. It may probably be due to the damage inflicted to the roots by nematode infestation resulting in the non-availability of fresh roots for nematode infestation and multiplication. An increase in the population density occurs when the host plant permits a reproductive rate in exess of mortality rate. Results in the present study showed that the nematode caused severe damage to roots. In uninoculated plants there was a considerable increase in the root weight (wet). from 3.38 g at second month to 9.3 g at sixth month (Table 1). Plants inoculated with 50, 150 and 250 nematodes showed significant reduction in the root growth from third month whereas the reduction was significant from fourth month onwards in plants inoculated with 10 nematodes. The reduction was more than 75 per cent in all inoculum levels over uninoculated plants at sixth month. Maximum reduction (86.02 per cent) was in plants inoculated with 250 nematodes. The results indicated that the damage caused to roots by *R. similis* infestation was severe and hence could not support nematode population further resulting in the low rate of nematode multiplication and decrease in the total nematode population from fourth month onwards. In *Heterodera schachtii* also the increase in population was more or less at the same level whatever was the initial population and Goffart (1952) attributed

Table 1. *Root growth, root lesion index and nematode build up in black pepper rooted cuttings inoculated with* Radopholus similis. (*Mean of four replications*)

Inoculum level	Root weight (wet) g*	Root lesion index	Nematode population			
			Soil	Root	one g of root	Build up (Pf/Pi)**
Two months after inculation						
Control	3.38	—	—	—	—	—
10 nematodes	3.70	0.25	7.50	60.40	16.32	6.7
50 ,,	3.17 (6.21)	0.50	18.75	93.25	29.41	2.2
150 ,,	3.23 (4.43)	0.75	22.50	142.45	44.19	1.1
250 ,,	4.02	1.25	112.50	282.20	70.19	1.5
C.D. at 5%	NS					
Three months after inoculation						
Control	5.89	—	—	—	—	—
10 nematodes	5.23 (9.67)	0.50	15.00	105.70	20.21	12.0 (1.7)
50 ,,	4.83 (17.99)	0.50	22.50	280.90	58.15	6.6 (2.7)
150 ,,	4.20 (28.69)	2.25	540.50	633.05	150.72	7.8 (7.0)
250 ,,	4.28 (27.33)	1.75	288.75	1016.75	237.55	5.9 (3.3)
C.D. at 5%	1.03					
Four months after inoculation						
Control	7.85	—	—	—	—	—
10 nematodes	3.75 (52.35)	1.00	131.25	686.45	183.54	81.7 (6.7)
50 ,,	3.36 (57.19)	1.50	138.75	799.50	237.94	18.7 (3.0)
150 ,,	3.11 (60.38)	3.25	213.75	569.00	182.95	5.2 (—1.4)
250 ,,	3.12 (60.25)	3.50	217.50	689.60	221.02	3.6 (—1.4)
C.D. at 5%	1.62					
Five months after inoculation						
Control	8.48	—	—	—	—	—
10 nematodes	3.30 (61.08)	1.50	116.24	393.10	119.12	50.9 (—1.6)
50 ,,	2.66 (68.63)	2.75	142.30	311.80	117.21	9.0 (—2.0)
150 ,,	2.46 (70.99)	3.25	228.75	336.00	136.58	3.7 (—1.3)
250 ,,	1.68 (80.18)	4.00	292.50	225.70	134.34	2.0 (—1.7)
C.D. at 5%	1.61					
Six months after inoculation						
Control	9.30	—	—	—	—	—
10 nematodes	2.29 (75.37)	3.00	131.25	386.20	128.73	51.7 (1.0)
50 ,,	1.81 (80.53)	3.75	142.50	197.50	52.66	6.8 (—1.3)
150 ,,	1.70 (81.72)	4.25	116.25	194.60	45.78	2.0 (—1.8)
250 ,,	1.30 (86.02)	4.75	127.50	161.15	33.93	1.1 (—1.7)
C.D. at 5%	1.20					

Figures in parentheses denote * Per cent reduction over control plants;

** Nematode build up/reduction over the population of preceding month.

Table 2. *Root growth, root-knot index and nematode build up in black pepper rooted cuttings inoculated with* Meloidogyne incognita (*Mean of five replications*)

Inoculum level	Root weight (wet) g*	Root knot index	Nematode population		
			Root	One gram of roots	Build up (Pf/Pi)**
Two moths after inoculation					
Control	1.73	—	—	—	—
100 nematodes	1.64	0.4	208.32	128	2.08
250 ,,	1.81	1.4	258.52	144	1.03
500 ,,	1.59	1.8	318.60	200	0.63
1000 ,,	1.55	1.4	395.72	264	0.39
2000 ,,	1.81	2.0	732.48	408	0.36
C.D. at 5%	NS				
Three months after inoculation					
Control	2.63	—	—	—	—
100 nematodes	2.99	0.8	1279.68	440	12.79 (6.14)
250 ,,	2.95	1.6	1186.16	412	4.74 (4.58)
500 ,,	3.31	1.6	1562.92	468	3.12 (4.90)
1000 ,,	3.31	2.2	2502.64	768	2.50 (6.32)
2000 ,,	3.79	3.0	4682.00	1228	2.34 (6.39)
C.D. at 5%	0.59				
Four months after inoculation					
Control	4.38	—	—	—	—
100 nematodes	4.58	2.4	7716.64	1684	77.16 (6.03)
250 ,,	3.98 (9.13)	2.0	6945.28	1718	27.78 (5.85)
500 ,,	2.91 (33.56)	2.6	5946.60	2044	11.89 (3.80)
1000 ,,	2.99 (31.73)	4.0	13864.84	4722	13.86 (5.54)
2000 ,,	2.85 (34.93)	4.0	14480.20	5108	7.24 (3.09)
C.D. at 5%	0.62				
Five months after inoculation					
Control	4.97	—	—	—	—
100 nematodes	3.86 (22.33)	2.4	9163.96	2348	91.63 (1.18)
250 ,,	4.13 (16.90)	3.0	8815.56	2176	35.20 (1.26)
500 ,,	3.38 (31.99)	3.4	7465.08	2248	14.93 (1.25)
1000 ,,	3.53 (28.97)	4.0	16801.36	4760	16.08 (1.21)
2000 ,,	2.95 (40.64)	4.2	14207.92	4736	7.10 (0.98)
C.D. at 5%	0.86				
Six moths after inoculation					
Control	5.87	—	—	—	—
100 nematodes	4.56 (22.31)	3.0	14793.32	3364	147.93 (1.61)
250 ,,	4.16 (29.13)	4.2	18735.00	4556	74.94 (2.12)
500 ,,	3.02 (48.55)	4.4	12965.52	4548	25.93 (1.73)
1000 ,,	2.73 (53.49)	4.4	12551.88	4748	12.55 (0.74)
2000 ,,	2.78 (52.64)	5.0	11214.08	4160	5.60 (—1.26)
C.D. at 5%	0.58				

Figures in parentheses denote * Per cent reduction over control plants.
** Nematode build up/reduction over the population of preceeding months.

this phenomenon due to volume of root available for nematode infestation. The results showed that with lower inoculum levels a longer period is required to R.L.I of 4.0 and above, a highly susceptible reaction of the host plant to the nematode. An inoculum of 250 nematodes and recording R.L.I five months after inoculation or an inoculum level of 150 nematodes and recording R.L.I six months after inoculation is optimum for studying the reaction of black pepper germplasm to *R. similis*.

Meloidogyne incognita : Root knot index (R.K.I) and nematode population in the roots at monthly intervals are given in Table 2. The R.K.I increased gradually with increase in the period of exposure of the host plant to nematodes and also correspondingly with increase in the inoculum level. Highest R.K.I (5.0) was recorded at sixth month in plants inoculated with 2000 nematodes. Highly susceptible reaction of the host plant (R.K.I 4 and above) was recorded at fourth month with an inoculum of 1000 and 2000 nematodes. Nematode population in roots also increased with the increase in the period of exposure of the plants to nematodes in lower inoculum levels (100, 250 and 500 nematodes). However, there was a decrease in the nematode population at higher inoculum levels (1000 and 2000 nematodes) at sixth month. Nematode population in one gram of roots also showed similar trend. Maximum population in one gram of roots (5108) was in plants inoculated with 2000 nematodes followed by 1000 nematodes (4772) at fourth month indicating that nematode population reached its peak level and there was no further increase in subsequent months. In lower inoculum levels nematode population was in increasing trend even at sixth month. Nematode build up (Pf/Pi) in higher inoculum levels gradually decreased from fifth month onwards and it was negative at sixth month in plants inoculated with 2000 nematodes. There was a considerable increase in root weight (wet) in control plants from 1.73 g at second month to 5.87 g at sixth month. In the initial stage inoculated plants had root weight more than that of uninoculated plants. From fourth month onwards root weight decreased in all inoculated plants over control plants and the reduction was significant in plants inoculated with 500, 1000 and 2000 nematodes which were on par. Maximum reduction (53.49 per cent) was with 1000 nematodes followed by 2000 nematodes (52.64 per cent) at sixth month. The study indicated that an inoculum of 1000 second stage juveniles and assessing R.K.I four months after inoculation is optimum for screening black pepper germplasm to *M. incognita*.

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