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ROLE OF PLANT PARASITIC NEMATODES IN THE SLOW WILT DISEASE COMPLEX OF BLACK PEPPER (*PIPER NIGRUM* L.) IN KERALA

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Abstract : High populations of *Radopholus similis* occurred more frequently in disease affected vines (4 out of 5) than in healthy vines (1 out of 5). High populations of *Meloidogyne incognita* and *Trophotylenchulus piperis* occurred in equal frequency in healthy and diseased vines. Further, the discriminant analysis indicated that presence of *R. similis* alone can influence overall differences between healthy and diseased state of the vines. The results clearly indicated that *R. similis* plays a major role in causing slow wilt disease in black pepper.

Key Words : Black pepper, *Piper nigrum*.

In recent years, 'slow wilt' disease of black pepper (*Piper nigrum* L.) is rampant in many of the pepper growing tracts of Kerala, India and is one of the major constraints in pepper production. The disease is characterised by the appearance of mild to severe foliar yellowing. The leaves occasionally show tip burn symptoms and the root system of the affected vines shows root-knots and varying degrees of necrosis and degeneration (Nambiar & Sarma, 1977). Some of the affected vines recover with the onset of the monsoon, but others lose vigour, productivity and finally succumb to the disease.

Many workers had attributed the disease to infestation by plant parasitic nematodes and have reported that primarily the root-knot nematode, *Meloidogyne incognita* and burrowing nematode, *Radopholus similis* are responsible for the 'pepper yellows' (Bridge, 1978; Christie, 1959; Ichinohe, 1976; Mustika, 1978; Ting, 1975 and Venkitesan & Setty, 1977). *Trophotylenchulus piperis* was also reported on black pepper from Kerala (Mohandas *et al.*, 1985).

Meloidogyne incognita and *Radopholus similis* were believed to be the primary cause of 'pepper yellows'. Butler (1906) and D'Souza *et al.* (1970) reported the association of root-knot and burrowing nematodes respectively with black pepper in India. Van der Vecht (1950) and Christie (1957) reported that *R. similis* was responsible for the 'pepper yellows' and this nematode was attributed to the death of millions of black pepper plants in Banka Islands of Indonesia (Christie, 1959). Venkitesan & Setty (1977) mentioned *R. similis* as the primary incitant of slow wilt disease in Kerala. Yellowing and gradual decline of pepper vines in Brazil and Indonesia (Ichinohe, 1975; 1976) and in Malaysia (Ting, 1975) was attributed to severe infestation by root-knot nematodes.

Hubert (1957) and Bridge (1978) were of the view that *R. similis* was primarily responsible for the disease but an associa-

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tion with fungus like *Fusarium* sp. was necessary to cause yellows disease. However, Mustika (1978) reported that there was a close relationship between the population levels of *M. incognita* and *R. similis* and onset of lethal yellowing of black pepper in Indonesia.

de Waard (1969) attributed 'pepper yellows' in Sarawak to nutritional deficiency and suggested that nematodes were only of secondary importance. The disease could be effectively controlled by application of fertilizers (de Waard, 1979). There were indications that deficiency of plant nutrients like N, P and K and soil moisture have roles in the disease incidence (Harper, 1974 and Wahid *et al.*, 1982). According to Winoto (1972) plants infested with root-knot nematode were more susceptible to infection by *Phytophthora*. Nambiar and Sarma (1979) attributed 'slow wilt' disease in Kerala to fungal-nematode complex, nutritional deficiency and moisture stress. A comprehensive study was undertaken in nine pepper growing districts of Kerala to study in detail the association of plant parasitic nematodes with root system of both healthy and 'slow wilt' affected black pepper vines and the role of nematodes in the disease incidence.

MATERIALS AND METHODS

The root samples were collected for a period of five years (1980–84) during September-November months, when nematode population in black pepper was found to be maximum. A total of 177 gardens were selected at random for the survey in Calicut, Wynad, Cannanore, Kasaragod, Idukki, Kottayam, Pattanamthitta, Quilon and Trivandrum districts. In each garden five healthy vines and five vines showing foliar yellowing (diseased) were selected at random. About 25 g roots (composite sample) were collected both from the healthy and diseased vines separately in each garden. Samples from healthy vines could be collected only in gardens where there was no disease incidence.

Root samples were washed free of soil particles, fibrous roots were cut into bits and the populations of three nematode species were estimated in one gram roots. The nematode populations were grouped into five grades on the basis of population for the purpose of statistical analysis (grades 1 = < 100; 2 = 101–250; 3 = 251–500; 4 = 501–1000 and 5 = > 1000 for *R. similis* and *T. piperis* and grades 1 = < 500; 2 = 501–1000; 3 = 1001–5000; 4 = 5001–10,000 and 5 = > 10,000 for *M. incognita*).

Statistical analysis was carried out to study whether there was any variation in the distribution patterns of five grades of nematode populations in roots of healthy and diseased plants. The data on three nematode species for each district were analysed separately and the heterogeneity x^2 values were compared to test whether there was any variation in their distribution pattern (Mather, 1977). A similar analysis was done by combining the frequencies of lower grades (1 and 2) and higher grades (3, 4 and 5) of nematode populations. The probabilities of occurrence of higher grades of nematode population in the roots of healthy and diseased plants for individual nematode species were also estimated.

The frequency of occurrence of the three nematode species at higher population grades either individually or in different combinations (i.e., the frequency of 0, 1, 2 and 3 nematode species in healthy and diseased plants) was tabulated. Fitting of binomial distribution was done to study the distribution pattern. This helped in estimating the mean number of nematode species (among the 3 under consideration) that occur in high population grades, in healthy and diseased plants.

To study the role of the individual nematode species in the incidence of the disease, when they occur jointly in roots of healthy and diseased plants, the population grades of three nematode species were tabulated for healthy and diseased samples. In all 177 and 155 sets of root samples from healthy and diseased plants, respectively, were considered for analysis. Based on the 'within group' sum of squares and sum of products matrix of population grades, a linear discriminant function of the form:

$Y = l_1 x_1 + l_2 x_2 + l_3 x_3$ where l_1, l_2 and l_3 were discriminant coefficients and x_1, x_2, x_3 were population grades of *R. similis*, *M. incognita* and *T. piperis*, respectively, was evaluated. This function was evaluated to help in discriminating the roots of healthy plants from that of diseased plants based on observed levels of population grades of three nematode species. If *di* is the difference in the mean population grades between the roots of healthy and diseased plants for the i^{th} nematode species, then a dispersion parameter representing the overall difference between roots of healthy and diseased plants can be evaluated as:

$D^2 = 3 \Sigma\, li\; di$ where i = 1 to 3 are for the 3 nematode species.

However, to test whether any single nematode species alone was sufficient to represent the overall difference between the status of healthy and diseased plants, D^2 was calculated for that particular nematode species alone and a suitable 'F-test' was made to test the hypothesis based on the difference in D^2 values (Rao, 1974).

RESULTS AND DISCUSSION

The frequency distribution pattern of different population grades of *R. similis*, *M. incognita* and *T. piperis* in the roots of healthy and diseased plants for nine districts is presented in Table 1. The heterogeneity x^2 values evaluated to study the variation in the distribution frequency of five population grades of *R. similis* in roots of healthy and diseased plants were significant for all the major pepper growing districts (Calicut, Cannanore, Kasaragod, Idukki and Pattanamthitta) except for Wynad district, indicating that the distribution pattern of *R. similis* is not the same in healthy and diseased plants. However, the x^2 values for *M. incognita* and *T. piperis* were not significant for most of the districts indicating that the grades of these nematode populations did not vary in healthy and diseased plants.

Further, the frequencies of the low population grades (1 and 2) and high population grades (3, 4 and 5) were combined to study the pattern of occurrence of low and high populations. For *R. similis*, the distribution pattern of high and low population grades was not the same for diseased and healthy plants in all the major pepper growing districts except Wynad. It should, however, be mentioned that in Wynad the incidence of the disease was low and also the population of *R. similis* was very low. The heterogeneity values for *M. incognita* and *T. piperis* were not significant indicating that there was no variation in the pattern of occurrence of low and high grades of nematode populations in healthy and diseased plants.

Taking into consideration, the frequency of occurrence of higher grades of nematode populations only (3, 4 and 5) in roots of healthy and diseased plants and the non-signficant heterogeneity x^2 values, it was estimated that higher grades of *R. similis* populations occurred in 1 out of 5 healthy plants and 4 out of 5 diseased plants. In case of *M. incognita* and *T. piperis* higher population grades occurred at equal frequency in healthy and diseased plants. In majority of the cases where the populations of the three nematode species were low, the symptoms of the disease were not expressed. This consistent association of high population of *R. similis* with root system of diseased vines clearly indicated its definite role in 'slow wilt' syndrome of black pepper.

TABLE 1. *Distribution pattern of five population grades of three plant parasitic nematodes in the roots of healthy and diseased plants in Kerala.*

District/State of the plant	No. of samples which yielded different population levels														
	R. similis					*M. incognita*					*T. piperis*				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Calicut															
Healthy (21)	11	6	2	2	—	11	2	8	—	—	10	4	6	1	—
Diseased (21)	4	5	4	5	3	6	4	5	5	1	4	4	8	3	2
Heterogeneity χ^2_{4df}	8.31					8.82					5.85				
Wynad															
Healthy (15)	15	—	—	—	—	5	4	6	—	—	14	—	—	1	—
Diseased (15)	15	—	—	—	—	4	3	3	5	—	11	4	1	2	—
Heterogeneity χ^2_{4df}	0.00					6.25					2.69				
Cannanore															
Healthy (15)	8	3	2	1	1	6	5	4	—	—	13	1	1	—	—
Diseased (11)	1	—	2	4	4	4	1	6	—	—	5	1	4	1	—
Heterogeneity χ^2_{4df}	11.70					2.92					5.87				
Kasaragod															
Healthy (25)	18	4	1	1	1	19	5	1	—	—	16	—	6	1	2
Diseased (22)	7	3	7	—	5	17	4	1	—	—	10	2	2	3	5
Heterogeneity χ^2_{4df}	13.01					0.03					7.50				
Idukki															
Healthy (51)	37	9	2	1	2	44	2	5	—	—	27	12	6	2	4
Diseased (51)	17	7	8	8	11	41	4	6	—	—	37	5	4	3	2
Heterogeneity χ^2_{4df}	22.93					0.86					5.71				
Kottayam															
Healthy (9)	5	1	1	2	—	8	1	—	—	—	9	—	—	—	—
Diseased (1)	—	—	—	1	—	1	—	—	—	—	1	—	—	—	—
Heterogeneity χ^2_{4df}	2.59					0.12					0.00				
Pathanamthitta															
Healthy (22)	20	1	—	1	—	17	3	1	1	—	17	2	2	1	—
Diseased (15)	7	5	1	2	—	12	1	1	—	1	11	1	1	1	1
Heterogeneity χ^2_{4df}	9.26					2.63					1.68				
Quilon															
Healthy (6)	6	—	—	—	—	3	1	2	—	—	5	—	—	—	—
Diseased (6)	5	—	1	—	—	4	1	1	—	—	6	—	—	—	—
Heterogeneity χ^2_{4df}	1.09					0.47					1.09				
Trivandrum															
Healthy (13)	12	1	—	—	—	12	1	—	—	—	8	2	3	—	—
Diseased (13)	13	—	—	—	—	13	—	—	—	—	8	4	1	—	—
Heterogeneity χ^2_{4df}	1.04					1.04					1.67				

Figures in parantheses denote number of root samples examined.

The frequency distribution of nematode species with higher levels of nematode population was well fit by binomial distribution for healthy and diseased plants (Table 2). In healthy plants, the mean value was 0.45 and in diseased plants it was 0.86 indicating that all the three nematode species need not occur in higher population simultaneously to cause the disease and that on the average one nematode species in higher population level could induce the disease. It was also found that higher population of *R. similis* occurred in 66 per cent diseased plants while higher populations of *M. incognita* and *T. piperis* occurred in 19 and 15 per cent diseased plants, respectively.

The parameters of discriminant analysis are presented in Table 3. The analysis yielded a dispersion parameter $D^2 = 1.96 \times 10^{-3}$ representing the overall difference between the healthy and diseased plants, when population levels (namely 1 to 5) of all the three nematode species were taken into consideration. The D^2 value based on population levels of *R. similis* alone was equal to 1.79 ×

TABLE 2. *Frequency distribution of number of nematode species (k) with high population grades in the roots of healthy and diseased plants.*

No. of nematode species (k)	Healthy plants		Diseased plants	
	Observed frequency	Expected* frequency	Observed frequency	Expected* frequency
0	110	108	62	56
1	55	58	54	68
2	11	10	37	27
3	1	1	2	4
TOTAL (N)	177	177	155	155
MEAN (np)	0.4520		0.8645	
χ^2_{2df}	0.283 (N.S.)		5.590 (N.S.)	

*The expected frequencies are fitted using binomial distribution.

$N \times n_{c_k} \quad P^k \quad Q^{(n-k)}$ where $P = 0.1507$; $Q = 0.8493$ for healthy plants,
and $P = 0.2882$; $Q = 0.7118$ for diseased plants
$n = 3$

TABLE 3. *Parameters of Discriminant analysis*

Nematode species	Mean grades of population		Difference vector of mean grades	Discriminant coefficient
	Healthy	Diseased		
Radopholus similis	1.4576	2.4129	0.9553	1.8890 E—03
Meloidogyne incognita	1.4576	1.6516	0.1940	7.9525 E—04
Trophotylenchulus piperis	1.5819	1.7742	0.1923	6.4642 E—06

10^{-3}. The appropriate 'F-test' indicated that D^2 values did not differ significantly indicating that the population level of *R. similis* alone was sufficient to represent the overall difference between healthy and diseased plants. Earlier field control trials with nematicides against 'slow wilt' disease of pepper in Kerala showed remission of foliar yellowing indicating the indirect evidence for the role of nematodes in the slow-wilt disease complex (Nambiar and Sarma, 1977). Inoculation of pepper rooted cuttings of hybrid Panniyur-I with *R. similis* resulted in severe foliar yellowing and death of the plants in pot culture studies. However, similar symptoms were not observed in plants inoculated with *M. incognita* (Mohandas and Ramana, unpublished) corraborating the results of the present investigation.

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