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Pathogenicity of *Heterodera oryzicola* (Nemata : Tylenchina) towards banana (*Musa* AAB cv. Nendran)

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Summary – Banana suckers grown in cement tubs buried into soil in an open field were inoculated with an initial inoculum of 0, 100, 200, 400, 800, 1000 and 2000 cysts of *Heterodera oryzicola* and the plants were kept under observation until harvest. The nematode attack induced precocious flowering and bunch maturation. Fresh weight of main, thick fleshy and thin secondary, tertiary and higher order roots, pseudostem height and girth at flowering as well as leaf production and leaf width were lower in the inoculated plants than in non-inoculated ones. The bunch weight and fruit traits such as number of hands, finger weight and volume were drastically reduced at all inoculum levels. The fruits exhibited a characteristic curvature of fingers in the inoculated plants. The nematode multiplied well on banana with heavy build up of population. *H. oryzicola* increased the concentration of nitrogen in leaves. Nematode infection also increased reducing sugars in fruit, while non-reducing sugars decreased. Total sugar content was less in fruit on infected plants.

Résumé – ***Pathogénie d'*Heterodera oryzicola *(Nemata : Tylenchina) envers le bananier (*Musa *AAB cv. Nendran)*** – Des rejets de bananier sont placés, à l'air libre, dans des tubes en ciment enfoncés dans le sol et inoculés avec 0, 100, 200, 400, 800, 1000 et 2000 kystes d'*Heterodera oryzicola;* les plants sont observés jusqu'à la récolte. L'attaque du nématode provoque une précocité de la floraison et de la maturation du régime. Chez les plants inoculés, le poids frais des racines charnues, celui des racines secondaires, tertiaires, etc., la hauteur et la circonférence du pseudo-tronc au moment de la floraison, le nombre et la largeur des feuilles, sont plus faibles que chez les plants non inoculés. Le poids du régime et certaines caractéristiques des fruits (nombre de mains, poids et volume de chaque banane) sont nettement réduits pour toutes les valeurs de l'inoculum. Chez les plants inoculés, les bananes présentent une courbure caractéristique. Le nématode se multiplie bien sur bananier, avec des augmentations importantes de population. *H. oryzicola* provoque un accroissement du taux d'azote dans les feuilles; en ce qui concerne les fruits, le taux des sucres réducteurs augmente alors que celui des non-réducteurs diminue; le contenu total en sucre est plus faible chez les fruits provenant de plants infestés.

Key-words : *Heterodera,* banana.

Charles and Venkitesan (1984) reported the occurrence of the cyst nematode *Heterodera oryzicola* Rao & Jayaprakash, 1978 on banana (*Musa* AAB) cv. Nendran from the state of Kerala, India. Before its isolation from banana, this nematode was considered to be one of the major pests of rice in Kerala (Usha, 1980). Although the association of *H. oryzicola* with banana cultivated in paddy lands in several areas of Kerala was recorded by Venkitesan and Charles (1985), its role as a pathogenic organism has not been investigated. The present studies were undertaken to study the pathogenicity of *H. oryzicola* on banana cv. Nendran with respect to plant growth and yield.

Materials and methods

Forty cement tubs (75 cm × 120 cm) were buried in the soil, in pits opened at 2 m × 2 m spacing at the experimental fields of the Banana Research Station, Kannara, Kerala in such a way that the rim remained 10 cm above the general soil surface. Each tub was filled with 0.5 m^3 of potting mixture previously denematized with 5.0 % formaldehyde. Pared banana suckers of the banana of size 250 g were given hot water treatment (55 °C for 10 min) and planted in each tub. One month after planting, fresh cysts of uniform size, containing an average of 120 viable eggs per cyst were hand picked from pure culture of the nematode and were maintained on live banana plants. Five replications of seven treatments were established viz. : supernatant from cyst suspensions, associated check, *Heterodera oryzicola* cysts at 100, 200, 400, 800, 1000 and 2000. The required number of cysts for each treatment was added near the feeder root zones in 1 ml water suspension. The plants were fertilized with N, P_2O_5 and K_2O at 190, 115 and 300 g per plant per annum, respectively, in two equal split doses, the first, at 2 months after planting and the second at 4 months after planting (Anon., 1983). Non-chemical or organic pesticides were applied during the test period for control of other pests and diseases. The plants were observed periodically for symptom development. Pseudostem height, girth at flowering, total number of leaves produced, number of days required

for flowering, and days from flowering to harvest were recorded. The length and width of D-leaf (third youngest leaf) were also recorded. At harvest, the bunch weight, number of fruit and hands per bunch and length, girth, weight, volume and curvature of fingers were recorded. The banana bunches were kept for quality analysis and the D-leaf was sampled for nutrient uptake studies. The fresh corm weight and weights of primary and lateral roots were also recorded. The lateral roots were sliced into small bits of less than 1 cm in length and 1 g samples were weighed out for staining with 0.1 % boiling acid fuchsin in lactophenol. The roots were then washed with tap water to remove excess stain and kept overnight in lactophenol. The number of cyst and female stages were counted. Soil from each tub was mixed well and a 100 ml sample from each tub was screened for cyst population.

At harvest, the D-leaf, the third youngest leaf (Hewitt, 1955) was dried, powdered and analysed for content of N, P, K and S. Nitrogen was estimated by the method of Wolf (1982). For determination of phosphorus, potassium and sulphur, the leaf samples were first digested with nitric or perchloric acid (1:1 acid v/v) mixture. Phosphorus concentration in the digest was determined colorimetrically by the vanadomolybdophosphoric yellow colour method, potassium and sulphur concentrations were determined by flame photometer and turbidimetrically respectively by the method of Jackson (1958).

The fruit collected from ripe bunches were used for fruit quality analysis. The middle fruit in the second hand was selected as the representative sample. Samples were taken from each fruit from three portions, namely, top, middle and bottom and their pulp was then pooled and macerated in waring blender. Triplicate samples from these were used for the estimation of acidity and content of reducing, non-reducing and total sugars, following procedures described in Anon. (1960).

The relation of all observations including vegetative characters, yield attributes and final nematode population with initial inoculum levels of cyst population was investigated through the following model :

$$y = a + b \ ln \ (x + 1)$$

where y = response variable; x = inoculum level (no/plant).

Results

Plant characteristics

The vegetative growth traits were impaired in proportion to the initial nematode inoculum per plant (Table 1). The duration from flowering to harvest showed a similar trend (Table 1). Even the initial inoculum load of 100-200 cysts per plant had affected the vegetative growth characters and duration for flowering and harvest. All the nematode inoculum levels have induced precocity in flowering.

The estimates of the coefficient of regression model fitted for growth and maturity traits of banana are presented in Table 2. All characters except D-leaf length (cm) and duration from flowering to harvest (days) showed significant relationship with initial load of cyst inoculum, provided to the plants. The important plant growth parameters showed a significant negative relationship. For the growth and maturity traits showing significant relationship with inoculum level, the adjusted r^2 varied from 0.8648-0.9699.

Table 1. Growth and maturity traits of banana cv. Nendran as influenced by *Heterodera oryzicola* at various inoculum levels.

Inoculum level	Pseudo-stem height [1] (cm)	Pseudo-stem girth [2] (cm)	Number of leaves	D-leaf [3] width (cm)	D-leaf length (cm)	Duration from planting to flowering (days)	Duration from flowering to harvest (days)
AO [4]	297.4	56.2	36.2	60.2	164.4	228.4	81.8
100	267.2 *	50.6 *	32.6 *	52.4 *	153.4 *	186.4 *	80.6
200	267.6 *	51.6 *	30.4 *	55.4 *	154.4 *	193.0 *	79.8
400	260.0 *	49.8 *	32.0 *	52.6 *	153.6 *	187.6 *	81.6
800	258.8 *	50.2 *	30.4 *	50.2 *	135.8 *	185.2 *	81.0
1000	251.8 *	48.2 *	29.8 *	50.0 *	135.0 *	187.4 *	67.8 *
2000	246.2 *	48.0 *	29.0 *	49.0 *	138.0 *	177.8 *	60.2 *

* Data are means of five replications.
* = Significantly different from the check (AO) at 0.05 by the LSD test.
(1) Height from the ground level to the axis of the youngest leaf.
(2) Girth at 20 cm above the ground level.
(3) Third youngest leaf.
(4) Associated check : No nematode inoculum, supernatant from nematode suspensions added.

Table 2. Estimates of coefficients of regression model fitted for growth and maturity traits of banana var. Nendran on cyst nematode, *Heterodera oryzicola*.

Sl. No.	Character	a	b	adj-r^2	Significance
1.	Pseudostem height (cm)	298.2291	– 6.4299	0.9699	*
2.	Pseudostem girth (cm)	56.2020	– 1.0460	0.9170	*
3.	Number of leaves	36.3075	– 0.9096	0.8985	*
4.	D-leaf width (cm)	60.4612	– 1.4398	0.8648	*
5.	D-leaf length (cm)	167.9351	– 3.7982	0.6534	NS
6.	Duration from planting to flowering (days)	225.2334	– 6.2205	0.8963	*
7.	Duration from flowering to harvest (days)	86.0242	– 1.8694	0.1682	NS

* Significant at 1 % level; NS denotes non-significant value of the character.

Table 3. Effect of different inoculum levels of *Heterodera oryzicola* on bunch and fruit characters of banana CV. Nendran.

Inoculum level	Bunch characters			Finger characters #				
	Bunch weight (1) (kg)	Number of hands	Number of fingers	Finger length (2) (cm)	Finger girth(3) (cm)	Finger weight (g)	Finger volume (4) (cc)	Finger curvature (5) (ratio)
AO @	7.7	4.2	36.6	20.5	11.6	171.1	159.0	1.07
100	6.0 *	3.8	32.6	20.2	9.2 *	147.0 *	142.2 *	1.19
200	5.5 *	3.6 *	36.4	20.4	10.7	139.7 *	130.6 *	1.18
400	5.5 *	3.2 *	31.0 *	19.9 *	10.3 *	130.6 *	128.2 *	1.26 *
800	4.7 *	3.2 *	27.4 *	19.9 *	9.4 *	117.2 *	112.2 *	1.26 *
1000	3.3 *	3.0 *	22.4 *	18.4 *	8.8 *	102.4 *	102.0 *	1.39 *
2000	3.4 *	2.8 *	23.8 *	18.8 *	8.3 *	106.4 *	96.0 *	1.34 *

Data are means of five replications

* = significantly different from the check (AO) at 0.05 by the LSD test.

The middle finger in the second hand was chosen for recording observations.

(1) Weight with 10 cm length of the peduncle above the first hand and 5 cm length of the male axis below the last hand.

(2) Length from the base of the pedicel to apex along the dorsal curve using a fine non-elastic thread and scale.

(3) The girth was measured at the middle using a non-elastic thread and scale.

(4) The volume of the finger was recorded using water displacement method.

(5) Outer length/inner length.

@ Associated check : No nematode inoculum, supernatant from nematode suspensions added.

YIELD CHARACTERISTICS

The observations on different yield attributes are presented in Table 3. The data show that most of the traits were influenced even at the lowest population level of 100 cysts per plant. The maximum bunch weight of 7.7 kg was recorded in the associated controls compared with 3.3 kg in plants inoculated with 1000 cysts. An initial inoculum of 100-1000 viable cysts per plant at planting time reduced bunch weight by 22.1-57.1 %. The curvature of fingers seemed to be a striking pathogenic manifestation, induced by the nematode and this trend of fruit bending increased from the initial to final inoculum per plant. The increase of finger curvature over control ranged from 10.3-29.9 %.

The estimates of the coefficients of regression model fitted for bunch and fruit characters are presented in Table 4. All yield attributes except finger curvature showed negative relation with initial cyst population in-

Table 4. Estimates of coefficients of regression model fitted for bunch and fruit characters of banana var. Nendran on cyst nematode, *Heterodera oryzicola*.

Sl. No.	Character	a	b	adj-r^2	Significance
1.	Bunch weight (kg)	8.0885	– 0.5530	0.8068	*
2.	Number of hands	4.3489	– 0.1790	0.8373	*
3.	Number of fingers	39.0551	– 1.7027	0.4923	NS
4.	Finger length (cm)	20.8649	– 0.2144	0.3417	NS
5.	Finger girth (cm)	11.7215	– 0.3705	0.5944	NS
6.	Finger weight (g)	177.6893	– 8.8801	0.8371	*
7.	Finger volume (cc)	166.7853	– 8.0116	0.7894	*
8.	Finger curvature (ratio)	1.0438	– 0.0373	0.7486	*

* Significant at 1 % level; NS denotes non-significant value of the character.

Table 5. Effect of different inoculum levels of *Heterodera oryzicola* on root and corm development and population development.

Inoculum level	Fresh primary root weight (1) (kg)	Fresh lateral root weight (2) (kg)	Fresh corm weight (kg)	Final cyst population/g root	Final cyst population/0.5 m^3 soil (10^3 n_{os})
AO @	4.69	2.02	9.30	0	0
100	3.23 *	0.84 *	8.90	15.59 (4.07) *	5 840 (76.43) *
200	3.82 *	0.80 *	9.90	18.39 (4.40) *	9 090 (93.35) *
400	3.15 *	0.68 *	9.70	23.79 (4.98) *	10 785 (103.85) *
800	1.62 *	0.40 *	9.30	27.59 (5.35) *	13 000 (114.02) *
1000	1.72 *	0.32 *	10.30	30.39 (5.60) *	15 130 (123.01) *
2000	1.70 *	0.30 *	10.30	35.18 (6.02) *	15 940 (126.26) *

Data are means of five replications.
Values in parenthesis are *(x + 1)* transformed

* = Significantly different from the check (AO) at 0.05 by the LSD test.
(1) Main thick fleshy roots.
(2) Secondary, tertiary and higher order roots.
@ Associated check : No nematode inoculum, supernatant from nematode suspensions added.

oculated. The attribute of curvature of fingers showed a significant positive correlation with cyst inoculum.

Root weight

Root weights were drastically reduced by the nematode (Table 5). The nematode attacked the lateral roots consisting of secondary, tertiary and higher order feeder roots of the banana plant. *H. oryzicola* was not observed to attack the primary thick fleshy roots and the corm. Feeder roots infected by nematodes turned black in colour and were easily detached. The reduction of main thick fleshy and thin secondary, tertiary and higher order feeder root biomass ranged from 18.6-65.5 % and 58.1-85.1 % respectively. The corm weight was not affected by the nematode.

The estimates of the coefficients of regression model fitted for root and corm weight are presented in Table 6. The regression equation for fresh secondary, tertiary and higher order root weight at harvest recorded a very high adjusted r^2 value of 0.9851. The final corm weight was non significant.

Table 6. Estimates of coefficients of regression model fitted for root and corm weight and final cyst population in root and soil.

Sl. No.	Character	a	b	adj-r^2	Significance
1.	Fresh primary root weight (kg)	5.0265	– 0.4111	0.7084	*
2.	Fresh lateral root weight (kg)	2.0012	– 0.2331	0.9851	*
3.	Fresh corm weight (kg)	9.0627	0.1148	0.1585	NS
4.	Final cyst population/g root	– 2.1568	4.4741	0.9445	*
5.	Final cyst population/100 ml soil	– 257.0956	424.6119	0.9097	*

* Significant at 1 % level; NS denotes non-significant value of the character.

Table 7. Foliar nutrient status and qualitative variations in banana cv. Nendran at different inoculum levels of *Heterodera oryzicola.*

Inoculum level	Leaf nutrient status (%)				Fruit quality traits (%)			
	N	P	K	S	Acidity	Reducing sugar	Non reducing sugar	Total sugar
AO @	1.44	0.16	1.54	0.15	0.30	14.49	3.25	17.70
100	1.63	0.17	1.55	0.18	0.38 *	15.01	1.50 *	16.51
200	1.76	0.18	1.58	0.16	0.40 *	15.08	1.40 *	16.28
400	1.81	0.18	1.82	0.16	0.37 *	15.21	1.33 *	16.45
800	1.97	0.26	1.83	0.14	0.37 *	15.19	1.12 *	16.31
1000	2.10	0.27	1.90	0.15	0.38 *	15.23	1.06 *	16.29
2000	2.25	0.32	2.50	0.13	0.37 *	15.26	0.88 *	16.14

Data of leaf components are means of five replications and fruit components are means of 15 replications.

* = Significantly different from the check (AO) at 0.05 by the LSD test.

@ Associated check : No nematode inoculum, supernatant from nematode suspensions added.

Nutrient status

The data on percentage of nitrogen, phosphorus, potassium and sulphur in the leaves are presented in Table 7. The percentage of P, K and S in leaves did not show significant difference between the plants, at varying inoculum levels. The nitrogen and phosphorus were higher in the leaves of inoculated plants compared to the plants in non-inoculated control. The percentage of potassium was found to be more in plants with higher inoculum level. The percentage of sulphur was found to be lower at higher inoculum level.

The estimates of the coefficients of regression model fitted for leaf nutrient status is presented in Table 8. Nitrogen content in leaf at harvest showed significant relation with the initial load of cyst inoculum provided to the plant.

Fruit quality

The quality of banana fruit as affected by the pathogenic effect of the different inoculum levels are presented in Table 7. The fruits of the nematode inoculated plants recorded higher acidity which increased with increasing level. The percentage of reducing sugars was lower in plants without the nematode inoculum than in the nematode inoculated plants. The percentage of non-reducing sugar was significantly reduced in the fruit of inoculated plants. This was found to decrease with the amount of initial inoculum per plant. The total sugar content of fruit was also less in inoculated plants compared to healthy plants. The pulp of fruits of nematode inoculated plants were hard and non-palatable.

The estimates of the coefficient of regression model fitted for quality traits of fruit are presented in Table 8. Reducing, non-reducing and total sugar contents in fruits showed significant relation with the initial cyst inoculum. The non-reducing and total reducing sugars showed negative relationship while reducing sugar showed a significant positive relation with cyst inoculum.

Table 8. Estimates of coefficients of regression model fitted for leaf nutrient status and fruit quality traits in banana on cyst nematode, *Heterodera oryzicola.*

Sl. No.	Character	a	b	adj-r^2	Significance
1.	Nitrogen (%)	1.3349	0.0974	0.7555	*
2.	Phosphorus (%)	0.1258	0.0178	0.4292	NS
3.	Potash (%)	1.3565	0.0869	0.3194	NS
4.	Sulphur (%)	0.1629	– 1.8917	0.0919	NS
5.	Acidity (%)	0.3152	9.7916	0.5520	NS
6.	Reducing sugar (%)	14.5113	0.1049	0.9762	*
7.	Non-reducing sugar (%)	3.1508	– 0.3103	0.9740	*
8.	Total Sugar (%)	17.5946	– 0.2016	0.9172	*

* Significant at 1 % level; NS denotes non-significant value of the character.

POPULATION DEVELOPMENT

The final nematode populations in root and soil increased in relation to the initial inoculum levels. Final population densities ranged from 15.6-35.2 cysts/g root and from 5 840 000-15 940 000 cysts/0.5 m^3 soil (Table 5).

The estimates of the coefficients of regression model fitted for final cyst population in root and soil are presented in Table 6. The regression equation of nematode population in root and soil at harvest recorded adjusted r^2 of 0.9445 and 0.9097 respectively.

Discussion

H. oryzicola at initial densities of 100 viable cysts per banana plant inoculated one month after planting adversely affected growth and yield (Tables 1, 3). The nematode reduced plant height, girth at flowering, leaf width and leaf production. Nematode infection led to precocious flowering and early bunch maturity. This is an uncommon phenomenon. The adverse effect of the nematode on banana growth extends the host pathogenicity of *H. oryzicola,* which was first reported on rice by Rao and Jayaprakash (1977) and by Usha and Kuriyan (1981). The nematode infection was observed to affect bunch and fruit characters also. The curvature of the fingers (fruit) was very much influenced by the nematode attack, compared to the normal fingers which showed a straight disposition. The increase in curvature was noticed in fingers at increased load of the nematode inoculum (Table 3). This pathogenic manifestation of finger curvature seemed to be unique. The phenomenon is explainable on the basis of some physiological imbalance in infected plants.

The adverse effects on the above-ground plant parts are probably a direct effect of feeder root damage. The reduction in the secondary and tertiary feeder root weight was higher (58.1-85.1 %) than the corresponding reduction in the primary lateral fleshy root weight (18.6-66.5 %). However, there is no significant reduction in the weight of corms. This may be due to the fact that the nematodes do not attack and feed on the tissues in the corm. The reduction of feeder roots might cause precocity of flowering, early bunch maturation and reduction in bunch weight. Such adverse growth traits have been reported by Brown (1969) in the case of *Globodera rostochiensis* in potato and also in maize (Yadav & Verma, 1971) infected by *H. avenae.*

The nutrient accumulation in the nematode infested plants might result from impairment in root physiology due to nematode infection mainly through suppression in the synthesis of cytokinins which in turn might have signalled the termination of the storage activity in the stem. The total sugar content of fruit was considerably less in inoculated plants. It has been generally observed in many crops that the rate of storage of nutrients remains fairly constant over the greater part of the storage period, provided there is not much fluctuation in temperature. In such a situation, the duration of storage is a powerful determinant of yield as reported by Watson (1971). In the infected plants, there was a significant reduction on the duration of the maturation period. Thus there might have been a reduction in the transport of photosynthate from leaves to the bunch. This phenomenon may also have contributed to the accumulation of nutrients in the leaves in the nematode inoculated plants.

Nitrogen and phosphorus in banana leaves were higher in proportion to the nematode inoculum level, while sulphur was inversely related to inoculum level.

Densities of nematode populations in roots and soil increased in accordance with increasing nematode inoculum (Table 5). This clearly shows that the nematode is capable of multiplying uninterruptedly on banana cv. Nendran under favourable circumstances. The present studies have clearly established that the nematode is potentially a severe pest of the cv. Nendran which is commercially very important in the State of Kerala.

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