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Relationship between mycelial growth rate and virulence of the isolates

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Abstract: The virulence of the 25 geographical isolates of *Macrophomina phaseolina* significantly varied for their disease incidence. The isolate Mp8 collected from Kovilpatti was the most virulent with maximum (88.3%) incidence of charcoal rot while Mp27 from Sankarankoil was the least virulent with the minimum (38.3%) diseases incidence. The most virulent isolate showed significant faster growth (41.3 mm) on PDA even 24 hours after inoculation while the least virulent isolate exhibited slowest growth (19.7 mm). Thus the virulence of the isolates was directly correlated with the initial growth rate of the isolates. *(Key words: Macrophmoina phaseolina, Virulence, Sunflower).*

The ubiquitous fungus *Macrophomina phaseolina* (Tassi.) Goid, has a host range of over 500 plant species. *M.phaseolina* is responsible for economically significant damage to a wide variety of crops worldwide. As a result, variations among its isolates in respect of morphological, cultural and pathological characters were frequently observed. Earlier workers correlated the variation in virulence with the morphological, cultural and physiological characterstics of the isolates. Dhingra and Sinclair (1973) and Gosh and Sen (1973) observed a direct correlation between the mycelial growth and the virulence of the isolates. Srivastava and Dhawan (1980) reported significant pathogenic variations among the 23-crucifer isolates on *Brassica juncea*. In the present investigation, relationship between mycelial growth rate and virulence of the isolates of *M.phaseolina* causing charcoal rot of sunflower was studied and the results are discussed hereunder.

Materials and Methods

Collection of M.phaseolina isolates.

Sunflower plants showing the typical symptoms of charcoal rot disease incited by *M.phaseolina* were collected from 25 conventional sunflower grown areas of Tamil Nadu. The pathogen was isolated from the roots of these infected plants by tissue segment method on potato dextrose agar (PDA) medium. Axenic culture of the pathogen was obtained by monosclerotial method and the purified cultures of the isolates were maintained on PDA slants for use in the studies.

Virulence of the isolates on sunflower plants

The sand maize inoculum of each of the isolates was thoroughly mixed with the sterilized (1.4 kg cm^{-2} pressure for 2 h) pot culture soil filled in earthern pots (30 cm) at 1:19 ratio, a week prior to sowing. Five surface sterilized seeds of the sunflower cv. Co_2 were sown in each pot. The pots were maintained in the glass house with uniform, regular and judicious watering. Three replications were maintained for each isolate. The observations on germination, root length, shoot length and seedling virour were made 15 DAS and the disease incidence was recorded 75 DAS.

Growth rate of the isolates

Sterilized and cooled (warm) PDA medium was poured into sterilized petri plates (90 mm) @ 20 ml and allowed to solidify. The isolates were inoculated separately at the centre of the plates by placing an eight mm actively growing PDA culture disc of the each isolate cut from a five day old culture by means of a sterilized cork borer. The plates were incubated at room temperature 28±2°C for 60 h. Three replications were maintained for each isolate. The radial growth of the mycelium was measured at six h interval to estimate the rate of growth.

Results and Discussion

Of the 25, the isolate, Mp8 collected from Kovilpatti was the most aggressive (88.3% disease) while Mp27 of Sankarankoil was the least virulent

Table 1. Virulence of *Macrophomina phaseolina* geographical isolates on sunflower plants (artificial inoculation)

Isolates	Germination* (per cent)	Root length (cm)	Shoot length (cm)	Seedling vigour index*	Disease incidence* (per cent)
Mp_1	(76.7) 61.14	9.63	21.13	2358	(85.0) 67.40
Mp_2	(81.7) 64.69	10.77	23.33	2784	(78.3) 62.48
Mp_3	(85.0) 67.40	12.13	24.50	3117	(61.7) 51.78
Mp_4	(85.0) 67.27	12.43	25.63	3236	(66.7) 54.75
Mp_5	(81.7) 64.69	11.37	24.30	2912	(56.7) 48.87
Mp_7	(80.7) 63.55	12.33	23.30	2848	(68.3) 55.77
Mp_8	(71.7) 57.86	8.60	18.07	1909	(88.3) 70.12
Mp_9	(83.3) 65.95	11.07	20.77	2650	(61.7) 51.78
Mp_{10}	(73.3) 59.00	8.80	18.50	2101	(86.7) 68.66
Mp_{11}	(81.7) 64.69	11.47	20.37	2600	(83.3) 65.95
Mp_{12}	(85.0) 67.40	13.03	22.37	3013	(80.0) 63.55
Mp_{13}	(83.3) 66.26	11.47	20.60	2673	(75.0) 60.00
Mp_{14}	(86.7) 65.95	12.73	22.53	2943	(70.0) 56.79
Mp_{15}	(83.3) 68.86	13.07	24.40	3242	(61.7) 51.78
Mp_{16}	(86.7) 68.66	13.00	23.83	3194	(70.0) 56.84
Mp_{18}	(85.0) 67.40	14.30	24.53	3431	(51.7) 45.96
Mp_{19}	(81.7) 65.19	12.80	25.50	3131	(48.3) 44.04
Mp_{20}	(85.0) 67.71	13.63	25.37	3317	(46.7) 43.09
Mp_{21}	(80.0) 63.55	13.50	24.50	3035	(48.3) 44.04
Mp_{22}	(83.3) 65.95	13.07	25.03	3175	(50.0) 45.00
Mp_{23}	(81.7) 65.00	12.67	25.30	3105	(58.3) 49.82
Mp_{24}	(78.3) 62.29	11.67	20.77	2541	(71.7) 57.86
Mp_{25}	(88.3) 70.12	13.60	24.57	3371	(41.7) 40.18
Mp_{26}	(78.3) 62.29	11.17	21.27	2543	(73.3) 58.93
Mp_{27}	(83.3) 65.95	14.43	25.13	3295	(38.3) 38.22
Control	(93.3) 75.24	17.87	29.90	4457	(0.0) 0.00
CD (P=0.05)	6.17	1.75	1.60	346	4.81

* Mean of three replications

Figures outside the parentheses in germination and disease incidence are arc sine transformed values.

(38.3%). The virulence of the isolate also reflected as the adverse effect on the seed germination and the seedling vigour of sunflower (Table 1). The earlier workers *viz*. Jimenez-Diaz *et al.* (1983) also reported similar variations in the virulence of the pathogen-affecting sunflower. In respect of other crops, Ali and Dennis (1992) indicated the existence of a large number of local pathotypes of *M.phaseolina* possesing considerable variations in the pathogenicity. Sobti and Sharma (1992) from Rajasthan recorded 13 to 63 per cent root rot incidence of groundnut with different isolates of *R.bataticola* collected from different locations. They also reported that the geographical distances did not have any relation to the virulence of the isolates. The pathogenicity index of the isolates of *M.phaseolina* from sunflower was not related to climatic area in Italy and the great variability

Table 2. Rate of growth of *M.phaseolina* isolates on PDA

Isolates	Mycelial growth (mm)*					
	24 h	% growth	48 h	% growth	60 h	% growth
Mp_1	32.7	36.3	77.3	85.9	90.0	100.0
Mp_2	33.3	37.0	81.0	90.0	90.0	100.0
Mp_3	25.7	28.6	74.3	82.6	90.0	100.0
Mp_4	39.3	43.7	90.0	100.0	90.0	100.0
Mp_5	32.0	35.6	74.0	82.2	90.0	100.0
Mp_7	31.3	34.8	83.7	93.0	90.0	100.0
Mp_8	41.3	45.9	90.0	100.0	90.0	100.0
Mp_9	27.3	30.3	72.0	80.0	90.0	100.0
Mp_{10}	40.0	44.4	90.0	100.0	90.0	100.0
Mp_{11}	24.0	26.7	72.0	80.0	90.0	100.0
Mp_{12}	27.0	30.0	72.0	80.0	87.7	97.4
Mp_{13}	29.0	32.2	72.7	80.8	90.0	100.0
Mp_{14}	25.0	27.8	68.0	75.6	90.0	100.0
Mp_{15}	31.0	34.4	69.0	76.7	90.0	100.0
Mp_{16}	27.7	30.8	67.3	74.8	88.3	98.1
Mp_{18}	28.3	31.4	74.3	82.6	90.0	100.0
Mp_{19}	28.7	31.9	73.7	81.9	90.0	100.0
Mp_{20}	28.0	31.1	69.0	76.7	90.0	100.0
Mp_{21}	34.0	37.8	72.7	80.8	90.0	100.0
Mp_{22}	35.7	39.7	83.0	92.2	90.0	100.0
Mp_{23}	28.3	31.4	70.3	78.1	89.3	99.2
Mp_{24}	31.0	34.4	73.0	81.1	90.0	100.0
Mp_{25}	34.0	37.8	81.3	90.3	90.0	100.0
Mp_{26}	25.3	28.1	72.0	80.0	90.0	100.0
Mp_{27}	19.7	21.9	63.0	70.0	84.0	93.3
CD (P=0.05)	5.1	–	6.1	–	1.1	–

* Mean of three replication

in pathogenicity in all the climatic areas suggested good adaptation to the host by the pathogen according to Manici *et al.* (1992). Grezes-Besset *et al.* (1996) also reported that the virulence of the *M.phaseolina* isolates was highly variable. Therefore, the difference in the charcoal rot incidence caused by different isolates of *M.phaseolina* could be well attributed to the highly variable nature of their virulence prevalent in the respective areas. Kovilpatti from where the most virulent isolate (Mp8) was collected is a conventional sunflower belt where the continuous and intensive sunflower cultivation might have contributed to the existence of the most virulent pathogenic type.

The most virulent isolate Mp8 showed significantly faster growth (41.3 mm) while Mp27 the least virulent isolate exhibited very slow growth (19.7 mm) (Table 2) even in 24h. Dhingra and Sinclair (1973) correlated the growth rate of various soybean isolates with their pathogenicity. Gosh and Sen (1973) correlated the initial growth rate of isolates from different crops with their virulence. Srivastava and Dhawan (1980) suggested that the cultural characteristics and the growth

rate of the 23 cruciferous isolates were related to their pathogenicity. Byadgi and Hegde (1985) found that the virulent isolate from bean grew significantly faster. For successful infection, rapid initial growth and pre-colonization of the host by the pathogen is a prerequisite. This aggressive isolate Mp8 thus fulfill the above requirement. However, Sobti and Sharma (1992) found no correlation between the mycelial growth and the pathogenic behaviour of the isolates. The isolates of *M.phaseolina* from sunflower in the present study exhibited wide variation in the mycelial growth as also similarly observed by Raut and Ingle (1989) and virulence.

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