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POPULATION STUDY OF THE SCLEROTIA OF *MACROPHOMINA PHASEOLINA* IN COTTON FIELDS*

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Abstract

A technique was developed for the isolation and enumeration of *Macrophomina phaseolina* from soil. After wet sieving through 120 mesh screen the soil residue retained on 300 mesh screen was suspended in 0.5% Calcium hypochlorite solution and distributed on PDA containing Penicillin, Streptomycin, Demosan and rose bengal. *M. phaseolina* colonies were detected within 5 days giving 94% recovery.

Naturally infested soils averaged 0-29 sclerotial propagules per gram of soil. More sclerotia were present in the surface soil than at 6" depths. A substantially higher sclerotial population was detected in certain root rot infested patches as compared to healthy fields. In general no significant difference in the population of sclerotia between the healthy and root rot infested portions of cotton fields was observed.

Introduction

Macrophomina phaseolina (Tassi) Goid., is known to produce root rot, stem rot or pod rot on over 305 host plants in different parts of the world of which atleast 40 economic hosts have so far been recorded from Pakistan (Young, 1949; Ghaffar et al, 1964). The fungus is believed to persist in soil in the form of sclerotia formed on infected host tissue and subsequently released in soil during decaying process (Smith, 1969). *M. phaseolina* is widely distributed in different parts of the world indicating its persistence in diverse environmental conditions with variable soil types. As a preliminary to the biological control of *M. phaseolina*, a technique for the direct isolation and enumeration of sclerotial propagules from soil was developed. The technique was subsequently used to study the population density of the sclerotia of *M. phaseolina* in cotton fields where root rot was found to be severe.

Materials and Methods

M. phoseolina, isolate KUMH 54, previously isolated from the root rot specimen of cotton was used in this study. This was the same isolate used by Ghaffar et al (1969) in their investigations.

The fungus was grown on corn meal sand medium (5% w/w) for two weeks at 30°C and the sclerotia separated by successive floatation in distilled water and decantation. The sclerotia were dried at room temperature and mixed in soil (0.005: 100g; w/w, sclerotia: soil). More or less similar technique as used for the separation of sclerotia of *Sclerotium cepivorum* (Papavizas, 1971) and *M. phaseolina* (Papavizas and Klag, 1974) was used in this experiment.

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Twenty g of artificially infested soil were wet sieved through 120 mesh (125 μ pore size) and 300 mesh (53 μ) screen. The sclerotia of *M. phaseolina* upto 120 μ (Haigh's 'C' strain of *R. bataticola*) are retained on 300 mesh screen. The residue obtained on 300 mesh screen were washed in running tap water for 1 minute and transferred into a beaker containing 0.5% CaOCl which was made up to 100 ml to obtain a 1:5 dilution. The sclerotial suspension was continuously agitated by magnetic stirrer and one ml aliquot were distributed on the strip of filter paper to count the number of sclerotial propagules. One ml aliquot were also removed and pippetted on to the surface of 3 day old agar plates and evenly spread The plates were incubated at 30°C. Grayish to black colonies of *M. phaseolina* were detected and easily identified within 5 days.

Experimental Results

I. Isolation and enumeration of sclerotia from soil:

Of the different media tried, PDA containing Penicillin, Streptomycin (each @ 60mg/litre) and Demosan (@ 300mg/litre) with or without rose bengal (@100mg/litre) gave consistently good results, (Fig. 1). Use of CaOCl was essential since it prevented the fast growing mucorales and other fungi. It may be mentioned that we did not use the various selective media as suggested by Papavizas & Klag (1974) and do not remove the CaOCl from sclerotial surface before its transfer on the agar medium. Approximately 6-10 *M. phaseolina* colonies were obtained on the agar plates. Of the 47-50 sclerotial propagules/g of soil as recovered on the strip of filter paper, 44-47 *M. phaseolina* colonies were obtained on the agar plates giving approximately 94% recovery. It would appear that non removal of CaOCl did not result in any appreciable loss of germinability of sclerotia. It may be mentioned that sclerotia of *M. phaseolina* are rindless and all the cells of the sclerotia have thick wall (Bryant & Wyllie (1970).

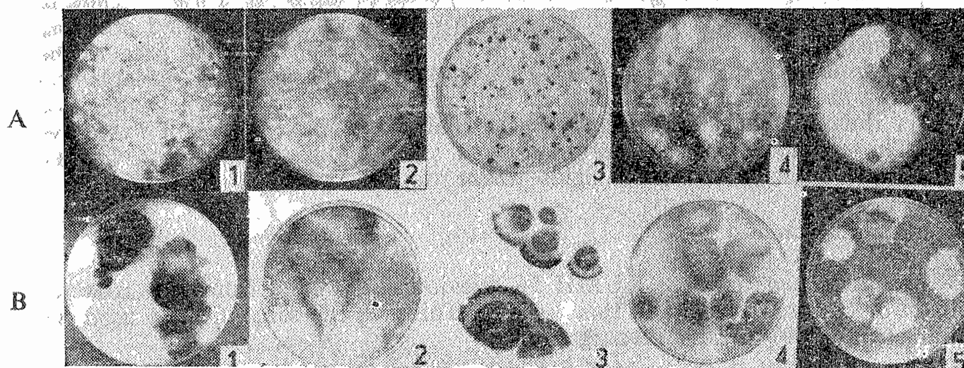

Fig. 1. Receovery of *M. phaseolina* on dilution plates containing PDA with different antimicrobial agents. A—Soil suspension. B—Soil suspension in Calcium hypochlorite.

1. PDA.
2. PDA + Penicillin + Streptomycin.
3. PDA + Penicillin + Streptomycin + Demosan.
4. PDA + Penicillin + Streptomycin + Rose bengal.
5. PDA + Penicillin + Streptomycin + Demosan + Rose bengal.

II. Population of *M. phaseolina* in soils of cotton field:

The above technique was used for isolating sclerotia of *M. phaseolina* from soil and estimating its inoculum density. Soil samples were taken at random from upto 2″ and 6—9″ depth of cotton fields of Rajawala and Risalewala (Lyallpur), Multan, Setharja and Tandojam where root rot is a problem and the plants showed wilting. Sampling was carried out during the 1974 cotton growing season. For comparison soil from the base of healthy plants were also obtained. The pH of the soil samples ranged from 7.2-8.3. Soil was kept in polythene bags and were air dried before analysis.

Of the 154 soil samples analysed, the population of sclerotia of *M. phaseolina* exhibited a substantial degree ot variability since a population of 0-29 sclerotial propagules per g of soil was detected (Table 1). The mean population counts varied significantly with the localities ($p \angle 0.001$). Considerably large population of sclerotia existed in Setharja, Tandojam and Rajawala and relatively small population in Risalewala and Multan. It is interesting to note that *M. phaseolina* sclerotia were detected from certain healthy cotton fields as well. In general no signifiant difference in the population of sclerotia between the healthy and root rot infested portions of the cotton fields was detected. In Setharja fields, however, a substantially higher sclerotial count was obtained from the root rot infested patches as compared to the healthy parts of the fields at both the depths examined.

TABLE 1. Sclerotial population of *Macrophomina phaseolina* in soil.

Locality	Patches	No. of Samples	Soil depth (inches)	pH range	Population/g of soil	
					Range	Average
1. Rajawala	Healthy	15	2	7.4-7.6	9—29	16.2
		15	6	7.2-7.6	2—12	5.8
	Root rot	10	2	7.4-7.6	5—25	15.1
		10	6	7.4-7.6	1—13	6.2
2. Risalewala	Healthy	4	2	7.4-8.0	0—1	0.5
		4	6	7.3-8.0	0—11	3.0
	Root rot	8	2	7.6-7.9	0—1	0.1
		8	6	7.6-8.3	0—0	0.0
3. Multan	Healthy	3	2	7.8-7.9	1—8	3.7
		3	6	7.7-7.9	1—4	2.3
	Root rot	5	2	7.8-7.9	0—23	5.6
		5	6	7.8-7.9	0—1	0.6
4. Setharja	Healthy	11	2	7.5-8.0	1—13	4.9
		11	6	7.5-7.9	0—14	3.8
	Root rot	13	2	7.5-7.9	2—21	6.7
		13	6	7.6-7.9	0—15	5.2
5. Tandojam	Healthy	3	2	7.7-7.8	10—25	15.7
		3	6	7.7-7.8	6—11	8.5
	Root rot	5	2	7.7-7.8	6—15	10.8
		5	6	7.6-7.8	3—19	9.8
	Total:	154				

Analysis of variance

(Based on 3 randomly chosen replicates)

Source of variation	SS	df.	M.S.	F
Main effects				
Localities (L)	948.5	4	237.1	9.34***
Healthy Vs Disease (H)	43.0	1	43.0	1.69 n.s.
Observations at different depths (D)	191.0	1	191.0	7.53**
First order interactions:				
LxH	75.5	4	18.87	0.74 n.s.
DxH	0.5	1	0.5	0.020 n.s.
DxL	210.3	4	52.5	2.071 n.s.
Second order interaction:				
L x D x H	197.2	4	49.3	1.945 n.s.
Treatments:	1666.0	19	87.68	3.45**
Error:	1014.0	40	25.35	—
Total:	2680.0	59	—	—

Levels of significane:
**p. 0.01
***p. 0.001
n.s. Non-significant.

The population of sclerotia of *M. phaseolina* varied significantly with the depth ($p \angle 0.01$). Population counts in most localities were considerably higher in the surface soil (2" depth) in comparison to soil collected from 6—9" depth.

Conclusion

The occurence of relatively greater number of sclerotial propagules of *M. phaseolina* in the surface soil would indicate that the chances of colonization of plant roots soon after emergence would be more. This observation is further substantiated by Hussain & Ghaffar (1975) who found greater degree of colonization of cotton roots by *M. phaseolina* in the proximal portion as compared to the distal part. It would suggest that deep ploughing and turning over of the soil may aid considerably in reducing the sclerotial population of *M. phaseolina* in the surface soil.

The numerical threshold for infection by *M. phaseolina* on cotton is not known although the sclerotial densities in soil have been correlated with increased disease incidence in bean (Watanabe et al, 1967). The presence of 0-29 sclerotial propagules in the healthy portions of the cotton fields as compared to 0-25 sclerotial propagules in the diseased portions would indicate that infection on roots may be present without obvious above ground symptoms and presumably certain edaphic factors interact with fungal propagules to produce wilting. Water stress has been attributed to be a more important predisposing factor in *Macrophomina* infection of sorghum (Hsi, 1961; Edmunds, 1964) and cotton (Ghaffar & Erwin, 1969). On the other hand symptoms of charcoal rot (*M. phaseolina*) development in soybean (*Glycine max*) have been ascribed to enzyme release and toxin production (Bryant & Wyllie, 1970; Dhingra & Sinclair, 1974).

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