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Role of inoculum level on diseases incidence of dry root rot caused by *Macrophomina phaseolina* in groundnut

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Abstract : Active role of *Macrophomina phaseolina* incolum level in dry root rot incidence of groundnut was found. Maximum disease incidence was recorded in unsterilized and sterilized soil at 15-45 days after sowing. Significant increase in disease incidence (48.7 and 98.5 per cent) with increase in inoculum level from 500 to 1000 mg/kg. of unsterilized and sterilized soil was noted. In unsterilized soil, 50 per cent reduction in root incidence due to the activity of bacteria and actinomycetes was observed compared to sterilized soil. *(Key Words) : Disease incidence, Inoculum level, Macrophomina phaseolina, Sterilized soil, Unsterilized soil).*

Root rot disease caused by *Macrophomina phaseolina* is an important problem in many crop plants. It produces abundant inoculum in the soil. Filiho and Dingra (1980) found that the presence of sufficient organic nutrients in the soil may enhance the *M. phaseolina* multiplication and such soil acts as reservoir of inoculum. Under such situation assessment of the inoculum level is an important concern to decide the management strategies like biological control of the pathogen. In the present study attempts were made to find out the role of inoculum level in root rot incidence of ground nut.

Pathogen behaviour of *Macrophomina phaseolina* isolates also vary with the quantity of inoculum (Meyar *et al.*, 1979). Inoculum dose of 1:5 (Pathogen:Soil) was found to cause maximum mortality due to *Macrophomina phaseolina* in groundnut (Subramanian, 1969; Shanmugam, 1971). Murugesan and Mahadevan (1989) reported that 93 per cent of root rot infection in groundnut plants by the direct use of pathogen propagules grown on ground nut stabbles and in broth culture but when inoculum was diluted 50 times with vermiculite, the root rot development was negligible.

Materials and Methods

Multiplication of sclerotial inoculum

The pathogen *Macrophomina phaseolina* was isolated from infected groundnut roots by tissue segment method on PDA medium. Mycelial discs from pure culture was transferred to PD broth (Murugesan and Mahadevan, 1989) and incubated for 15 days. The fungal mass consisting of mycelium and sclerotia was separated and homogenised with sterile water. Fungal suspension was filtered through filter paper (Whatmam No.42). The residue containing sclerotia were dried at 40° C for 24 h, ground in pestle and mortor and passed through 80 mesh sieve for inoculation.

Effect of inoculum levels on disease incidence

Garden soil was sieved and sterilized. The sterilized and unsterilized soil were distributed (2.5 kg./pot). The sclerotia of pathogen were incorporated in sterilized and unsterilized soil at two levels viz., 500 and 1000 mg/kg of soil (Elad *et al.*, 1980). Groundnut seeds were sown in all pots. Seeds sown in uninoculated soil served as control. The germination per centage was recorded after 10 days of sowing. Presence of pathogen in ungerminated seeds and seedlings was tested. In sterilized soil the rhizosphere population of *M. phaseolina* and per cent of root rot incidence were recorded. In unsterilized soil in addition to rhizosphere population of pathogen other fungi, bacteria, actinomycetes were also recorded at 15 days interval.

Assessment of rhizosphere population of Macrophomina Phaseolina and other microbes

The colony forming units of *Macrophomina phaseolina,* fungi, bacteria, actinomycetes in their respective medium were estimated by dilution plate technique (Pramer and Schmidt, 1956) Rose bengal agar for fungi (Martin and Nicolas, 1970), Soil extract agar for bacteria (Allen, 1957) and Kenights agar for actinomycetes (Allen, 1953) were used for enumeration. One hundred micro litre of soil suspension at their respective dilutions were dispersed over the sterile medium in petri plates and were incubated for 3 days for bacteria, 5 days for fungus and 7 days for actinomycetes. Colonies were counted in colony counter. The selective medium Potato-200g, Glucose-20g, Agar-15g, Mercuric chloride-0.008g, Rose bengal-0.1g, Chloroneb-0.3g, Streptomycin sulphate-0.1g, Sodium propionate-0.05g described by (Samiyappan, 1988) was used to enumerate the pathogen.

Results and Discussion

The results of the present investigation indicated that generally root rot incidence due to *Macrophomina phaseolina* increased for 15-45 days after sowing. However after 45 days, decline in disease incidence was noticed. Increase in mortality of groundnut plants resulted when the inoculum level was raised from 500-1000mg/kg of soil both in sterilized and in unsterilized soil. Higher rhizosphere population of 33.8 x 10^3 cfu^{-g} of soil was observed at inoculum level of 1000 mg as against 22.2 x 10^3 cfu^{-g} of soil in 500 mg of *M. phaseolina* inoculum level, which might have influenced the disease expression (Table 1). Baker (1971) also indicated that an increase in inoculum density of soil borne plant pathogen usually resulted increase in disease incidence until a plateau is reached.

In sterilized soil, the inoculum level of 500 and 1000 mg/kg of soil resulted 73.3 per cent and 98.5 per cent mortality of groundnut plants respectively (Table 2), where as the same inoculum level could cause only 23.0 per cent and 48.7 per cent disease incidence in unsterilized soil. This can be expected since the pathogen *M. phaseolina* in sterilized soil grew freely without any competition (Indra and Grover, 1988). To find out the role of the other factors on the disease incidence in unsterilized soil, an analysis of the soil microflora at different stages of crop growth indicated an initial higher population of bacteria and actinomycetes, while the fungal population showed a build up at later stages (Table 3). Initial population of *Macrophomina phaseolina* was low whereas population of actinomycetes and bacteria were more. Colonization of *M. phaseolina* sclerotia by soil bacteria has also been suggested by Kavoor (1954).

Table 1. Influence of inoculum levels on disease incidence and rhizosphere population of *Macropnomina phaseolina* in unsterilized soil.

S. No	Sampling intervals (Days)	*Rhizosphere population of Macrophomina phaseolina* (cfu x 10^3/g of soil)			Root-rot disease incidence (%)		
		Inoculum level - mg/kg of soil			Inoculum level-mg/kg of soil		
		500	1000	mean	500	1000	mean
1	15	7.0	10.8	8.8	0.0	2.7	1.3
2	30	11.5	18.8	15.1	6.3	14.9	10.6
3	45	16.5	30.3	23.4	16.6	37.8	27.2
4	60	20.5	32.0	26.3	20.4	45.9	33.2
5	75	22.2	33.8	28.1	23.0	48.7	35.9
	Mean	15.6	25.1	--	19.9	31.5	

CD (p=0.05)
Treatment =0.8
Interval = 1.3
Interaction = 1.8

Treatment =1.9
Interval = 3.0
Interaction = 4.3

Reduction in *Macrophomina* root rot of cotton has been attributed to an increase in population of actinomycetes and bacteria (Ghaffer and Erwin, 1969., Samiyappan, 1988). The stimulation of microbial activity would appear to affect the sclerotial propugules in unsterilized soil. Thus in the absence of a build up of the rhizosphere actinomycetes population and increase in pathogen propagules resulted in higher disease incidence in sterilized and unsterilized soil.

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Table 2. Influence of inoculum levels on diease incidence and rhizosphere population of *Macropnomina phaseolina* in sterilized soil.

S. No	Sampling intervals (Days)	*Rhizosphere population of Macrophomina phaseolina* (cfu x 10^3/g of soil)			Root-rot disease incidence (%)		
		Inoculum level - mg/kg of soil			Inoculum level-mg/kg of soil		
		500	1000	mean	500	1000	mean
1	15	15.3	22.0	26.9	5.8	7.5	6.5
2	30	23.3	30.5	26.9	26.9	32.6	29.8
3	45	32.3	40.3	36.3	52.1	73.7	52.7
4	60	35.8	44.5	40.2	67.7	87.7	77.7
5	75	37.5	46.8	42.2	73.3	98.5	85.9
	Mean	28.9	36.8	--	45.4	59.7	--

CD (p=0.05)
Treatment =1.7
Interval = 2.6
Interaction = NS

Treatment=1.8
Interval = 2.9
Interaction = 4.0

Table 3. Rhizosphere population of fungi, bacteria actionmycetes in unsterilized soil

S. No	Sampling intervals (Days)	Rhizosphere population of fungi (Cfu x 10^4/g of soil)			Rhizospher population of bacteria (Cfu x 10^6/g of soil)			Rhizospher population of bacteria (Cfu x 10^5/g of soil)		
		Inoculum level - mg/kg of soil			Inoculum level - mg/kg of soil			Inoculum level - mg/kg of soil		
		500	1000	mean	500	1000	mean	500	1000	mean
1	15	11.0	12.5	11.8	37.3	27.0	32.1	16.5	13.5	15.0
2	30	15.0	18.5	16.8	31.5	23.8	27.6	11.5	7.8	9.6
3	45	19.8	32.3	26.0	22.5	17.5	20.0	7.0	4.8	5.9
4	60	21.5	33.8	27.5	19.5	16.5	18.0	6.5	2.3	4.4
5	75	23.5	37.5	30.5	17.0	13.3	15.1	5.8	2.3	4.0
	Mean	18.2	26.9	--	25.6	19.6	--	9.5	6.1	--

CD (p=0.05)
Treatment =1.8
Interval = 1.1
Interaction = 2.5

Treatment=1.0
Interval = 1.5
Interaction = 2.2

Treatment=0.7
Interval = 1.0
Interaction = NS

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