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Population dynamics of microflora in the rhizosphere of *Macrophomina phaseolina* resistant and susceptible varieties of Mungbean

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ABSTRACT: *Eighteen fungi and one bacterium showed different trends in population in initial infested soil and in rhizosphere at seedling and flowering stages of resistant MSJ-118 and susceptible RMG-62 varieties. The frequency and abundance (%) of microorganisms also showed different trends.*

Key words: *Macrophomina phaseolina, Mungbean, rhizosphere, microflora.*

INTRODUCTION

Mungbean [*Vigna radiate* (L.) Wilczek] is an important pulse crop in Indian continents. Mungbean is being infected by several fungal, bacterial and viral diseases but dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid. is considered as the most devasting disease in all the mungbean growing areas of Rajasthan.The disease is quite wide spread across the Rajasthan state due to congenial weather conditions and causes considerable yield losses (Philip *et al*., 1969, Grewal, 1988). The pathogen may infect almost all parts of plants i.e. root, stem, branches, petioles, leaves and pods. Seed infection due to *Macrophomina phaseolina* ranges from 2.2 to 15.7 per cent which may cause losses in grain yield to the extent of 10.8 per cent and protein content of 12.3 per cent (Kaushik *et al*., 1987). The infected seeds act as an important source of primary inoculum for new areas. Plant stand is affected due to pre and post-emergence infection of the crop. In pre-emergence stage, the fungus causes seed rot and mortality of germinating seedlings while in post emergence stage seedlings get blightened due to soil and seed-borne infection. In later stages of crop growth decay of secondary roots and shredding of the cortex region of the tap roots are commonly observed. Rhizosphere microflora plays an important role in the manifestation of diseases, especially the soilborne ones. It is characterized by greater microbial activity which plays a role in biological control. The present study was carried out to compare the rhizosphere microflora of resistant MSJ-118 and susceptible RMG-62 varieties of mungbean at different stages of plant growth. The extent of changes in population of micro organisms in rhizosphere and in soil is to be determined by the enumeration of microflora. For soil-borne diseases the knowledge of microbial activity in the root zone (rhizosphere) is of prime importance. In rhizosphere the micro organism are known to synthesize metabolites which in some cases may be antagonistic to root pathogens. The antagonists may be effective in reducing the population of pathogen.

MATERIALS AND METHODS

Soil from fields of Bikaner district of Rajasthan, where mungbean is mainly grown was collected and to increase the bulk soil from Agronomy Farm, College of Agriculture, Bikaner with farm yard manure was mixed. The soil was filled in two wooden boxes which were rinsed with formalin and dried. The highly pathogenic isolate Bikaner was multiplied on sand maize flour medium and added to wooden box soil. The infested soil was irrigated and left for ten days. For sampling, surface soil about 1 cm was removed and randomly three soil samples taken diagonally from each wooden box. The soil samples were air dried in laboratory and passed through 2 mm sieve. For enumeration of fungi 'Soil plate method' (Warcup,

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1950 and Parkinson, 1958) was followed. A microspatula was standardized for placing the required quantity of soil in Petri dishes (0.0035 g/ microspatula). A drop of sterilized distilled water was placed in the centre of Petri dish and on this drop soil with the help of microspatula of the sample was placed to disperse it uniformly in the medium. Martin's peptone dextrose agar medium just above the solidifying temperature was poured in these dishes. Thornton's standardized medium was used for the isolation of *Bacillus* bacterium following 'The Soil Dilution and Plate Count Method' (Waksman, 1927). In each Petri dish 1 ml of prepared dilution was transferred aseptically to which 15-20 ml medium just above the solidifying temperature was poured. To disperse the soil or soil/water dilution uniformly, the medium was rotated inside the Petri dish clockwise as well as anti clockwise with swirling motions of the dish. The poured Petri dishes were incubated at 28 ± 1°C for four to five days. The recording of colonies for different microorganisms was continued for fifteen days or until new colonies ceased to develop. After studying soil samples from initial infested soil, seeds surface sterilized with 0.1% mercuric chloride of dry root rot resistant MSJ-118 and susceptible RMG-62 varieties were sown in the boxes separately for rhizosphere studies at seedling, flowering stages. The boxes were watered at intervals depending upon the dryness of soil and care was taken to break the upper crust of soil. In order to prevent lumping of soil about roots, water was not given 24 hours prior to sampling. At seedling stage three plants of each variety were removed from the soil with roots intact, taking care that the roots were not injured. The roots of these plants were shaken gently to dislodge the adhering soil which was collected in sterilized Petri dishes for further dispersal in different media. The same process was repeated at flowering stage. For enumeration and isolation of fungi and bacteria the techniques employed for initial infested soil were followed.

$$\text{Frequency}(\%) = \frac{\text{Number of Petri dishes in which colony of a species appeared}}{\text{Total number of Petri dishes}} \times 100$$

$$\text{Abundance}(\%) = \frac{\text{Total number of colonies of a species in all the Petri dishes}}{\text{Total of colonies of all species in all Petri dishes}} \times 100$$

RESULTS AND DISCUSSION

Different trends were observed in population of each species estimated in 1 g rhizosphere soil of resistant MSJ-118 and susceptible RMG-62 varieties (Table 1 and 2). *Aspergillus clavatus, Aspergillus flavus, Aspergillus niger, Chaetomium globosum, Cochliobolus lunatus, Fusarium oxysporum, Macrophomina phaseolina, Penicillium aurantiogriseum, Penicillium javanicum, Rhizopus stolonifer, Trichoderma atroviride, T. harzianum* and *T. viride* were present in initial infested soil and rhizosphere soil of two stages of plant (seedling and flowering) of resistant MSJ-118 variety of mungbean. The population of *Aspergillus clavatus, Aspergillus flavus, Fusarium oxysporum, Macrophomina phaseolina* and *Rhizopus stolonifer* decreased at flowering as compared to seedling stage. *Aspergillus niger, Cochliobolus lunatus, Penicillium aurantiogriseum, Penicillium javanicum, Trichoderma harzianum, T. atroviride* and *T. viride* had increasing trend from seedling to flowering stage. The population of *Aspergillus clavatus, Aspergillus flavus, Aspergillus niger, Chaetomium globosum, Cochliobolus lunatus, Fusarium oxysporum, Rhizopus stolonifer, Trichoderma atroviride, T. harzianum* and *T. viride* increased at seedling stage as compared to initial infested soil. *Macrophomina phaseolina, Penicillium aurantiogriseum* and *Penicillium javanicum* had decreasing trend from initial infested soil to seedling stage. The population of *Chaetomium globosum* increased from initial infested soil to seedling stage but remains constant from seedling to flowering stage. Similarly, *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Chaetomium globosum, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Penicillium javanicum, Rhizopus stolonifer, Tricoderma atroviride* and *Trichoderma harzianum* were present in initial infested soil and rhizosphere soil at seedling and flowering stages of susceptible RMG-62 variety of mungbean. The population of *Alternaria alternata, Aspergillus niger, Penicillium javanicum, T. atroviride* and *T. harzianum* increased from seedling to flowering stage. The population of *Aspergillus flavus, Chaetomium globosum, Curvularia lunata, Fusarium oxysporum* and *Rhizopus stolonifer* decreased at flowering stage as compared to seedling stage. Population of *Alternaria alternata, Aspergillus flavus, Chaetomium globosum, Fusarium oxysporum, Penicillium javanicum* and *Rhizopus stolonifer* increased from initial infested soil to seedling stage. Population of *Aspergillus niger, Curvularia lunata* and *Macrophomina phaseolina* decreased at seedling stage as compared to initial infested soil.The population of *Tricoderma atroviride* and *Trichoderma harzianum* were constant at initial infested soil and seedling stage but had increasing trend from seedling to flowering stage. *Bacillus subtilis* appeared at all three stages of resistant MSJ-118 as well as susceptible RMG-62 variety of mungbean. The population of *Bacillus subtilis* was abundant in initial infested soil,

decreased at seedling stage and further increased at flowering stage in rhizosphere soil of resistant MSJ-118 variety. The population of *Bacillus subtilis* was abundant in initial infested soil and decresed at seedling and flowering stage of rhizosphere soil of susceptible RMG-62 variety. The trend of frequency (%) of microorganisms in rhizosphere varied. In *Aspergillus niger, Cochliobolus lunatus, Penicillium aurantiogriseum, Penicillium javanicum, Rhizopus stolonifer, Trichodrema harzianum, T. atroviride* and *T. viride* frequency had increasing trend from seedling to flowering stage of resistant MSJ-118 variety. In *Aspergillus clavatus, Fusarium oxysporum and Macrophomina phaseolina* frequency had decreased at flowering stage as compared to seedling stage. In *Aspergillus flavus* and *Chaetomium globosum* frequency remains constant from seedling to flowering stage. In *Aspergillus clavatus, Aspergillus flavus*, *Aspergillus niger*, *Chaetomium globosum, Cochliobolus lunatus, Fusarium oxysporum*, *Rhizopus stolonifer, Trichodrema harzianum* and *T. viride* frequency had increasing trend from initial infested soil to seedling stage. In *Macrophomina phaseolina*, *Penicillium aurantiogriseum* and *Penicillium javanicum* had decreasing trend from initial infested soil to seedling stage. The frequency of *T. atroviride* remains constant from initial infested soil to seedling stage. Similarly, *Alternaria alternata*, *Aspergillius niger, T. atroviride* and *Trichoderma harzianum* frequency had increasing trend from seedling to flowering stage of susceptible RMG-62 -variety of mungbean. In *Chaetomium globosum, Curvularia lunata, Penicillium javanicum* and *Rhizopus stolonifer* frequency had decreased at flowering stage as compared to seedling stage. In *Alternaria alternata*, *Aspergillus flavus, Chaetomium globosum*, *Fusarium oxysporum*, *Penicillium javanicum, Rhizopus stolonifer* and *Tricoderma atroviride* frequency had increasing trend from initial infested soil to seedling stage. In *Aspergillius niger* and *Curvularia lunata* frequency decresed from initial infested soil to seedling stage. The frequency of *Trichoderma harzianum* remains constant at initial infested soil and seedling stage but increased at flowering stage. The frequency of *Aspergillus flavus* and *Fusarium oxysporum* increased from initial infested soil to seedling stage but remains constant at seedling and flowering stage. The frequency of *Macrophomina phaseolina* remains constant at all the three stages. The frequency of *Bacillus subtilis* decreased from initial infested soil to seedling stage and further increased from seedling to flowering stage of resistant MSJ-118 variety. The frequency of *Bacillus subtilis* decreased from initial infested soil to flowering stage of susceptible RMG-62 variety. *Aspergillus niger*, *Cochliobolus lunatus, Rhizopus stolonifer*, *Trichodrema harzianum, T. atroviride* and *T. viride* had the increasing abundance (%) from initial infested soil to flowering stage of resistant MSJ-118 variety. In *Aspergillus clavatus, Chaetomium globosum* and *Fusarium oxysporum*, abundance had increased from initial infested soil to seedling stage but further decreased seedling to flowering stage. In *Penicillium aurantiogriseum* and *Penicillium javanicum* abundance had decreased from initial infested soil to seedling stage but further increased seedling to flowering stage. The abundance of *Macrophomina phaseolina* decreased from initial infested soil to flowering stage. Abundance of *Alternaria alternata* and *Trichoderma atroviride* was increasing from initial infested soil to flowering stage of rhizosphere of susceptible RMG-62 variety. *Aspergillus flavus, Chaetomium globosum*, *Fusarium oxysporum* and *Rhizopus stolonifer* increased from initial infested soil to seedling stage but declined at flowering stage. The abundance of *Aspergillus niger*, *Macrophomina phaseolina*, *Penicillium javanicum* and *Trichoderma harzianum* decreased from initial infested soil to seedling stage but steadily increased from seedling to flowering stage. The abundance of *Curvularia lunata* decreased from initial infested soil to flowering stage of susceptible RMG-62 variety. The abundance of *Bacillus subtilis* decreased from initial infested soil to seedling stage but further increased at flowering stage of resistant MSJ-118 variety. The abundance trend of *Bacillus subtilis* had decrease from initial infested soil to flowering stage of susceptible RMG-62 variety. *Alternaria alternata*, *Aspergillus japonicus, Aspergillus nidulans* and *Curvularia lunata* were present in the rhizosphere at initial infested soil and seedling stage of resistant MSJ-118 variety but missing at flowering stage. The population frequency and abundance of *Alternaria alternata*, *Aspergillus japonicus* and *Aspergillus nidulans* increasing from initial infested soil to flowering stage whereas the population frequency and abundance of *Curvularia lunata* decreasing from initial infested soil to seedling stage. In susceptible RMG-62 variety *Aspergillus clavatus*, *Cochliobolus lunatus* and *Penicillium aurantiogriseum* present at initial infested soil and seedling stage but did not appear at flowering stage. The population frequency and abundance of *Aspergillus clavatus* increased from initial infested soil to seedling stage but these were decreased for *Penicillium aurantiogriseum*. The population and frequency of *Cochliobolus lunatus* remains constant but abundance decreased from

Table 1
Microorganisms isolated from initial infested soil and rhizosphere soil of resistant MSJ-118 variety of mungbean

Fungi	*Initial infested soil*			*Rhizosphere at seedling*			*Rhizosphere at flowering*		
	P	*F*	*A*	*P*	*F*	*A*	*P*	*F*	*A*
Alternaria alternata (Fr.) Keissler	42.32	14.81	1.19	53.46	25.92	1.96	-	-	-
Aspergillus clavatus Desm.	21.16	7.40	0.58	105.81	33.33	2.28	95.21	23.23	1.79
Aspergillus flavus Link Fr.	497.35	81.47	14.04	770.89	100	18.80	634.91	100	14.76
Aspergillus japonicus Saito	31.74	11.11	0.92	52.90	18.51	1.26	-	-	-
Aspergillus nidulans (Eidam) Winters	21.07	7.40	0.58	31.74	11.11	0.69	-	-	-
Aspergillus niger Van Tieghem	253.96	74.06	7.29	603.17	89.26	14.24	783.06	100	18.36
Chaetomium globosum Kunze	52.90	18.51	1.55	84.65	29.62	2.03	84.65	29.62	2
Cochliobolus lunatus Nelson and haasis	31.74	11.11	0.90	116.39	18.51	1.96	169.30	51.84	3.97
Curvularia lunata Wakkar & Boedijn	253.96	81.47	7.28	169.30	48.17	4.02	-	-	-
Drechslera halodes Subram & B.L.Jain	-	-	-	137.56	48.14	3.18	10.58	3.70	0.24
Fusarium oxysporum (Sachlecht) Snyd. and Hans.	328.04	68.95	9.48	857.14	100	19.99	444.44	85.17	10.41
Macrophomina phaseolina (Tassi) Goid.	740.73	100	21.18	264.54	77.09	6.28	211.63	70.36	4.83
Penicillium aurantiogriseum Dierckx	529.1	88.88	15.17	328.03	81.47	7.64	761.90	96.29	17.77
Penicillium javanicum V. Beyma	275.12	66.66	15.17	42.32	14.81	0.99	95.23	33.33	2.23
Rhizopus stolonifer (Ehrenb.) Vuill.	137.56	40.73	3.90	539.67	59.25	4.25	317.46	85.17	7.35
Trichoderma atroviride	31.74	11.11	0.91	63.49	11.11	1.21	95.23	18.32	1.89
Trichoderma harzianum Rifai	84.65	29.62	2.44	179.89	62.95	4.25	359.78	81.47	8.32

Table 2
Microorganisms isolated from initial infested soil and rhizosphere soil of susceptible RMG-62 variety of mungbean

Fungi	*Initial infested soil*			*Rhizosphere at seedling*			*Rhizosphere at flowering*		
	P	*F*	*A*	*P*	*F*	*A*	*P*	*F*	*A*
Alternaria alternata (Fr.) Keissler	42.32	14.81	1.19	74.07	25.92	1.60	306.87	96.29	7.03
Aspergillus clavatus Desm.	21.16	7.40	0.58	31.74	11.11	0.70	-	-	-
Aspergillus flavus Link Fr.	497.35	81.47	14.04	952.37	100	21.22	666.66	100	15.25
Aspergillus japonicus Saito	31.74	11.11	0.92	-	-	-	63.49	22.22	1.45
Aspergillus nidulans (Eidam) Winters	-	-	-	232.80	44.44	5.14	1079.35	85.18	8.23
Aspergillus niger Van Tieghem	253.96	74.06	7.29	232.80	62.95	5.16	444.44	96.29	10.18
Chaetomium globosum Kunze	52.90	18.51	1.55	243.38	70.33	5.42	211.63	66.66	4.85
Cochliobolus lunatus Nelson and haasis	31.74	11.11	0.90	31.74	11.11	0.70	-	-	-
Curvularia lunata Wakkar & Boedijn	253.96	81.47	7.28	137.56	48.14	3.05	84.65	25.92	1.93
Drechslera halodes Subram & B.L.Jain	-	-	-	42.32	14.81	0.93	158.72	55.55	3.62
Fusarium oxysporum (Sachlecht) Snyd. and Hans.	328.04	68.95	9.48	740.73	100	16.48	634.92	100	14.56
Macrophomina phaseolina (Tassi) Goid.	740.73	100	21.18	550.26	100	12.31	550.26	100	12.64
Penicillium aurantiogriseum Dierckx	529.1	88.88	15.17	285.71	85.17	6.36	-	-	-
Penicillium javanicum V. Beyma	275.12	66.66	15.17	306.87	100	6.85	402.11	92.59	27.65
Rhizopus stolonifer (Ehrenb.) Vuill.	137.56	40.73	3.90	359.78	92.58	8.03	285.71	85.17	6.56
Trichoderma atroviride	31.74	11.11	0.91	31.74	18.51	1.21	63.49	21.18	1.79
Trichoderma harzianum Rifai	84.65	29.62	2.44	84.65	29.62	1.87	137.53	48.14	3.15
Trichoderma viride Pers. ex Fr.	42.32	14.81	1.23	-	-	-	10.58	3.70	0.24
Total	3375.43			4359.68			5110.99		
Bacteria									
Bacillus subtilis	7835.23	100	60.32	6243.37	90.58	52.67	5845.68	70.27	48.11
Total	7835.23			6243.37			845.68		

P = Mean population in one 'g' soil, F = Frequency in per cent, A = Abundance in per cent, ¯ = No population

Table 3
Total population of microorganisms per 'g' soil enumerated in initial infested soil and at rhizosphere of resistant MSJ-118 and susceptible RMG-62 varieties of mungbean

Microorganisms	*Initial infested soil*	*Rhizosphere of resistant MSJ-118*		*Rhizosphere of susceptible RMG-62*	
		Seedling	*Flowering*	*Seedling*	*Flowering*
Fungi	3375.73	4570.35	4359.66	4359.68	5110.99
Bacteria	7835.23	6542.21	9873.56	6243.37	5845.68
Total	11210.96	11112.56	14233.22	10603.05	10956.67

initial infested soil to seedling stage. *Aspergillus japonicus* and *T. viride* were present at initial infested soil and flowering stage but absent at seedling stage. The population frequency and abundance of *Aspergillus japonicus* increased from initial infested soil to flowering stage whereas these were decreased for *T. viride, Aspergillus nidulans* and *Drechslera halodes* were present at rhizosphere of seedling and flowering stage but absent at initial infested soil. The population frequency and abundance of *Aspergillus nidulans* and *Drechslera halodes* increased from seedling to flowering stage. Total population in 1g soil in initial infested soil, rhizosphere at seedling, flowering of resistant MSJ-118 variety was 3375.73, 4570.35, 4359.66 for fungi, 7835.23, 6542.21, 9873.56 for bacteria, respectively (Table 3). Similarly, total population in 1g soil in initial infested soil, rhizosphere at seedling, flowering of susceptible RMG-62 variety was 3375.73, 4359.68, 5110.99 for fungi, 7835.23, 6243.37, 5845.68 for bacteria, respectively. The total population of fungi in rhizosphere of resistant MSJ-118 increased from initial infested soil to seedling stage but decreased to flowering stage. In rhizosphere of susceptible RMG-62 the total population increased from initial infested soil to flowering stage. The total population of *Bacillus subtilis* decreased from initial infested soil to flowering stage of rhizosphere of susceptible RMG-62 variety. The total population of *Bacillus subtilis* decreased from initial infested soil to seedling stage but further increased at flowering stage of rhizosphere of resistant MSJ-118 variety.The population of microorganisms was maximum at flowering in rhizosphere of resistant MSJ-118 i.e 14233.22 and minimum at seedling i.e 11112.56 stage whereas the population of microorganisms was maximum at initial infested soil i.e 11210.96 and minimum at seedling stage i.e 10603.05 of susceptible RMG-62 variety of mungbean.

Quite a large number of species of fungi were present in initial infested soil and in the rhizosphere of resistant MSJ-118 and susceptible RMG-62 varieties of mungbean but they varied in their population per 'g' soil, frequency (%) and abundance (%). These species showed certain trends in their behavior, in a few either a steady rise or decline was noted in rhizosphere at seedling and flowering of the both varieties or it was maximum or minimum at flowering and a few others. Eighteen fungi and one bacterium were found in initial infested soil and in rhizosphere of resistant MSJ-118 and susceptible RMG-62 variety at different stages. The total population of fungi was maximum at seedling stage but minimum at initial infested soil in rhizosphere of resistant MSJ-118 variety whereas total population of fungi was maximum at flowering stage but minimum at initial infested soil in rhizosphere of susceptible RMG-62 variety. The total population of bacteria was maximum at flowering stage but become minimum at seedling stage in rhizosphere of resistant MSJ-118 variety whereas total population of bacteria was maximum at initial infested soil and become minimum at flowering stage in rhizosphere of susceptible RMG-62 variety. The total population of microorganisms (fungi and bacteria) was minimum at seedling stage of both the varieties but became maximum at flowering stage in MSJ-118 and at initial infested soil of RMG-62 variety. Wright (1956), Odunfa and Oso (1979), Ahmad and Baker (1987), Dhedhi *et al.* (1990), Singh *et al.* (1993), Mane and Gaikwad (2008), Pan and Bhagat (2008), Youssef *et al.* (2008), Baviskar and Padghan (2009) isolated fungi inclusive of Trichoderma spp., *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp. *Macrophomina phaseolina* etc. and bacteria viz. *Bacillus spp., Pseudomonas spp.* from the rhizosphere and rhizoplane of different crops. The variation in rhizosphere effect may be due to the crop, plant, age and the type of microorganisms. The rhizoshere microflora of resistant MSJ-118 and susceptible RMG-62 varieties appear to be mainly under the influence of the root exudates. The constituents of root exudates seems to have the stimulating effect which promote the growth of fungi and bacteria in rhizosphere at seedling and flowering stages of plant growth.

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