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Population Dynamics of Microfungi in Root Region of Soybean With Reference to Growth Stages and Environmental Factors

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Fungi were screened from the root region of soybean at seedling, preflowering, flowering, postflowering and sensecent stages of growth and they differed both quantitatively and qualitatively. The average number of fungi/g soil gradually increased from seedling to flowering stage, thereafter their frequency declined at senescent stage. Aspergilli were dominant followed by penicillia and fusaria in the non-rhizosphere and rhizosphere regions. In the early vegetative growth stages Phycomycetes were very frequent, but their population declined after flowering stage. However, the frequency of *Macrophomina phaseolina* and *Neocosmospora vasinfecta* gradually increased on rhizoplane with the onset of senescence. Total number of fungi showed a negative correlation with soil pH, total organic carbon and organic matter.

Key Words Frequency Fungi Phycomycete Rhizoplane Rhizosphere Soybean

Dynamism of Metabolic interactions between microorganism and root system of green plants is complex, since the population varies with the type of plant and even with the same plant at different growth stages. Microbial population in the root region increases with age of a plant and reaches maximum with the vegetative development, and with the onset of senescence the population gradually declines to the level present in the surrounding soil following the death of plant and decomposition of its tissues Katznelson 1965, *et al.* 1980, Antique *et al.* 1982). Moreover, moisture status, temperature and pH of the soil, and host exudation affect the activity of saprophytes and pathogens at different root zones of a plant (Griffin 1972, Rovira 1965).

These studies provide some understanding of saprophytic and pathogenic microorganisms developing in different regions of the root and help in the manipulation of the rhizosphere population for biological control of soil-borne pathogens causing root diseases. Besides host susceptibility, climatic and edaphic factors govern the disease incidence. Hence the present investigation has been designed to study the population dynamics of non-rhizosphere, rhizosphere and rhizoplane fungi of soybean (*Glycine max* (L.) Merr.) at different growth stages in relation to environmental factors.

MATERIALS & METHODS

Soybean seeds (cv Punjab-1) were sown in rows (15 x 15cm)in an experimental plot of the Botanical Garden, Banaras Hindu University after the first heavy rains in June, 1983. Fungal population and soil characteristics were determined at seedling, preflowering, flowering, post-flowering and senescent stages of plant growth.

Determination of physico-chemical properties of soil Soil texture (sand, silt and clay) was determined as suggested by Piper (1944). Water holding capacity, moisture content and temperature of the soil were measured (Misra 1968). Soil pH was measured by a digital pH meter using soil-water extract (1:10 w/v). Total organic carbon was determined by rapid titration method (Walkley & Black 1934), whereas total organic matter content was estimated by multiplying the total organic carbon with 1.724 (Robinson 1952).

Isolation of non-rhizosphere fungi Soil samples were collected near plants free from the influence of root systems in sterilized polythene bags using sterile spatula. Soil samples (10 g) were transferred to 250 ml Erlenmeyer flasks containing 100 ml sterilized (1.4 Kg/cm2 for 1 h) distilled water. The flasks were shaken vigorously on a horizontal electric shaker (120 throws/min. 1.5 cm displacement/throw) and suitable dilutions ($1:10^2$ and $1:10^3$) were prepared in sterile distilled water. One ml aliquot of the soil suspension from $1:10^3$ dilution was poured in Petri plates containing 20 ml Markins medium. Five replicates were maintained. The fungal colonies were counted after incubation at 25±1°C for 1 week).

Isolation of rhizophere fungi

The root system of soybean plants was carefully removed from the soil and gently tapped to remove the adhering soil particles. The roots cut into bits (2 cm long) were transfered to a 250 ml Erlenmeyer flask containing sterile distilled water. Appropriate dilutions ($1:10^2$ and $1:10^3$) were prepared. (The fungal population was assessed as described above. Dry weight of soil of the stock solution was determined according to Timonin (1940). R:S ratio (a ratio of microbial number per unit weight of rhizosphere soil (R) to that of adjacent non-rhizosphere soil (S)) was determined (Katznelson 1946).

Isolation of plane fungi The method of Harley & Waid (1955) was employed for the isolation of intimately associated fungi with the root surface. The root pieces used for the isolation of rhizosphere fungi were collected and washed (8-10 times) with sterile distilled water. The surface mositure of root bits was removed by blotting with sterile blotting paper. Five root bits were transferred to Petri plates containing Martins' medium. Five replicates were maintained. The plates were incubated for 1 week at 25+1 C, and the colonies growing on the bits were recorded.

RESULTS The meteorological observations and physico-chemical characteristics of soil collected during the experiment are presented in the Fig. 1 and Table 1 respectively. A total of 54 fungi were isolated from the non-rhizosphere, rhizosphere and rhizoplane regions of soybeen at seedling, preflowering, flowering, post flowering and senescent stages of plant growth (Table 2). Of the fungi recorded, aspergilli were dominant followed by penicillia, fusaria and other forms. The dominant fungi in the non-rhizosphere soil were *Alternaria alternata* (Fr.) Keissl., *Aspergillus flavus* Link ex Fr., *A.fumigatus* Fres., *A.luchensis* Inui., *A. niger* van Tiegh., and *A.Terreus* Thom, whereas from the rhizosphere they were *A. flavus* *A. fumigatus, A. luchuensis, A. niger, A. terreus, Cladosporium cladosportioides* (Fres.) de Vries, *Curvularia lunata* (Wakk.) Boed., and *Fusarium oxysporum* Schlecht. Moreover, rhizoplane also harboured some dominant fungi., *A. niger Cladosporium herbarum* (Pers.) Link ex Gray, *F. oxysporum, f. solani* (Mart.) Sacc., *Macrophomina phaseolina* (Tassi) Gold., *Neocosmospora vasinfecta* Smith and *Rhizoctonia solani* kuhn.

Drechslera australiensis (Bug.) Subram. & Jain ex Ellis, *Myrothecium roridum* Tode ex Fr., *Nigrospora sphaerica* (Sacc.) Mason and *Periconia byssoides* Pers present in the rhizosphere, and *M.phaseoilina* and *N. vasinfecta* present in the rhizoplane regions were not detected in the non-rhizosphere soils. *N. vasinfects* was frequently isolated from all the growth stages of the plant except from preflowering stage, while *M. phaseolina* and *R. solani* were detected after seedling and preflowering stages, repectively.

The members of Moniliales were dominant followed by Mucorales, Mycelia sterilia and Hypocreales. The fungal taxa included 5 Phycomycetes, 1 Ascomycete, 45 Deuteromycetes and 3 Mycelia sterilia.

Average number of fungi per unit dry wt of soil and R;S ratio gradually increased from seedling to flowering stage, thereafter declined with the commencement of plant senescence (Table 3). Total number of fungi of non-rhizosphere soil showed positive correlation with temperature (r = 0.84) and negative correlation with pH (r = -0.94), total organic carbon (r = -0.83) and total organic matter (r = -0.97) (Table 4). similarly, aspergilli and penicillia of the rhizosphere soil revealed negative correlation with total organic carbon (r = -0.92 and -0.91 respectively).

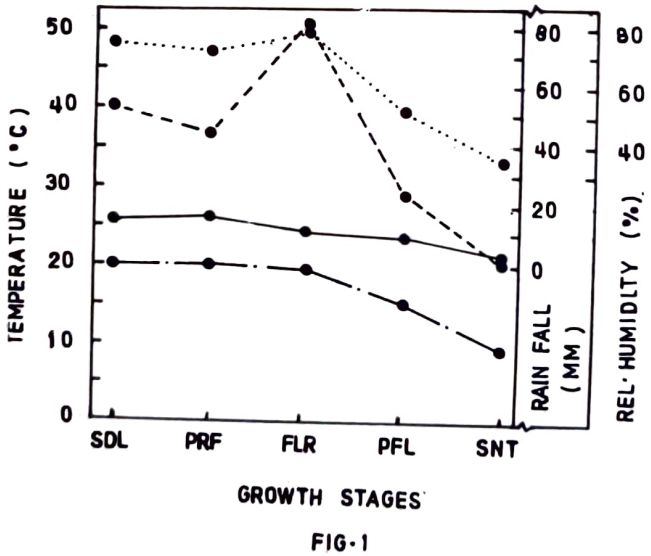

Fig. 1: Meteorological data obtained from the Department of Geophysics, B.H.U., Varanasi (July (seedling) - Nov. (senescent stage), 1983). ● – – –●, maximum temperature (°C); ●———●, minimum temperature (°C); ●———●, average rain fall (mm); ●.....●, average relative humidity(%)

Table 1. Physico-Chemical Properties of Soil Collected During Various Growth Stages of Soybean Plants

Soil Properties*	Growth stages (months)**				
	SDL (July)	PRF (Aug)	FLR (Sept)	PFL (Oct.)	SNT (Nov.)
Moisture (%)	15	13	19	9	11
Temperature (°C)	31	31	30	24	19
pH	7.5	7.4	7.3	7.4	7.4
Total organic carbon (%, w/w)	0.082	0.064	0.057	0.059	0.064
Total organic matter (%, w/w)	0.142	0.111	0.099	0.102	0.109

* Mechanical composition - Sand, 67.50%; Silt, 21.25%; Clay, 11.25%; Water holding capacity, 32%

** Samples were collected at 30 day interval.

SDL, Seedling; PRF, preflowering; FLR, flowering;

PFL, postflowering; Snt, Senescent

DISCUSSION Qualitative and quantitative variation of fungal population in the non-rhizosphere and rhizosphere of plants as influenced by environmental factors have been reported earlier (Roy *et al.* 1980, Antique *et al.* 1982, Upadhyay & Rai 1982). Stimulation of rhizosphere fungi with the vegetative growth of soybean might be due to rhizosphere effect which is caused by root exudates and sloughed off root tissues (Ramchandra Reddy 1959, Rouatt & Katznelson 1961). Rovira (1965) described that the rhizosphere and rhizoplane microflora may be regarded as selective sieve, absorbing some exudate constituents and, in turn, releasing exudates from their own cells.

In soybean rhizosphere some fungi were restricted to a particular root region and growth stage of soybean (Table 2). Selective action and specificty of root exudates on rhizosphere and rhizoplane microflora with a considerable stimulation of certain micro organisms and/or the group of microorganisms and suppression of others have been reported (Yoshida & Sakai 1962,). *F. solani, F. oxysporum, M. phaseolina, N. vasinfecta* and *R. solani* were frequently observed on the rhizoplane of soybean (Table 2). These fungi are considered as root pathogens of soybean (Nyvall 1979).

The frequency of *M. phaseolina* gradually increased with growth of soybean; moreover, its incidence is favoured by host senescence (Sabet *et al.* 1961). Wyllie & Calve (1969) reported that the sclerotial formation of *M. phaseolina* was conditioned solely by flowering and pod set and other variables such as soil moisture and temperature were subordinate in their effect. It may be explained that the plants' aging cause decline in resistence which allows colonization by root pathogens. A linear increase in recovery of *M. phaseolina* from the rhizoplane of soybean plants with the sampling age has been reported by Mueller *et al.* (1985).

The succession of micro organisms in root regions of green plants depends upon the plant age and environmental factors. No definite pattern in variation of fungal species was observed. However, the sugar loving fungi such as *Mortierella subtilissima, Mucor spinosus, Rhizoctonia solani, Rhizopus nigricans*, etc., were frequently detected in the early growth stages in the rhizosphere and rhizoplane regions followed by the members of deuteromycetes. Moreover, variation in the non-rhizosphere fungi at different growth stages may be attributed to the seasonal fluctuation of edaphic factors such as moisture, temperature, pH, aeration, organic mater, available nutrients, etc. (Griffin 1963, 1972). Menszies (1970) described that production of passive propagules by several root pathogens on substrates in soil in high rain fall areas was usually much less than in the dry irrigated areas.

Table 2. Per cent Frequency and Occurrence of Non-Rhizosphere, Rhizosphere and Rhizoplane Microfungi of Soybean

	Growth Stages														
	SDL			PRF			FLR			PFL			SNT		
	NR*	RS*	RP**	NR	RS	RP	NR	RS	RP	NR	RS	RP	NR	RS	RP
Acrophialophora fusispora (Saksena) Samson	5	5	—	3	—	—	4	—	—	—	—	—	—	—	—
Alternaria alternata (Fr.) Keisal.	3	2	—	5	—	—	5	4	—	5	—	—	—	5	—
A. humicola Oudem.	—	—	15	–	—.	—	—	—	—	—	4	—	—	—	10
Aspergillus candidus Link ex Fr.	—	—	—	4	—	—	2	—	—	—	—	—	5	—	—
A. flavus Link ex Fr.	9	7	—	10	7	—	8	5	—	7	—	—	14	—	—
A. fumigatus Fres.	—	7	—	7	—	—	6	8	—	8	15	—	—	—	—
A. tuchuensis Inui	8	12	—	5	11	—	5	—	—	8	19	—	7	12	—
A. nidulans (Eidam) Winter	3	6	—	—	—	—	—	—	—	7	—	—	—	—	—
A. niger van Tiegh.	19	7	17	20	7	12	17	13	28	—	24	—	—	9	23
A. sulphureus (Fres) Thom & Church	—	—	—	—	—	—	7	—	—	3	5	—	—	—	—
A. sydowi (Bain. & Sart.) Thom & Church	—	—	—	—	—	—	2	4	—	—	—	—	6	—	—
A. terreus Thom	30	12	—	24	16	—	19	18	—	23	16	—	13	15	20
A. ustus (Bain) Thom & Church	—	—	—	—	—	—	3	—	—	—	5	—	—	—	—
A. variecolor (Berk. & Br.) Thom & Rap.	—	—	—	—	—	—	5	10	—	5	—	—	—	—	—
Black sterile mycelia	3	—	—	3	—	—	1	—	—	—	—	—	—	—	—

	Growth Stages														
	SDL			PRF			FLR			PFL			SNT		
	NR*	RS*	RP**	NR	RS	RP	NR	RS	RP	NR	RS	RP	NR	RS	RP
Cephalosporium roseo-griseum Saksena	1	4	—	—	—	—	—	—	—	—	—	—	—	—	—
C. acremonium Corda	—	—	—	2	—	—	3	—	—	1	2	—	—	—	—
Cladosporium cladosporioides (Fres.) de Vries	—	5	—	4	—	—	—	—	—	—	7	—	—	6	—
C. herbarum (Pers.) Link ex Gray	—	—	15	—	—	10	2	—	20	—	—	—	8	—	23
Cunninghamella echinulata Thaxt.	—	—	—	—	—	—	—	—	—	—	—	—	—	1	5
Curvularia lunata (Wakk.) Boed	—	—	—	—	5	13	—	4	—	—	6	13	8	—	—
C. pallescens Boed.	—	—	—	—	—	—	—	—	10	—	—	—	—	8	—
Dark sterile mycelia	—	6	—	—	2	—	1	—	18	—	—	15	5	3	—
Drechslera australiensis (Bug.) Subram. & Jain ex Ellis	—	5	—	—	—	—	—	3	—	—	7	—	—	—	—
Fusarium moniliforme Sheld	—	5	—	4	—	—	—	—	—	5	—	—	5	8	—
F. oxysporum Schledht.	—	—	15	—	6	15	3	4	—	—	7	18	7	—	—
F. semitectum Berk. & Rav.	6	—	—	—	—	18	3	—	—	—	—	—	3	6	—
F. solani (Mart) Sacc.	—	—	28	—	—	—	—	1	15	—	—	—	—	5	—
Gliomastix convoluta (Mar.) Mason	—	—	—	—	—	—	—	—	—	2	—	—	4	3	—
Humicola grisea Traaen	—	—	—	—	—	—	—	—	—	6	3	—	—	—	—

	Growth Stages														
	SDL			PRF			FLR			PFL			SNT		
	NR*	RS*	RP**	NR	RS	RP	NR	RS	RP	NR	RS	RP	NR	RS	RP
Macrophomina phaseolina (Tassi) Goid.	—	—	—	—	—	10	—	—	20	—	—	33	—	—	35
Mortierella subtilissima Oudem.	—	—	13	—	2	18	—	—	8	—	5	—	—	—	—
Mucor spinosus van Tiegh.	—	—	10	1	2	5	—	2	—	—	—	—	—	3	—
Myrothecium roridum Tode ex Fr.	—	—	—	—	4	—	—	2	—	—	7	—	—	—	—
M. inudatum Toad	—	—	—	5	—	—	—	—	—	—	—	—	—	—	—
Neocosmospora vasinfecta Smith	—	—	8	—	—	—	—	—	10	—	—	15	—	—	15
Nigrospora sphaerica (Sacc.) Mason	—	—	—	—	1	—	—	1	—	—	—	—	—	—	—
Penicillium chrysogenum Thom	—	—	—	—	—	—	—	—	—	—	—	—	6	—	—
P. citrinum Thom	2	5	—	—	—	—	—	4	—	5	4	—	2	6	—
P. granulatum Bain.	—	—	—	—	4	—	—	—	18	—	5	—	—	—	—
P. javanicum Van Beyma	—	—	—	—	5	—	—	—	—	7	3	—	—	4	—
P. rubrum Stole	—	—	—	—	—	8	—	—	10	3	—	—	—	—	—
Periconia byssoides Pers.	—	—	—	—	2	—	—	3	—	—	—	—	—	—	—
Rhoma humicola Gill & Abb.	—	—	—	—	4	—	—	1	—	—	7	18	—	—	8
Populaspora sp	—	—	—	—	9	—	—	—	13	—	9	—	—	—	15
Rhizoctonia solani Kuhn	—	—	—	—	—	—	—	—	20	—	1	20	—	1	13
Rhizopus nigricans Ehrenb	—	—	10	—	3	8	—	3	—	—	—	—	—	3	—

	Growth Stages														
	SDL			PRF			FLR			PFL			SNT		
	NR*	RS*	RP**	NR	RS	RP	NR	RS	RP	NR	RS	RP	NR	RS	RP
Scopulariopsis sp	2	5	—	—	3	—	—	3	—	—	—	—	—	—	—
Spicaria silvatica Oudem	—	—	—	2	—	—	—	2	—	4	—	—	—	—	—
Stysanus stemonites (Pes.) Corda	—	—	—	—	2	—	—	—	—	2	—	—	—	—	—
Syncephalastrum racemosum (Cohn) Sch.	1	2	—	1	3	—	—	1	—	—	—	—	2	—	—
Trichoderma viride Pers. ex Gray	—	—	—	—	—	—	2	—	—	2	—	—	3	—	—
Verticillium candelabrum Bon	—	4	—	—	—	—	—	—	3	—	—	—	6	3	—
White sterile mycelia	5	—	—	—	6	—	—	3	—	—	—	8	—	—	—
Total number of species	14	17	9	16	21	10	19	23	13	18	21	8	17	18	10

* Per cent occurrence; ** Per cent frequency; NR= Non-rhizosphere; RS= Rhizosphere; RP= Rhizoplane;
SDL = Seedling; PRF = Preflowering; FLR = Flowering; PFL = Postflowering; SNT = Senescent.

Table 3. Average Number of Fungi Present in the Non-Rhizosphere and Rhizosphere Soil of Soybean at Differen Growth Stages.

Soil	Average number of fungi (x 10^4/g)				
	Growth stages				
	SDL	PRF	FLR	PFL	SNT
Non-rhizosphere	5.037	5.064	7.060	6.622	5.187
Rhizosphere	8.460	12.770	20.019	17.406	15.076
R:S ratio	1.679	2.521	2.835	2.629	2.906

SDL, seedling; PRF, preflowering;
FLR, flowering; PFL, post-flowering;
SNT, senescent.

Table 4 Correlation Coefficient (r) Values Between Edaphic Factors and Population of Fungi in the Non-Rhizosphere and Rhizosphere of Soybean.

Edaphic factors	Fungal Population							
	Total number		Aspergilli		Penicillia		Fusaria	
	NR	RS	NR	RS	NR	RS	NR	RS
Moisture	0.27	0.11	0.66	0.35	-0.65	-0.59	-0.12	-0.32
Temperature	0.84	-0.32	0.60	-0.01	-0.58	-0.88*	-0.80	-0.84
pH	-0.84	-1.0**	-0.43	-0.94*	-0.38	-0.62	-.049	-0.40
Total organic carbon	-0.83	-0.94**	-0.35	-0.73	-0.27	-0.64	-0.14	-0.31
Total organic matter	-0.97**-	1.19**	-0.43	-0.92*	-0.07	-0.01*	-0.27	-0.49

* Signaficant at P = 0.05;
** Significant at P = 0.01.

A significant correlation between the total number of rhizosphere fungi, and pH and total organic carbon may be explained by the fact that on increasing the microbial community the organic susbrates are rapidly utilized resulting in decrease in pH and total organic matter. Chaturvedi & Dwivedi (1984) have also reported lowering of total organic carbon and pH, and release of nutrients from the substrates in large amount with subsequent increase in microbial community of soil.

We propose that variation in the population of non-rhizosphere microfungi occurred with respect to the environmental factors whereas. that of rhizosphere and rhizoplane fungi would have been due to stimulation by host exudates. A gradual increase in the frequencies of *M. phaseolina* and *N. vasinfecta* with the onset of senescene clearly explains the effect of plant age on their incidence.

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