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STUDY OF RHIZOSPHERE, NON-RHIZOSPHERE AND RHIZOPLANE MYCOFLORA AT DIFFERENT STAGES OF Vicia faba L.

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Key words : *Vicia faba*, Mycoflora, Non-rhizosphere, Rhizosphere, Rhizoplane.

Isolation of rhizosphere, non-rhizosphere and rhizosplane mycofora was done at early, pre-flowering, flowering and fruiting stages of plant growth. The number of fungi/g dry soil and fungal species were maximum at early stage. A slight increase in number of fungi at the late fruiting stage was observed.

INTRODUCTION

The term "Rhizosphere" was coined by Hiltner (1904) to describe the portion of soil where microorganism mediated processes are under the influence of the root system. Clark (1949) proposed that ecological niche or habitat provided for microorganism by root surface be designated as the rhizoplane. Their growth and excretion influence microorganisms present on the root surface of the plant.

Many workers observed stimulation of fungi in the rhizosphere such as Agnihothrudu (1955), Sosova and Vasin (1961), Babeva and Seveleva (1963), Rangaswamy and Vasantharajan (1962), Bharadwaj (1970), Mohammad (1985) and Jain (2001). Commonly, fungi in the rhizosphere soil are several times greater than those of root free soil.

MATERIAL AND METHODS

For the isolation of rhizosphere mycoflora, plants from each plot was dug out with the help of sterilized trowel and was gently tapped so as to remove loosely adhering soil, and roots were cut off with sterilized scissors and were placed in 250 ml flask containing 100 ml sterilized distilled water. The flask was shaken vigorously to get uniform soil water suspension. One ml of this suspension was transferred to each petridish and then 15 ml sterilized Czapek's medium of the following composition was poured in each one: KH_2P0_4 1.0g; $MgS0_4$, $7H_2O$ 0.5g; KcI 1.0g; $FeS0_4$ trace; yeast powder O.5g; $NaN0_3$ 2.0g; Dextrose 10.0g; Agar - Agar 15.0g and distilled water 1000 ml (cooled to 40°C). Five replicates were used for the rhizosphere. The remaining soil suspension after removing root was dried in an electric oven at 105°C for 24 hrs and the weight of the oven dried soil was calculated. The number of fungi appearing in one ml solution was counted and the average of colonies calculated.

For the isolation of non-rhizosphere mycoflora, soil was taken away from the root system. 10g of the soil sample was taken in a 250 ml flask containing 90 ml sterilized distilled water.

The flask was then shaken vigorously to make uniform soil suspension, and dilution series of 1:100 , 1:1000, 1:10000, were prepared. One ml suspension of each dilution was transferred in plates and nutrient medium. Separate pipettes were used for each dilution. Moisture content of the soil was determined. For calculation of fungi/g of dry soil in the non-rhizosphere, the average number of fungal colonies of 5 plates were multiplied by particular dilution and the average was calculated.

The moisture content of soil was taken into consideration while calculating the fungi/g of dry soil. The percentage occurrence of fungi in rhizosphere and non-rhizosphere was calculated.

The method of Harley and Waid (1995) was employed to study the rhizoplane fungi. Roots were taken out from the flask and washed thoroughly with several changes of sterilized distilled water. Roots were first dried with sterilized Whatman's filter paper No. 44 and then small root bites of 10mm size were cut off and five bites were placed on sterilized Czapek's agar medium in sterilized petridishes. Five replicates were used for the rhizoplane study. Inoculated plates were incubated for 5-6 days at 25°C and thereafter fungi were isolated and identified.

RESULTS AND DISCUSSION

Rhizosphere, Non-Rhizosphere and Rhizoplane mycoflora :

RHIZOSPHERE

In all 35 fungal species were isolated from the rhizosphere soil out of which 4 species were from Phycomycetes, 2 from Ascomycetes and 29 from Deuteromycetes. The latter dominated the rhizosphere mycoflora with 83% of the total fungal population. The dominant species in the rhizosphere were *Rhizopus nigricans, Syncephalastrum racemosum, Paecilomyces fusisporus, Aspergillus flavus, A.terreus, A.niger, P.citrinum, Fusarium udum*, White sterile mycelium whereas *Mucor luteus, Cunninghamella echinulata, Phoma glomerata,Botryodiplodia theobromae, Cephalosporium coremioides, Trichoderma lignorum, A.luchuensis, Trichothecium roseum, Torula convoluta, Cladosporium cladospoioides, Curvularia lunata, Fusarium nivali* and *Phylostica sp.* were of rare occurrence and were obtained once or twice. They were rare also because their % occurrence was very low (Table-1).

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TABLE -1 : Percentage occurrence of rhizosphere, non-rhizosphere and rhizoplane fungi at different stages of plant growth. (Mean of three plots lying very close to one).

	Rhizosphere				Non-Rhizosphere				Rhizoplane			
Name of fungi	**E**	**PF**	**FL**	**FR**	**E**	**PF**	**FL**	**FR**	**E**	**PF**	**FL**	**FR**
Rhizopus nigricans	6	4	2	3	7	-	14	6	-	-	-	-
Mucor luteus	-	-	2	-	4	4	-	-	-	-	-	-
Cunninghamella echinulata	-	-	-	2	5	1	-	-	-	-	-	-
Syncephalastrum racemosum	21	5	40	-	3	-	10	-	-	-	7	-
Chaetomium globosum	-	-	6	-	3	-	-	-	-	-	-	-
Neocosmospora vasinfecta	-	-	7	-	-	-	-	-	-	-	-	-
Phoma glomerata	-	3	-	-	-	-	-	-	-	-	-	-
Botryodiplodia theobromae	1	-	-	-	-	-	-	-	-	-	-	-
Chaetomella horrida	-	-	5	-	-	-	-	6	-	-	-	-
Verticillium glaucum	-	-	-	5	-	-	-	-	-	-	-	-
Paecilomyces fusisporus	3	17	-	15	6	10	4	6	-	-	-	-
Cephalosporium coremioides	1	-	-	-	-	-	-	-	-	-	-	-
Trichoderma lignorum	-	-	-	1	-	6	1	12	-	-	-	--
Aspergillus flavus	37	4	9	7	6	7	7	9	-	-	-	-
A. terreus	1	17	3	10	-	16	10	7	-	-	-	-
A. luchuensis	1	3	-	-	6	4	9	9	-	-	-	-
A. niger	5	4	13	5	7	12	15	12	-	19	-	27
A. candidus	-	2	-	-	4	2	-	3	-	-	-	-
A. nidulans	-	-	2	3	-	4	1	-	-	-	-	13
Penicillium citrinum	1	11	-	5	4	4	5	10	-	-	-	7
Trichothecium roseum	-	-	-	-	4	-	-	-	-	-	-	-
Papulospora sp.	-	-	2	-	3	-	-	-	-	-	-	-
Torula convoluta	-	-	2	-	-	-	-	5	-	-	-	-
Humicola fuscoata	-	-	-	-	4	4	1	3	-	-	-	-
Cladosporium lignicolum	5	2	-	-	7	2	2	3	13	-	-	-
Cladosporium cladosproioides	1	-	-	-	4	-	-	-	-	-	-	-
Curvularia tetramera	1	5	-	-	4	2	-	-	7	-	-	-
Curvularia lunata	-	-	-	-	3	-	-	-	-	6	-	-
Alternaria tenuis	-	4	-	-	-	-	-	-	-	-	-	-
A.humicola	2	-	-	-	5	2	-	-	-	-	-	-
Fusarium udum	2	-	7	17	-	5	6	-	33	44	73	53
F.nivale	1	-	-	-	-	-	-	-	-	-	-	-
Myrothecium roridum	1	2	-	-	3	3	-	-	-	-	-	-
Phylostica sp.	1	-	-	-	-	-	-	-	-	-	-	-
White sterile mycelium	7	4	-	16	5	12	5	3	40	31	20	-
Black sterile mycelium	2	-	-	5	-	-	-	-	7	-	-	-
Pink sterile mycelium	-	-	-	6	-	. -	-	-	-	-	-	-
Total number of species isolated	20	16	13	14	22	18	15	15	5	4	3	4

E-Early stage, PF-Pre-flowering stage, FL-Flowering, FR-Fruiting stage.

TABLE-2 : Average number of fungi/g of dry soil (In thousand) in the rhizosphere (R), non-rhizosphere (NR) number of species isolated from R, NR and Rhizoplane (RP) and R/S ratio of the test plant.

State of Sampling	R Fungi/g of dry soil	No.of species in R	No.of species on RP	NR Fungi/g of dry soil	No. of species NR	R/S ratio
Early stage	197.31	20	5	27.26	22	7.23
Pre-Flowering Stage	132.21	16	4	32.24	18	4.10
Flowering Sage	101.57	13	3	24.03	15	4.22
Fruiting Stage	127.94	14	4	26.91	15	4.75

Amongst the common fungi present both in the rhizosphere and non-rhizosphere, % -age occurrence of *Syncephalastrum racemosum, Paecilomyces fusisporus, A.flavus, A.terreus* and white sterile mycelium was more in the rhizosphere. The % -age occurrence of *R. nigricans, A. luchuensis, A. niger, P. citrinum, Cladosporium lignicolum,* and *A. humicola* decreased in the rhizosphere in comparison to non-rhizosphere. 20,16,13, and 14 fungal species were isolated from early, preflowering and fruiting stages, respectively (Table-2).

Non-Rhizosphere

28 Species were isolated from non-rhizosphere soil, out of which 4 were Phycomycetes, 1 Ascomycetes and 23 Deuteromycetes like the rhizosphere. The Deuteromycetes were the dominant group covering 82%of the total soil mycoflora. The species isolated from the non-rhizosphere were mostly common to rhizosphere. *Trichothecium roseum, Humicola fuscoatra* and *Curvularia lunata* were confined only to non-rhizosphere. The dominant species were *R. nigricans, M. luteus, Cunninghamella echinulata, T.lignorum, A. luchuensis, A. niger, A. candidus, Cladosporium lignicolum* and *F. roseum* and *Chaetomium globosum*, Papulospora sp. were of rare occurrence. 22,18,15 and 15 fungal species were isolated from non-rhizosphere soil at early, pre-flowering, flowering and fruiting stages respectively (Table-1).

Rhizoplane

Fungi isolated from rhizoplane were comparatively less in number than those in the rhizosphere and non-rhizosphere (Table-1). In all, 7 species were isolated out of which one belonged to Phycomycetes and one belonged to Deuteromycetes. *A. niger*, *Fusarium udum* and white sterile mycelium were the dominant species. (Table-1).

Quantitatively the rhizosphere soil harboured higher number of fungi/g than the nonrhizosphere soil (Table-1). The enrichment of rhizosphere mycoflora was also evident by the high R/S ratio which was more than one (Table-2). Considering the different groups of fungi isolated from the rhizosphere and non-rhizosphere it was observed that the latter in comparison to the former showed higher percentage of Phycomycetes and Deuteromycetes and lower percentage of Ascomycetes. Though all the fungal species isolated from the rhizosphere of the plants were not isolated from the non-rhizosphere, yet such species, which could be isolated only from the rhizosphere of the plant, were not many and were of rare occurrence.

Starkey (1958), Papavizas and Davey (1961) and Neal *et al.* (1964) reported that the rhizosphere mycoflora differs both qualitatively and quantitatively from the general soil mycoflora. Singh (1967), Gupta (1971), Pandey (1970), Singh (1970), Kumar (1993), Jain (2001) and Deo (2006) also observed difference in the rhizosphere and non-rhizosphere mycoflora. Kumar and Gupta (2006) reported that the maximum number of fungi/g of dry soil was more at early stage. Mishra (1968) observed that fungi in the rhizosphere decreased as the age of the plant increased. Gupta and Paliwal (2009) reported maximum number of fungi/g of dry soil at the early stage. Rovira (1956) reported that qualitative as well as quantitative change take place in the root exudates with the growing age of the plant.

The present study revealed a significant quantitative difference between rhizosphere and non- rhizosphere mycoflora which is evident by the R/S ratio. The number of fungi/g of dry soil and fungal species were maximum at early stage. The number of fungi was found less than the early stage and a slight increase in the number of fungi at late fruiting stage was observed.

Reason for the presence of maximum number of fungi at early stage of plant growth may be either due to the presence of maximum number of amino acids and sugars in the root extract. Agnihotri (1964) reported an increase in the exudation of amino acids, glutamine, glucose, fructose and decrease in organic acids. Bhuvaneshwari and Subba Rao (1957) reported that root exudate is the main factor which influences the rhizosphere microflora. Its fluctuation with growing age of the plant has been correlated with the quality and quantity of root exudation which are supposed to change with age of the plants. The slight increase in R/S ratio and the number of fungi at late fruiting stage may be attributed to the availability of food by the death and decay of the roots of the plant and may be important factor in influencing the rhizosphere fungal flora at the fruiting stage.

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