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STUDY OF RHIZOSPHERE, NON-RHIZOSPHERE AND RHIZOPLANE MYCOFLORA AT DIFFERENT STAGES OF GROWTH OF *CICER ARIETINUM*

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Isolation of rhizosphere, non-rhizosphere and rhizoplane mycoflora 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 the number of fungi at late fruiting stage was observed.

Keywords : *Cicer arietinum*, Mycoflora; Non-rhizosphere; Rhizosphere; Rhizoplane.

Introduction

The term 'rhizosphere' was coined by Hiltner[1] to describe the portion of soil where microorganism mediated process are under the influence of the root system. Functions of the rhizosphere are of central importance for the plant nutritions, health and quality. The well studied rhizosphere effect describes the phenomenon that, in comparison with bulk of soil, the biomass and activity of microorganisms is enhanced as a result of exudation of compounds by the root[2,3]. Clark[4] proposed that the ecological niche provided for microorganisms present on the root surface be designated as the 'rhizoplane'. Microorganisms present on root surface of the plant and influenced by their growth and excretion. Many workers have observed stimulation of fungi in the rhizosphere[5-7].

Material and Methods

For the isolation of rhizosphere mycoflora, 3 plants, one plant from each plot was dugout and gently tapped so as to remove loosely adhering soil, and roots were cut off with a 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_2PO_4 1.0g.; $MgSO_4$-7 H_2O 0.5 g.; KCI 1.0g.; $FeSO_4$ trace; yeast powder 0.5g.; $NaNO_3$ 2.0g; Dextrose 10.0g.; Agar-agar 15. 0g and distilled water 1000ml (cooled to 40°C). Five replicates were used for the rhizosphere. The remaining soil suspension after removing root was dried in a electric oven at 105°C for 24 hrs and the weight of the oven dry 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-rhizoshere mycoflora, soil was taken away from the root system. 10gm of the soil sample was taken in a 250 ml flask containing 90 ml sterilized distilled water. The flask then, shaken vigorously to make uniform soil suspension and dilution series of 1 : 100; 1 : 1000; 1 : 10,000 were prepared. One ml suspension of each dilution was transferred in plates and the nutrient medium. Separate pipette 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 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[8] 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 firstly dried with sterilized Whatman's filter paper No. 44 and then small root bits of 10 mm size were cut off and five bites were placed on sterilized Czapek's agar medium in sterilized petridish. 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 32 fungal species were isolated from the rhizosphere, out of which, 4 species were from Phycomycetes, 2 from Ascomycetes and 26 from Deuteromycetes. The latter dominated the rhizosphere

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

Name of fungi	Rhizosphere				Non-Rhizosphere				Rhizosphere			
	E	PF	FL	FR	E	PF	FL	FR	E	PF	FL	FR
Rhizopus nigricans	1	1	2	7	6	4	-	-	-	-	-	-
Mucor luteus	2	1	-	-	5	2	-	-	22	-	-	-
Choanephora cucurbitarum	2	-	-	-	-	-	-	-	-	-	-	-
Cunninghamella echinulata	-	-	5	1	-	-	4	-	-	-	-	-
Chaetomium globosum	-	-	-	1	-	-	-	-	-	-	-	-
Neocosmospora vasinfecta	2	-	-	-	1	-	-	-	-	-	-	-
Cephalosporium coremioides	3	2	4	1	2	-	3	3	-	-	-	-
Chaetomella horrida	2	-	-	-	-	-	-	-	-	-	-	-
Trichoderma lignorum	2	1	2	2	2	3	6	5	-	-	-	-
Aspergillus flavus	8	15	27	20	5	4	-	10	33	34	38	33
A. niger	1	13	17	14	4	11	10	8	-	28	24	26
A. luchuensis	3	17	18	-	5	10	8	8	-	-	-	-
A. candidus	3	1	-	4	2	-	7	3	-	-	-	-
A. terreus	1	6	-	-	2	9	8	10	6	-	-	-
A. nidulans	-	3	-	-	5	4	-	-	-	-	-	-
Penicillium citrinum	2	1	4	6	6	5	5	8	-	-	-	-
Verticillium glaucum	-	-	-	-	-	-	-	3	-	-	-	-
Paecilomyces fusisporus	9	12	2	1	2	5	3	-	-	-	-	-
P. varioti	-	8	-	-	-	-	-	-	-	-	-	-
Trichothecium roseum	1	1	3	1	-	-	-	-	-	-	-	-
Stachybotrys atra	2	-	-	-	-	-	-	-	-	-	-	-
Nigrospora sphaerica	-	-	-	2	-	-	-	-	-	-	-	-
Humicola fuscoatra	-	-	-	-	-	2	8	-	-	-	-	-
Cladosporium herbarum	10	8	6	6	8	3	-	-	-	-	-	-
C. lignicolum	1	-	1	12	-	-	7	-	-	-	-	-
Curvularia lunata	2	1	2	4	7	3	-	-	-	-	-	-
C. tetramera	4	-	-	-	7	5	7	8	-	-	-	-
Helminthosporium sativus	2	1	-	-	-	-	-	-	-	-	-	-
Alternaria tenuis	6	-	-	-	2	-	-	-	-	-	-	-
A. humicola	3	1	1	4	2	3	-	-	-	-	-	-
Fusarium roseum	3	-	-	-	3	-	-	6	6	-	-	-
F. udum	8	4	2	8	5	4	3	5	33	32	19	5
Myrotheium roridum	9	1	-	-	5	3	3	-	-	-	-	-
Black sterile mycelium	4	-	2	2	7	8	8	10	-	-	-	-
White sterile mycelium	4	2	2	4	7	12	10	13	-	6	19	36
Total no. of species isolated	28	21	17	19	23	19	16	14	5	4	4	4

E = Early Stage; PF = Pre-flowering Stage; FL = Flowering Stage; FR = Fruiting Stage.

mycoflora with 81% of the total fungal population. The dominant species in the rhizosphere were *Rhizopus nigricans, Cephalosporium coremioides, Aspergillus flavus, A. niger, A. candidus, A. terreus, Penicillium citrinum, Paecilomyces fusisporus, Trichothecium roseum, Cladosporium herbarum, Curvularia lunata, Alternaria humicala, Fusarium udum,* White sterile mycelium. Whereas *Choanophora cucurbitarum, Chaetomium globosum, Stachybotrys atra, Nigrospora sphaerica, Cladosporium lignorum and Fusarium roseum* were of rare occurrence and were obtained once and twice.

Amongest the common fungi present both in the rhizosphere and non-rhizosphere, percentage occurrence of *Cephalosporium coremioides, Aspergillus flavus, A.*

Table 2. Average number of fungi/g of dry soil (in thousand) in the rhizosphere, non-rhizosphere, number of species isolated from rhizosphere (R), non-rhizosphere (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	187.73	28	5	27.26	23	6.8
Pre-flowering stage	129.47	21	4	30.24	19	4.28
Flowering Stage	107.89	17	4	24.03	16	4.28
Fruiting Stage	114.94	19	4	25.31	14	4.54

niger, A. luchuensis, Paecilomyces fusisporus, Cladosporium herbarum, C. lignicolum and Fusarium udum was more in the rhizosphere whereas the percentage occurrence of *Mucor luteus, Trichoderma lignorum, A. terreus, Curvularia tetramera,* White sterile mycelium and Black sterile mycelium was less in the rhizosphere in comparison to non-rhizosphere. 28, 21, 17 and 19 fungal species were isolated from early, pre-flowering, flowering and fruiting stages, respectively (Table 2).

Non-Rhizosphere : Twenty seven species were isolated from non-rhizosphere soil, out of which 3 secies were from Phycomycetes, 1 from Ascomycetes and 23 from Deuteromycetes. Thus, like the rhizosphere, the Deuteromycetes was the dominant group covering 88% of the total soil mycoflora. The species isolated from the non-rhizosphere were almost common to rhizosphere except *Verticillium glaucum* was confined only to non-rhizosphere soil. The dominant species were *A. niger, A. luchuensis, A. terreus, Penicillium citrinum and Curvularia tetramera,* White sterile mycelium and Black sterile mycelium whereas *Rhizopus nigricans, Cunninghamella echinulata, Neocosmospora vasinfecta, Verticillium glaucum and Cladosporium lignicolum* were of rare occurrence. 23, 19, 16 and 14 fungal species were isolated from non-rhizosphere soil at early, pre-flowering, flowering and fruiting stages, respectively (Table 1).

Rhizoplane : Fungi isolated from the rhizoplane were comparatively less in number than those present in the rhizosphere and non-rhizosphere. In all 7 species were isolated, one belongs to Phycomycetes and rest from Deuteromycetes. *Aspergillus flavus, A. niger, Fusarium udum* and white sterile mycelium were the dominant species (Table 1).

Quantitatively the rhizosphere soil harboured higher number of fungi/g as compared to the non-rhizosphere 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 showed lower percentage of fungi belonging to Phycomycetes, Ascomycetes and higher percentage of Deuteromycetes. Though, all the fungal species isolated from the rhizosphere of the plant were not isolated from the non-rhizosphre yet such species which could be isolated only from the rhizosphere of the plant were not many and were of rare occurrence.

Starkey[9], Papavizas and Davey[10] and Neal *et al.*[11] reported that the rhizosphere mycroflora differs both qualitatively and quantitatively from the general soil microflora. Gupta[12], Babu[13] and Deo[14] also observed difference in the rhizosphere and non-rhizosphere mycoflora. Kumar and Gupta[7] reported maximum number of fungi/g of drysoil was more at early stage. Mishra[15] observed that fungi in the rhizosphere decreased as the age of the plant increased. Deo[14] and Yadav[16] reported maximum number of fungi/g of dry soil at the early stage. Rovira[6] reported that qualitative as well as quantitative change take place in the root exudate with the age of plant.

The present study revealed a significant quantitative difference between the 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 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[16] or due to an increase in the exudation of amino acids, glutamine, glucose, fructose and decrease in organic acids[17]. Bhuvaneshwari and Subba Rao[18] reported that root exudate is the main factor which influences the rhizosphere microflora. Its fluctuation with age of the plant has been correlated with the quality and quantity of root exudation which are supposed to change with age of 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 the important factor in influencing the rhizosphere fungal flora at fruiting stage.

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