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Studies on ceratain aspects of root surface fungi. I
Fungi on living roots of Pennisetum typhoides (Burm f.) Stapf et Hubb. *

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The present paper deals with the succession of fungi on the root regions of living *Pennisetum typhoides*. The presence and amounts of amino acids and sugars in root exudates (July to October) and root extracts (July to November) have been studied.

INTRODUCTION

The succession of higher plants in relation to various environmental factors has been studied by various scientists. The succession of microorganisms on various substrates has also been extensively studied. The succession of fungi and on ageing roots of different plants has also been reported (e.g. Das 1953; Dix 1964; Gadgil 1965; Parkinson, Pearson 1967; Taylor, Parkinson 1965; Dickinson, Pugh 1965a, 1965b; Srivastava 1969; Srivastava, Mishra 1971a; Kanaujia 1973). The association of fungi to the root at different stages and at various depths for crop plants has not been properly worked out in tropics and need further exploration. In the present communication, the process of fungal succession

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on living roots of *Pennisetum typhoides* (Burm f.) Stapf et Hubb. has been described by author.

MATERIALS AND METHODS

Pennisetum typhoides (Burm f.) Stapf et Hubb. had been selected for the present study. The crop was raised in the experimental plot situated in the campus of the University of Gorakhpur. The mycoflora of seeds had been also assessed (Mishra, Kanaujia 1973a) before they were sown in the field. The seeds were sown on July 1, 1970. The first sampling was completed when the plants were 10 days old. The subsequent two samplings were done on July 15 and 25, 1970. Further samplings were done twice a month till November 1970. On July 10 and 15, when roots were quite small and their division into crown (RC), middle (RM) and distal (RD) regions was possible, the whole root system was considered as single entity. The three above-mentioned regions were considered on 25 July and later. The roots from crown, middle and distal regions and nonrhizosphere soil from corresponding regions were also separately collected as described by Kanaujia (1973). The process was repeated the following year from July 1971 to November 1971. The isolation of mycoflora of rhizosphere (RS), cortical portion of rhizoplane (RPC), steller portion of rhizoplane (RPS) and nonrhizosphere (NR) region of crown, middle and distal zones of the root was assessed by methods described by Kanaujia (1973). The fungi were identified (Gilman 1956; Clements, Shear 1931; Barnett 1960; Thom, Raper 1945) and preserved on sterilized Czapek's ager slants. The whole process was repeated the following year from July 1971 to November 1971. The fortnightly data for the months from August to November were consolidated every month for the presentation in this study. The moisture content and pH of nonrhizosphere of RC, RM and RD regions on every sampling day were determined by the Piper's method (1966). The fungal population was calculated on the basis of one g dry soil in the nonrhizosphere, one g dry soil and dry root in the rhizosphere and average number of colonies per plate in rhizoplane cortical and steler regions. The root exudates were collected monthly from 20 day old plants from July to October 1970. Alcoholic root extracts of crown, middle and distal regions were separately prepared on every sampling time. The amino acids and sugars present in the root exudates and extracts were detected by the undirectional paper chromatography (Smith 1960a, 1960b). The amount of amino acids and sugars was determined by colorimetrically (Peach, Tracey 1955). The effect of root-exudates

on 15 rhizosphere fungi, viz., *Rhizopus nigricans, Mucor hiemalis, Cunninghamella bertholletiae, Trichoderma viride, Aspergillus fumigatus, A. flavus, A. terreus, A. niger, A. aculeatus, A. tamarii, A. sydowi, Penicillium chrysogenum, Curvularia lunata, Alternaria alternata*, and *Fusarium nivale*, was studied in cavity slides by the hanging drop method. The cellulose, hemicellulose and lignin components of root of crown, middle and distal zones was estimated by the Peach and Tracey's method (1955).

RESULTS

Seed mycoflora. A considerable number of fungal species (4 of *Phycomycetes*; 3 of *Ascomycetes*; 19 of *Deuteromycetes*; 2 of sterile colonies) were found associated with seeds of *Pennisetum typhoides* before they were used to raise the crop. *Aspergilli* were predominant (Table 1).

Table 1

Dominant fungi associated with Pennisetum typhoides

Species	Isolation techniques		
	direct plating	moisture chamber techn.	surface wash method
Acrocylindrium granulatum Bon.	+		
Alternaria alternata Fr.	+	+	+
Aspergillus aculeatus Lizuka	+	+	+
A. flavus Link	+	+	+
A. fumigatus Fres.	+	+	+
A. nidulans /Eid./ Winter		+	+
A. niger v. Tiegh.	+	+	+
A. niger /n°2/	+	+	
A. terreus Thom	+	+	+
Botryotrichum piluliferum Sacc.	+		
Cephalosporium acremonium Corda		+	+
Chaetomium globosum Kunze ex Fr.	+	+	
Cladosporium herbarum Link ex Fr.	+	+	
Curvularia lunata /Wakker/ Boedijn	+	+	+
Fusarium nivale /Fr./ Ces.		+	
Lophotrichus sp.	+	+	
Mucor racemosus Fres.	+		
Papularia sphaerosperma /Pers./ v.Höhn.		+	
Penicillium chrysogenum Thom	+	+	+
P. humicola Oud.		+	+
Phoma hibernica Grimms, O'Con.et Cumm.	+	+	
Rhizophus nigricans Ehrenb.	+	+	+
Spicaria simplicissima Oud.		+	
Syncephaiastrum racemosum Cohn et Schroet.		+	+
Trichoderma viride Pers. ex Fr.	+	+	+
Zygorhynchus japonicus Kominami			+
White sterile cols /W2/		+	+
Brown sterile cols		+	
Total	18	24	16

Horizontal distribution of fungi

One hundred and ten fungi belonging to different taxonomic groups were isolated from the rhizosphere, cortical and steler regions of rhizoplane and nonrhizosphere during July to November 1970 and 1971.

Table 2

Distribution of fungi in horizontal /NR, RS, RPC, RPS/ and vertical /RC, RM, RD/ regions of Pennisetum typhoides

Genus	Horizontal region				Vertical region			Total
	NR	RS	RPC	RPS	RC	RM	RD	
Phycomycetes								
Rhizophus	3	3	1	1	3	3	3	3
Mucor	9	6	1	1	9	8	6	9
Others spp	8	6			7	7	6	8
Deuteromycetes								
Aspergillus	19	16	8	7	19	15	14	19
Penicillium	11	9	2	1	11	10	7	11
Cladosporium	3	3	2	2	3	3	3	3
Curvularia	4	4	2	1	4	4	3	4
Fusarium	6	6	5	4	6	5	6	6
Others spp	27	20	10	6	27	18	16	31
Ascomycetes								
Chaetomium	3	3	1		3	4	2	4
Others spp	2	1			2	2	1	2
Mycelia sterilia								
Rhizoctonia	1				1		1	1
Sterile colonies	6	7	5	4	8	6	6	9
Total	102	84	37	27	103	85	74	110

Fungi in the number of 102, 84, 37 and 27 were isolated from nonrhizosphere, rhizosphere, cortical and steler portions of rhivoplane respectively (Table 2). *Deuteromycetes* were the most numerous in all the horizontal regions followed by *Phycomycetes*, sterile colonies, *Ascomycetes* and *Mycelia sterilia*. *Aspergillus* followed by *Penicillium* in *Deuteromycetes*, *Mucor* followed by *Rhizopus* in *Phycomycetes*, *Chaetomium* in *Ascomycetes* and white sterile colonies in sterile colonies were predominant (Table 2). Out of 102 species of fungi isolated from NR region, only 78 of them could establish themselves in RS region (6 sp. were characteristic for rhizosphere region); 84 sp. of RS region decreased to 36 ones (one restricted to this region) in the cortical portion of rhizoplane, whereas there were only 27 arrivals in the steler region of rhizoplane and all of them were contributed by RPC region. Thus pattern of fungal arrival from NR → RS, RS → RPC → RPS was 102 → →78, 84→36, 37→27. *Aspergilli* — the most numerous — were followed by ssp. of *Penicillium*, *Mucor*, *Fusarium* and *Curvularia* (Tables 2 and 3). The number of fungal species in the four above stated root regions exhibited a regular pattern in crown, middle and distal zones. The fungi in all the horizontal regions of the root were the most numerous in the crown region and were followed by the middle and distal zones. However, in the steler region of distal zone the number of fungal species was appreciably higher (23) than in the corresponding region of the middle zone (18) but their number was lower than the crown region (25) (Table 3).

Rhizopus nigricans, *Mucor hiemalis*, *Trichoderma viride*, *Aspergil-*

lus aculeatus, A. flavus, A. fumigatus, A. niger, A. terreus, Penicillium chrysogenum, Verticillium albo-atrum, Cladosporium epiphyllum, Curvularia lunata, Fusarium oxysporum, white (Wl) and black sterile colonies were commonly associated with all the horizontal zones of crown, middle and distal zones. Ten other isolates were also recorded from NR, RS, RPC and RPS regions irrespective of vertical zones.

Table 3

Distribution of fungal species in different root regions /NR, RS, RPC, RPS/ of crown, middle and distal regions of Panicum typhoides

Species	Region											
	crown				middle				distal			
	NR	RS	RPC	RPS	NR	RS	RPC	RPS	NR	RS	RPC	RPS
Absidia spinosa Lendn.	+	+			+	+				+		
Rhizophus nigricans Ehrenb.	+	+	+	+	+	+	+	+	+	+	+	+
R. oryzae Went. et Prinsen Geerligs	+	+			+	+			+			
Mucor hiemalis Wehm.	+	+	+	+	+	+	+	+	+	+	+	+
M. hiemalis f. luteus /Lin./ Schipper	+				+							
M. mucedo L. ex Fr.	+				+							
M. pusillus Lindt.	+	+				+			+	+		
M. circinelloides v. Tiegh. et Le Monn.	+											
M. racemosus Fres.	+	+	+	+	+	+			+	+		
M. heterogamus Vuill.	+	+			+	+			+			
M. dispersus Hagem.	+	+			+				+			
M. heterosporus Fisch.	+	+			+				+			
Circinella simplex v. Tiegh.					+							
Zygorhynchus Aponicus Kominami	+	+			+	+			+	+		
Choanephora cucurbitarum /Berk.et Rav./Thaxter	+	+			+	+			+	+		
Cunninghamella bertholletiae Stod.	+	+			+	+			+	+		
C. echinulata Plapk	+	+			+	+			+	+		
Syncephalastrum racemosum Cohn.et Schroet.	+	+			+	+			+	+		
Helicostylum piriforme Bain.	+											
Chaetomium apiculatum B.C. Lodha	+				+							
C. globosum Kominami	+	+	+		+	+	+		+	+		
Cladosporium herbarum Link ex Fr.						+						
Chaetomium sp.	+				+	+				+		
Celasinospora cerealis	+				+				+			
Thielavia terricola /Gilm.et Abbott/ Emmons	+					+						
Thoma hibernica Grimms, O Connor et Cummins	+	+	+		+	+	+		+	+		
P. humicola Gilman et Abbott	+	+	+	+			+					
Botryodiplodia theobromae Pat.	+				+				+			
Monilia sitophila /Mont./ Sacc.	+	+			+	+	+		+	+		
Cephalosporium acremonium Corda		+					+					
Trichoderma viride Pers. ex Fr.	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus aculeatus Iizuka	+	+	+	+	+	+	+	+	+	+	+	+
A. carneus /v.Tiegh./ Bloch.	+	+			+	+			+	+		
A. fischeri Wehmer	+	+			+	+						
A. flavipes Berk. et Br.	+	+			+	+			+	+		
A. flavus Link	+	+	+	+	+	+	+	+	+	+	+	+
A. fumigatus Fres.	+	+	+	+	+	+	+	+	+	+	+	+
A. japonicus Saito	+											
A. lanosus Link	+											
A. nidulans /Eid./ Wint.	+	+			+	+	+		+	+		
A. niger v. Tiegh.	+	+	+	+	+	+	+	+	+	+	+	+
A. ochraceus Wilhelm	+	+		+	+				+	+		
A. rugulosus Thom. et Raper	+	+			+				+	+		
A. sulphureus /Fres./ Thom. et Church	+	+			+				+	+		
A. sydowi /Bain. et Sart./ Thom. et Church	+	+			+	+			+	+	+	
A. tamarii Kita	+	+			+	+			+	+		
A. terreus Thom	+	+	+	+	+	+	+	+	+	+	+	+
A. terreus var. africanus Penn. et Rap.	+	+										
A. ustus /Bain. Thom et Church	+	+		+	+		+	+	+		+	+
Aspergillus versicolor /Vuill./ Tirabeschi	+											
Penicillium chrysogenum Thom	+	+	+	+	+	+	+	+	+	+	+	+
P. humicola Oud.	+	+	+		+	+	+		+	+		
P. lanosum Westl.	+	+			+				+	+		
P. nigricans /Bain./ Thom.	+	+			+	+			+	+		
P. notatum Westl.	+	+			+				+	+		
P. oxalicum Curr. et Thom	+				+	+						
P. rugulosum Thom	+											
P. spiculisporum Lehm.	+	+			+	+			+			
P. terrestre Jens.	+	+			+							
Penicillium sp. 1	+	+			+				+			
Penicillium sp. 2	+				+							
Gliocladium roseum Link					+							
Acremonium vitis Cattaneo	+								+			
Sporotrichum roseum Link ex Fr.	+											
Verticillium albo-atrum Reinke et Berth.	+	+	+	+	+	+	+	+	+	+	+	+
V. terrestre /Link/ Lindau	+								+	+		
Verticillium glaucum Bonorden	+	+				+						
Acrocylindrium gracilentum Bon.						+						
Paecilomyces fusisporus Yaluoena	+	+	+	+	+	+	+	+	+	+	+	+
P. varioti Bain.	+	+			+	+			+	+		
Spicaria elegans /Corda/ Lindau	+				+	+			+	+		
S. simplicissima Odd.		+										
Papularia sphaerosperma /Pers./ v. Höhn.	+	+										
Pullularia pullulans /de Bary/ Berkhout	+											

Tab.3

Species	NR	RS	RPC	RPS	NR	RS	RPC	RPS	NR	RS	RPC	RPS
Torula allii /Hara/ Sacc.	+											
T. herbarum /Link/ ex Fr.					+							
Nigrospora sphaerica /Sacc./ Mason	+								+			
Humicola fuscoatra Traen	+			+	+		+		+			
Botryotrichum piluliferum Sacc. et Marchal	+	+										
Cladobotryium chlamydosporum C.G. Nees		+										
Cladosporium epiphyllum /Pers./ Martius	+	+	+	+	+	+	+	+	+	+	+	+
C.herbarum /Link ex Fr.	+	+	+		+	+	+		+	+	+	+
C.ligneroum	+	+			+	+			+			
Scolecobasidium constrictum Abbott	+	+			+							
Curvularia geniculata /Tracy et Earle/Boedijn	+				+				+	+		
C. lunata /Wakker/ Boedijn	+	+	+	+	+	+	+	+	+	+	+	+
C. pallescens Boedijn	+	+	+				+		+			
C. tetramera /McKinney/ Boedijn	+	+			+				+	+		
Helminthosporium sativum Pammel,King et Bakke	+	+			+				+	+	+	
Tetracoccosporium paxianum Szabo	+											
Alternaria alternata Fr.	+	+	+						+		+	+
Pestalotia sp.									+			
Ramenula effinis /Fautr.et Lamb./ Wollenw.	+	+			+							
Fusarium avenaceum /Fr./ Sacc.	+	+	+		+	+			+			
F. chlamydosporum Wr. et Rg.		+			+	+			+			
F. monilliforme Sheld.	+				+	+			+		+	+
F. nivale /Fr./ Ces.	+	+	+	+	+	+			+	+	+	
F. oxysporum Schlecht.em.Snyd.et Hans.	+	+	+	+	+	+	+	+	+	+	+	+
F. roseum Link.	+		+	+						+	+	+
Rhizoctonia solani /Kühn./	+								+			
White sterile cols /W1/	+	+	+	+	+	+	+	+	+	+	+	+
White sterile cols /W2/	+	+										
White sterile cols /W3/			+									
Grey st. cols	+	+	+	+	+		+		+		+	+
Creamy st. cols	+				+				+			
Brown st. cols		+										
Pink st. cols		+	+	+			+	+			+	+
Yellow st. cols					+	+			+			
Black st. cols	+	+	+	+	+	+	+	+	+	+	+	+
Total	95	73	30	25	76	54	29	18	69	50	27	22

Twenty four spp. to NR, 6 dpp. to RS, 1 sp. to RPC and none to RPS were found to be restricted in their distribution (Table 3). *Rhizopus nigricans, Mucor hiemalis, Trichoderma viride, Aspergillus flavus, A. aculeatus, A. fumigatus, A. niger, Cladosporium epiphyllum* and *C. herbarum* (NR, RS, RPC and RPS); *Paecilomyces fusisporus* (NR), *Aspergillus terreus* (NR, RS), *Penicillium chrysogenum* (NR, RS and RPC) and *Fusarium oxysporum* and white sterile fungus (Wl) were dominant

Table 4

Distribution of dominant fungi in horizontal /NR, RS, RP-RPC, RPS/ and vertical /RC, RM, RD/ regions of root

Species	Horizontal regions				Vertical regions		
	NR	RS	RPC	RPS	RC	RM	RD
Mucor hiemalis Wehm.	+	+	+	+	+	+	+
Rhizophus nigricans Ehrenb.	+	+	+	+	+	+	+
Trichoderma viride Pers. ex Fr.	+	+	+	+	+	+	+
Aspergillus aculeatus Iizuka	+	+	+	+	+	+	
A. flavus Link	+	+	+	+	+	+	+
A. fumigatus Fres.	+	+	+	+			+
A. niger v. Tiegh.	+	+	+	+	+	+	
A. terreus Thom	+	+			+	+	+
Penicillium chrysogenum Thom	+	+	+		+	+	+
Paecilomyces fusisporus	+				+		
Cladosporium epiphyllum /Pers./ Martius	+	+	+	+	+	+	+
C. herbarum Link. ex Fr.	+	+	+	+	+	+	+
Fusarium oxysporum Schlecht. em. Snyd. et Hudn.			+	+		+	+
White sterile cols /W1/			+	+			+

fungi at different stages of plant growth associated with regions indicated in the brackets (Table 4).

Vertical distribution of fungi

Fungi in the number of 103, 85 and 74 were respectively recorded from crown, middle and distal zones. Crown region mycoflora comprised 19 *Phycomycetes*, 5 *Ascomycetes*, 70 *Deuteromycetes*, 1 *Mycelia sterilia* and 8 sterile colonies. 18, 6, 55 and 6 species of *Phycomycetes*, *Ascomycetes*, *Deuteromycetes* and sterile forms respectively from middle; and 15 spp. of *Phycomycetes*, 3 of *Ascomycetes*, 49 of *Deuteromycetes*, one of *Mycelia sterilia* and 6 sterile colonies from distal zones were recorded. *Deuteromycetes* — among them *aspergilli* — always were present at all the depths. *Aspergilli* followed by species of *Penicillium*, *Mucor*, *Fusarium* and *Curvularia* were the most numerous ones. Species of *Rhizopus*, *Chaetomium* and *Cladosporium* appeared nearly in equal numbers in different vertical zones. The crown contained the highest number of species was followed by the middle and distal zones (Table 2). Fifteen species of *Phycomycetes*, 3 of *Ascomycetes*, 38 of *Deuteromycetes* and 5 of sterile fungi were commonly present in RC, RM and RD zones irrespective of horizontal regions; 15, 4 and 1 species of fungi were confined to crown, middle and distal zones respectively. The remaining forms were isolated from two of the three vertical zones (Table 3). *Mucor hiemalis*, *Rhizopus nigricans*, *Trichoderma viride*, *Aspergillus flavus*, *Cladosporium epiphyllum*, *C. herbarum* and *Penicillium chrysogenum* were dominantly associated with all the vertical zones. Other dominant fungi were *Aspergillus niger*, *A. aculeatus*, *A. terreus* (RC, RM), *Paecilomyces fusisporus* (RC), *Fusarium oxysporum* (RM), and *Aspergillus fumigatus* and white sterile fungus (RD) in the zones indicated in the brackets (Table 4).

Number of species at different stages of plant growth

The number of fungal species at seedling, preflowering, flowering, fruiting and senescent stages exhibited a regular pattern. Its number was always the biggest one in the nonrhizosphere and was followed by RS, RPC and RPS regions. The smallest number of fungi was recorded in the steler region. Generally, the number of fungi was greater in NR of crown than that of remaining two zones. There was no regular pattern of number of species and average number of colonies per plate in two regions of rhizoplane (Table 5). The variation in fungal popu-

Table 5
Number of fungal species in different root regions and in different months of 1970 and 1971

Months		Root regions											
		crown				middle				distal			
		NR	RS	RPC	RPS	NR	RS	RPC	RPS	NR	RS	RPC	RPS
1970	July	26	26	6/6	4/6	16	10	5/7	4/4	15	13	6/10	4/5
	August	35	17	9/8	8/8	24	14	8/8	4/5	22	18	11/11	8/8
	September	47	16	9/8	5/6	30	21	9/17	6/8	25	14	11/13	7/8
	October	41	16	14/17	8/8	26	22	13/25	6/8	25	20	10/10	8/8
	November	11	13	9/16	8/7	9	15	4/15	3/5	12	17	9/10	6/5
1971	July	32	28	11/10	5/4	17	13	7/10	5/6	18	11	11/10	5/5
	August	30	20	12/19	6/8	28	19	12/12	6/6	24	24	13/19	5/7
	September	39	23	15/17	13/13	29	21	11/10	6/5	26	18	14/13	8/11
	October	32	18	9/17	99/8	29	16	14/17	6/5	25	17	13/13	5/8
	November	20	16	10/12	6/5	19	13	12/7	7/8	11	16	8/13	6/6

Digits in denominator denote the numbers of colonies per plate in rhizoplane cortical and stelar regions

lation (expressed on the basis per g dry soil in NR and per g dry soil and dry root in RS) exhibited a regular sequence. The population in NR was always lesser than in RS and in RS region; the population per g dry soil was greater than that one calculated at per g dry root. In the NR region, the highest population was recorded in July during both years. It decreased gradually till November. In the RS region, the obtained variation pattern of population is presented below.

In the seedling stage (July) when plants were quite young, the population was also low. Ageing of plants resulted in an increase in the population which reached a maximum at the flowering stage. It decreased in the senescent stage (November) population in the three vertical zones; it also exhibited a definite pattern. It was always highest in the crown, followed by middle and distal zones in the NR region. During seedling (July) and preflowering (August), the highest population was in the crown zone followed by the middle and distal zones of rhizosphere. At flowering (September), fruiting (October) and senescent stages (November), the highest and lowest population was observed in distal and middle zones of rhizosphere (Fig. 1).

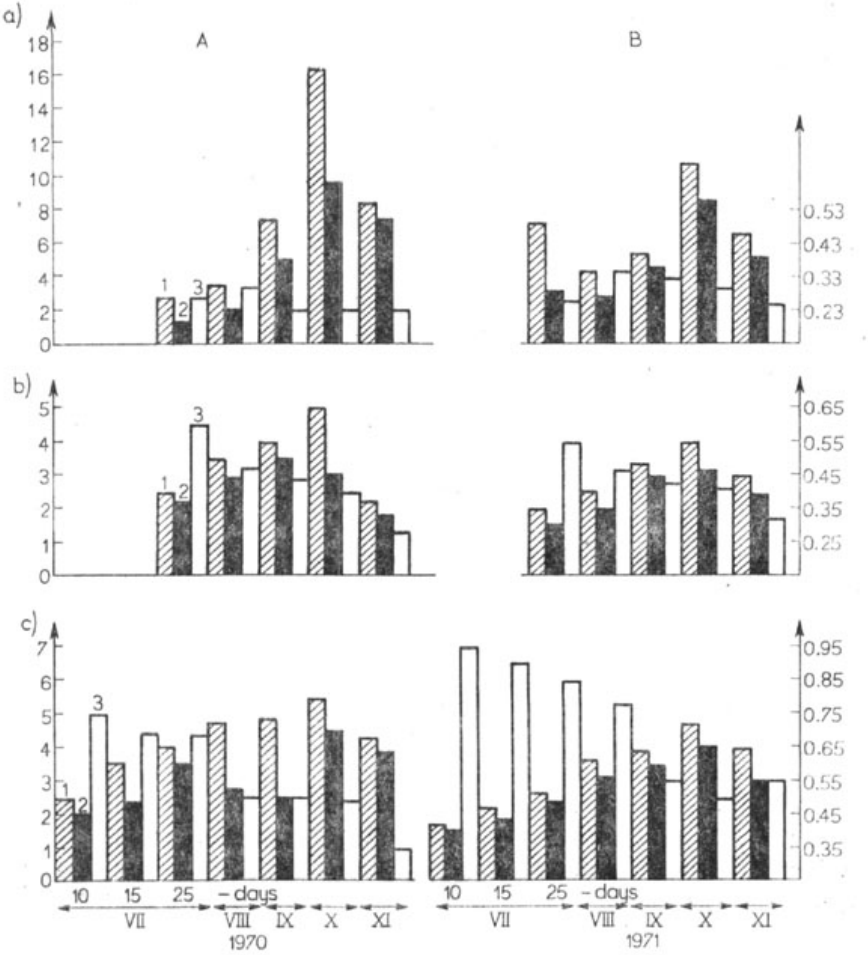

Fig. 1. Fungal population of nonrhizosphere (B) ($\times 10^5$) and rhizosphere (A) ($\times 10^5$) regions of *Pennisetum typhoides*

a — distal region, *b* — middle region, *c* — crown region; *1* — rhizosphere/g dry soil, *2* — rhizosphere/g dry root, *3* — nonrhizosphere/g dry soil

Amino acids and sugars in the root exudates and extracts of *Pennisetum typhoides*

Eight amino acids (Table 7) were found in the root-exudates collected from seedling to fruting stage of *P. typhoides*. At the seedling stage, a lower number of amino acids was exuded from the roots. The

number increased gradually till flowering when the highest number of acids was recorded. The maximum amount of amino acids was also recorded at this stage. The amount decreased at fruiting, however, the number was equal to that one obtained at preflowering. Alanine was traced till flowering. Leucine, methionine and valine were recorded at flowering and fruiting; and histidine, and threonine were only detected at preflowering. Cysteine, however, was exuded at the fruiting stage only (Table 6). Six sugars (Table 7) were found in the root exudates at different stages of plant growth. Glucose and rhamnose were present from seedling to fruiting stages. Mannose and sucrose were exuded at seedling, and flowering and fruiting stages respectively. The amount of total sugars increased gradually from seedling to fruiting stages (Table 6).

Table 6
Amino acids/sugars present in the root-exudates of Pennisetum typhoides

Amino acids/sugars	1971 July	August	September	October
Alanine/glucose	25/+	30/+	30/+	-/+
Arginine/mannose	50/+	75	90	0
Cystein/rhamnose	-/+	-/+	-/+	25/+
Histidine/ribose		30/+	-/+	-/+
Leucine/sucrose			30/+	45/+
Methionine/xylose		-/+	35/+	45/+
Threonine		25		
Valine			60	75
No. of amino acids/ sugars	2/3	4/4	5/5	5/5
Amount of acids/sugars /ug/10 plants in 120 h/	75/70	150/410	245/575	230/100

Sixteen amino acids (Table 7) were detected in the extracts of RC, RM and RD zones from seedling to senescence. Such amino acids as cysteine, histidine, arginine, leucine and threonine were frequently occurring in different root regions. Aspartic acid and glycine were detected at fruiting (RD) and preflowering (RC) stages respectively in zones indicated, whereas citrulline was also restricted to RC, RM and RD zones during fruiting (October). On July 10 and 15, when roots were very short and not differentiated in to the three regions, the number of amino acids obtained was 3 and 5 respectively. From July 15 till October (fruiting), the number of amino acids also increased gradually and maximum number was recorded at fruiting. It decreased at senescence. The highest and lowest amount of free amino acids was always recorded in the roots of distal and middle zones. Amount of individual amino acids varied at different stages of plant growth in 3 zones (Table 7).

Table 7
Amino acids and sugars present in the extracts from crown /RC/, middle /RM and distal /RD/ regions of Pennisetum typhoides roots

Amino acids/sugars	July					August			September			October			November		
	10	15	25														
			RC	RM	RD	RC	RM	RD	RC	RM	RD	RC	RM	RD	RC	RM	RD
Alanine Arabinose	25	40		50	− +		125	− +	− +			150	175	300 +	190	125	140 +
Aspartic acid Deoxyribose										− +				50			
Arginine/Glucose	− +	50 +	− +	− +	90 +	− +	− +	50 +	− +	150 +	300 +	200 +	210 +	100 +	160 +	200 +	125 +
Citrulline/Lactose												100	120	80 +			
Cystein/Mannose					100 +			100	125			500	225	800	50		150
Glutamic acid/ Raffinose					275						− +	− +	50 +				
Glycine/Rhamnose	− +	− +	− +	− +	− +	? +	− +	− +	− +	− +	− +	− +	− +	− +	− +	− +	− +
Histidine/Ribose	110	170 +	210		130	225 +	125	− +	90 +	100 +	180 +	280		100 +	80	70	125
iso-leucine/Sucrose			25			40		25	− +		− +	50 +	100 +	50 +	− +	− +	− +
Leucine/Trehlose	70	45		75	125												
Methionine/Xylose	− +	40 +	50 +	− +	75 +	50 +	− +	100 +	− +	− +	− +	50 +	90 +	80 +	40 +	75 +	75 +
Proline/unknown /Rf.0.204/			− +					180	210	150	50				60		
Serine						188			110	120	180				50		
Threonine			50	70	50	50	87	90	210	170	270	190	25	100			
Tryptophane							200		75	100	50	130		90	150		100
Valine			100	150		210	210		190	140	160	180	300	320			150
No. Amino acids Sugars	3 3	5 4	5 4	4 3	6 5	8 4	6 3	8 5	8 6	8 5	8 7	11 5	9 5	12 7	9 4	5 4	8 5
Amount - amino acids Sugars	204 200		435 775		570 690		847 850		1020 2000		1340 2300		1295 1000		270 875		995 970
/ug/2g fresh roots/	245 310	245 310		345 520		933 1340		1030 1500		1015 1100		1920 2175		2270 2500		660 550	

Twelve sugars (Table 7) were detected in the present study. The highest number of sugars in the roots RC, RM and RD zones was obtained at flowering and in RD zone at fruiting stage. The variation pattern in the amount of sugars in different stages was very similar to that one obtained for sugars. Glucose, xylose and rhamnose were present throughout in roots of all depths. On the other hand, trehalose (RD — September) and an unknown sugar (R_f 0.204) (RM — July 25) were confined to the months and regions indicated in the brackets (Table 7).

Moisture content and pH of nonrhizosphere soil and root and shoot growth of the plant. A narrow variation in moisture content and pH of the nonrhizosphere soil of 3 vertical zones at different stages of plant growth was observed (Fig. 2).

Cellulose, hemicellulose and lignin components of root

During July (seedling stage), when the plants were quite young, the cellulose, hemocellulose and lignin contents of the root were very

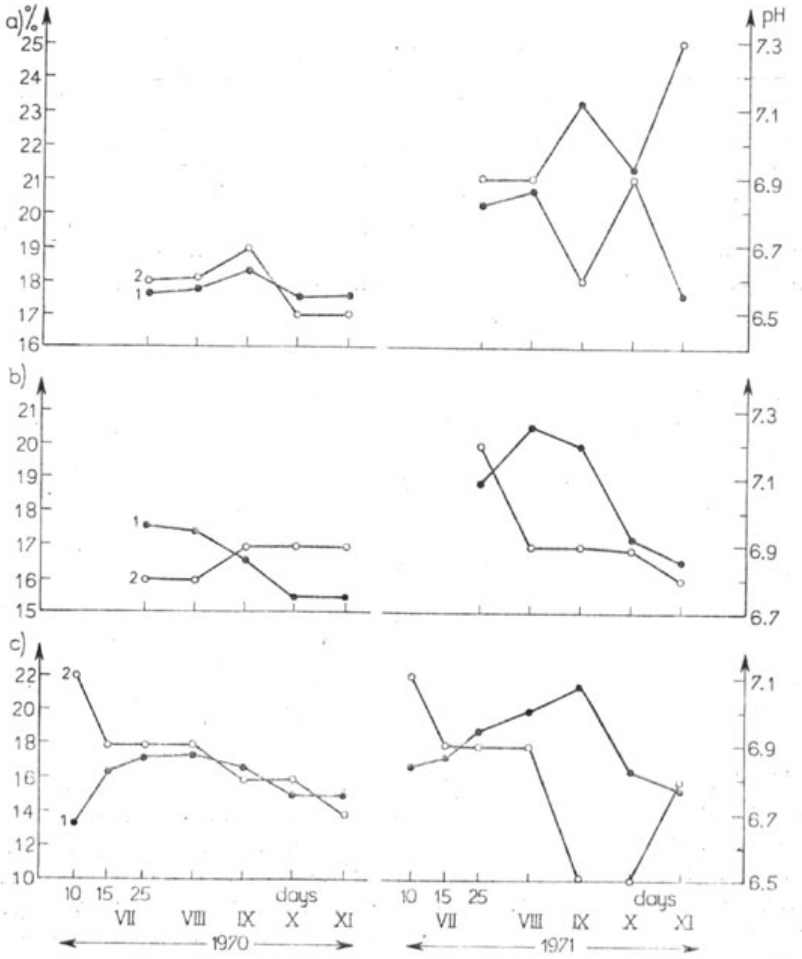

Fig. 2. Moisture content and pH of nonrhizosphere regions of *Pennisetum typhoides*
a — distal region, *b* — middle region, *c* — crown region; *1* — MC — moisture content (%) of soil, *2* — pH of soil

low. All the constituents increased with the ageing of the root. Cellulose in RC and RM regions increased from August to November. The trend of cellulose content was somewhat similar in the RD region to that one obtained in RD et RM. The highest amount, however, was obtained in October (fruiting). The highest and lowest amounts of cellulose were obtained in RC and RD regions respectively. Hemicellulose and lignin, on the other hand, exhibited an increasing tendency

in all the three regions of roots from seedling to senescent. Their highest and lowest values were obtained in crown and distal regions respectively (Fig. 3).

Effect of root exudates on certain rhizosphere fungi

The overall picture of this experiment revealed that root exudates

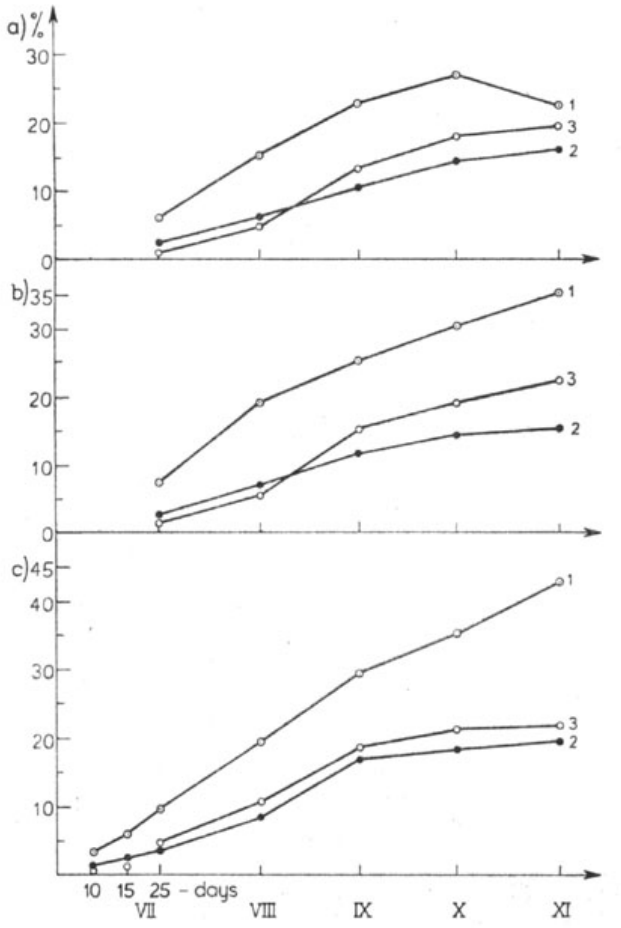

Fig. 3. Celluloses, hemicelluloses and lignins of crown, middle and distal regions of root of *Pennisetum typhoides* (expressed on initial dry weight)
a — distal region, *b* — middle region, *c* — crown region; *1* — cellulose, *2* — hemocellulose, *3* — lignin

exerted a selective behaviour against rhizosphere test fungi. The majority of the test fungi were stimulated by root exudates, however, a few species like *Aspergillus sydowi* and *A. tamarii* were adversely affected in the exudates of earlier stages. Their germination was slightly enhanced later on by exudates of ageing plants. The effect of exudates from different stages was not uniform, either. In most of the cases a pronounced favourable effect was noticed in August (preflowering) (Fig. 4).

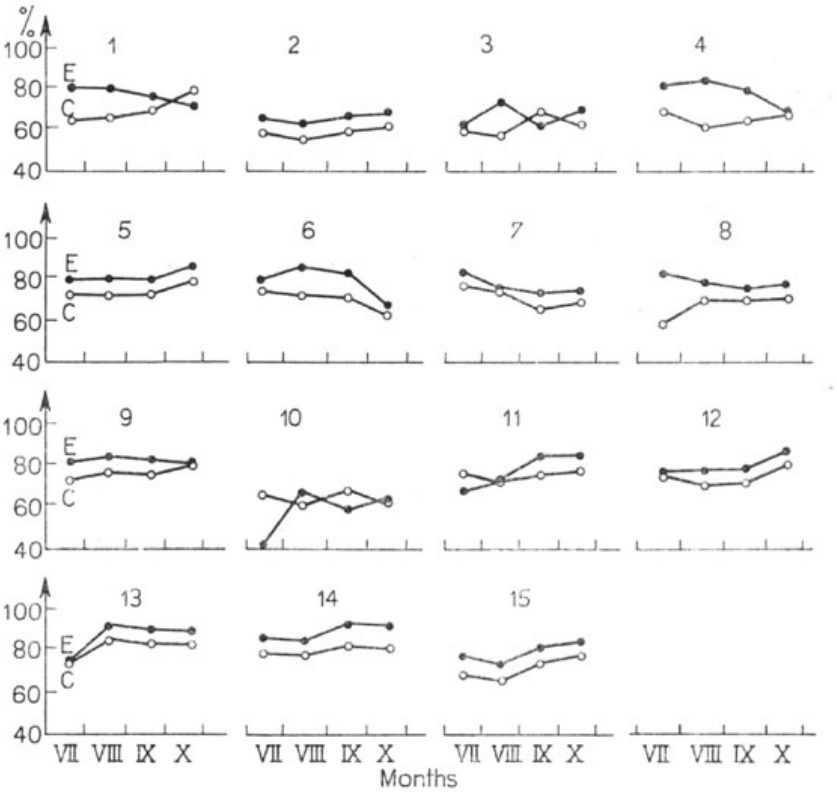

Fig. 4. Germination (%) of certain rhizosphere fungal spores in the root exudates of *Pennisetum typhoides* in July, August, September, October 1971

1. *Rhizopus nigricans*, 2. *Mucor hiemalis*, 3. *Cunninghamella bertholletiae*, 4. *Trichoderma viride*, 5. *Aspergillus flavus*, 6. *A. fumigatus*, 7. *A. terreus*, 8. *A. niger*, 9. *A. aculeatus*, 10. *A. tamarii*, 11. *A. sydowi*, 12. *Penicillium chrysogenum*, 13. *Curvularia lunata*, 14. *Alternaria tenuis*, 15. *Fusarium nivale*

E — exudate, *C* — control

DISCUSSION

The soil is a unique assemblage of various micro- and macroorganisms like fungi, bacteria, actinomycetes, algae, protozoans, nematodes and other living and non-living entities both in active and passive phases. The competition for space and nutrition amongst the individuals of similar species is more intense than between closely related species (Garret 1963).

A greater number of fungi was recorded from the nonrhizosphere of all the three zones than from the corresponding rhizosphere regions and the number of fungi in cortical region was bigger than in the steler or the rhizoplane (Tables 1-3). The mycopopulation in the rhizosphere region (both in quality and quantity) was lowest at seedling stage. It increased gradually resulting in the highest population at flowering and fruiting stages when plants were at full maturity and decreased at the senescent stage (Fig. 1). The maximum amount of sugars and amino acids in the root exudates and extracts (Tables 6, 7) at this stage accounted for the higher population during this period. The stimulating effect of root exudates upon the majority of rhizosphere fungin further was confirmed by spore germination studies (Fig. 4). A few forms were not stimulated in the earlier stages, but were stimulated by the root--exudates of ageing plants. The release of certain amino acids like leucine, methionine and valine, and some sugars like trehalose at the latter stages possibly stimulated the spore germination of few species. The maximum fungal population at the time of maximum growth of the plants has widely been reported (Rovira 1956a, 1956b; Tandon, Bilgrami 1957; Jackson 1960; Waid 1960; Vancura 1964; Bhat 1966; Srivastava 1969; Kanaujia 1973). Many amino acids and sugars present in root exudates and extracts exerted a selective effect upon various microfungi and thus accounted for better growth of many of them in various root regions.

The selective action of various amino ucids and sugars of different fungi by breaking the dormancy of spores has been reported by Jackson (1960). Amino acids in mixture have a more favourable influence on the growth of fungi than individual amino acids (Tandon, Bilgrami 1957).

From preflowering to senescence, the population was always higher in the distal area followed by the crown and middle zones (Fig. 1). In the distal zone roots being younger exuded more amino acids and sugars which in turn supported the highest fungal population. In older tissues the cells become considerably thickened and impregnated with lignin which is more resistant to decomposition by microorganisms than cellulose is (Garrett 1963). The soft tissues of distal region possessed

comparatively less lignin (Fig. 3) which made them more susceptible to fungi and thus they supported a richer mycoflora than the other two regions.

A. higher fungal population in the upper horizon of nonrhizosphere has been observed in the present study. The greater amount of organic matter in this region probably accounted for such behaviour (Saksena 1955; Thornton 1956; Dwivedi 1959; Srivastava 1969; Mishra, Kanaujia 1973; Kanaujia 1973).

The quality and quantity of fungi (number of species and average number of colonies per plate) was generally higher in the cortical region than in the steller one. During the seedling stage, the number of fungi associated with the two regions of rhizoplane was low due to the intact epidermis of the roots. Further a layer o thin mucilage-like substance around the newly formed adventitions roots as observed in the present plant might be an important barrier for many fungi. As the root ages, the epidermal and subepidermal tissues are acted upon by microorganisms. Fungi, hitherto, unable to colonise the root surface now get the opportunity to participate in the invasion and decomposition of root tissues. The aggregation of a large number of fungi in the cortical region is associated with the above mentioned fact. The cortical region being largely composed of thinwalled cells harboured more fungi than the harder tissues of the steler region.

Twenty four fungal species were exclusively present in the nonrhizosphere and 6 and 1 of them were characteristic of rhizosphere and cortical portion of rhizoplane (Tab. 3). The restricted occurrence of a large number of fungi in nonrhizosphere region may possibly be due to the poor competitive saprophytic ability of these forms. Garrett (1963) stated that in the course of normal competition the fungi best suited to the environment could survive and appeared more frequently on the substrate. Most of the species restricted to the rhizosphere region were actually absent in the soil (Table 3). They were introduced in it with seeds (Table 1). Moreover, the possibility of release of certain substances like hydrocyanic acid (Rangaswami, Balasubramanian 1963) and certain phytotoxins (Borner, 1960) from the roots and the antagonistic effect among microorganisms (Lockwood 1964) and antibiosis (Brian 1960; Brian et al. 1948) possibly eliminated certain forms from the rhizosphere.

The pH moisture content of the soil exhibited a range which seems not to have any significant effect on soil mycoflora in any particular region (Fig. 2).

Out of 110 species isolated, 102, 84, 37 and 27 of them were present in NR, RS, RPC and RPS regions respectively (Table 2). Eighty four

species recorded from RS region included 78 forms from nonrhizosphere and 5 introduced with seeds (Tables 1, 3). One of them, *Cladoboaryum chlamydosporum,* however, was neither present in NR nor in seeds. All but one fungal species recorded from cortical region of the rhizoplane were derived from the rhizosphere. These rhizosphere forms could reach the cortical region relatively in higher number (36) while the arrivals in steler region of rhizoplane numbered only 27 (Table 3).

The first group of fungi consisting of 14 dominant species particularly the genus of *Aspergillus, Penicillium* and *Trichoderma,* possess high saprophytic ability and appeared as dominant in different regions at different stages. Few of them perhaps also liberated certain substances toxic to other organisms (Barum 1924; Brian 1960; Brian et al. 1948; Hessayon 1953; Woods 1960; Write 1954). The second group of fungicontains species like *Absidia spinosa, Mucor racemosus, Cunninghamella bertholletiae, Syncephalastrum racemosum, Cephalosporium acremonium, Monilia sitophila, Acremonium vitis, Aspergillus carneus, A. terreus* var. *terreus* and var. *africanus, Cladosporium chlamydosporum, Spicaria elegans, S. simplicissima, Botryotrichum pilluliferum, Penicillium* sp. 1, *Stachybotrys atra* and brown sterile colonies. These were generally isolated during seedling and preflowering. Most of them were confined to rhizosphere and rhizoplane regions due to the sufficient leakage of nutrients.

The third group of fungi included a few species of *Phycomycetes,* the majority of *Deuteromycetes* — particularly the species of *Paeciolomyces* and *Fusarium* — which appeared in both rhizosphere and rhizoplane regions without any regular sequence. The dynamic changes in microbial balance (Garrett 1963), nonspecific nature of organisms in relation to nutrients, certain antagonistic factors (Lockwood 1964) and possibly the release of substance(s) toxic to these fungi may be assumed from their discontinuous occurrence in different root regions.

Occurrence of large number of *Phycomycetes* at seedling and preflowering was observed. Primarily these are called sugar fungi. Their number was comparatively reduced at flowering and later which may be due to the changed nature of root exudates antagonism among large numbers of fungi (Garrett 1963; Kanaujia 1973), by products of simultaneous decomposition of older tissues (Patrick, Toussoun 1965) and by decomposers themselves (Carter 1958). Simultaneous appearance of a large number of *Deuteromycetes* at seedling and their persistance througout may be due to their wide range of nutritional acceptance (Cochrane 1958; Siue, Reese 1973; Kanaujia 1973); comparatively large number of fungi appeared at senescence in all the root regions. Presumably, the decrease in nutri-

tional level led to the sterility of certain forms (Nicot 1960; Gadgil 1965).

Few species like *Cladosporia* appeared frequently with dominance during fruiting and senescence. The low temperature prevailing during October and November stimulated their growth and resulted in the appearance of such species.

Mishra and Srivastava (1970), Mishra and Kanaujia (1972, 1973a, b, 1974, Srivastava 1969), Srivastava and Mishra (1971a, b) and Kanaujia (1974) have also reported the appearance of Cladosporia dominantly associated with soil root, aerial surfaces of plants and air during the winter months.

SUMMARY

The present paper deals with the succession of fungi on the root regions of living *Pennisetum typhoides* (Burm f.) Stapf. et Hubb. from July to November of 1970 and 1971. A clear succession of fungi from nonrhizosphere → rhizosphere → → cortical portion of rhizoplane → steler portion of rhizoplane has been observed. The fungal population and number of species in the crown, middle and distal regions of roots in above mentioned four regions also exhibited a definite pattern. The presence and amounts of amino acids and sugars in root exudates (July to October) and root extracts (July to November) have been studied. The amount of cellulose, hemocellulose and lignin components, the pH and moisture content of nonrhizosphere soils from corresponding vertical zones have been determined and effect of root exudates of different age on 15 rhizosphere fungi has been studied.

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