ACTA MYCOLOGICA
VOL. XVIII (1): 45 - 60
1982

Studies on certain aspects of root surface fungi III. Effect of harvesting *

R. S. KANAUJIA **

Department of Botany, University of Gorakhpur, Gorakhpur (U.P.) India

Kanaujia R. S.: *Studies on certain aspects of root surface fungi. III. Effect of harvesting*, Acta Mycol. 18(1): 45-60, 1982.

The effect of harvesting of above ground part on the succession of fungi on crown, middle and distal regions of decomposing roots of *Pennisetum typhoides* (Burm f.) Stapf et Hubb. was investigated for a period of six months (January to June, 1971). The number of fungal species was generally lower in harvested plants than in standing plants whereas the fungal population exhibited the reverse trend. The amino acids, sugars, cellulose, hemicellulose and lignin components of the roots in different vertical regions were assessed and a correlation was established between the above factors and fungal succession in the two sets of plants. It was also noticed that roots in all the depths decomposed earlier than the set where aerial parts were left intact and no phytotoxins were detected in the harvested set against standing one where vanilic acid and 3-4 dihydroxy benzoic acids were chromatogrammed during March and April. The pH and moisture content exhibited a poor correlation with the fungal succession. *Deuteromycetes* along with few *Phycomycetes* in the beginning, *Deuteromycetes* with few *Ascomycetes* in the second phase and *Deuteromycetes* along with *Mycelia sterilia* in the third phase were isolated.

INTRODUCTION

Succession of fungi underground parts of the standing plants in relation to environmental conditions, physico-chemical nature of the soil, nutritional status of substrate, foliar application of certain substances

* A part of the Ph. D. Thesis, approved by University of Gorakhpur, India

** Present Address: Department of Botany, K. S. Saket Post-Graduate College (Awadh University), Faizabad (U.P.), India.

and competition among the microflora has extensively been studied (Agnihotri 1964; Das 1963; Dickinson, Pugh 1965; Kanaujia 1973; Mishra, Kanaujia 1973, 1974; Parkison et al. 1963; Srivastava 1969; Waid 1957). Variations in the quality and quantity of fungi, differences in the amino acids and sugars, hemicellulose, cellulose and lignin components of the roots of the harvested plants at different decomposition stages are poorly understood (Kanaujia 1973). This aspect has been studied in the present paper and an effort has been made to correlate the occurrence of fungi on the roots of harvested *Pennisetum typhoides* (Burm f.) Stapf et Hubb. at different decomposition stages and the nutrients released during the period.

MATERIALS AND METHODS

Pennisetum typhoides raised in the experimental plot situated on the campus of University of Gorakhpur (India) was selected in the present study. On December 25, 1970, the above ground parts of the plants of half of the plot were cut 5 cm above the soil surface and removed whereas the remaining half of the plot was left with intact above ground plants. The first sampling of the root (standing and harvested crop sets) from crown (RC), middle (RM) and distal (RD) regions and soils from respective nonrhizosphere regions were separately completed on January 25, 1971. Subsequent samplings were done on the 25th of each month till the roots were completely decomposed. The mycoflora associated with the nonrhizosphere soil (NR), rhizosphere (RS), cortical (RPC) and steler (RPS) portions of rhizoplane were assessed separately by methods described by Kanaujia (1973). The amino acids and sugars present in the roots of crown, middle and distal regions were separately investigated after preparing the root extracts by unidirectional paper chromatography (Smith 1960 a, b). The amounts of free amino acids and total sugars were determined colorimetrically (Peach, Tracey 1955). The cellulose, hemicellulose and lignin components of root at different stages was estimated and was expressed on the basis of initial dry weight of the root (Wise et al. 1945). The root-washings from crown, middle and distal regions were separately collected from standing and harvested sets on the day the sampling of roots for mycoflora was carried out. The root-washing was used for the detection of phytotoxins (Smith 1960c; Hathway 1960). The moisture content and pH of the nonrhizosphere soil was determined by using the methods suggested by Piper (1966).

RESULTS

Sixty-seven and 57 fungal species were isolated from all the three regions of root and corresponding nonrhizosphere soils of standing and harvested sets respectively. In the standing set, 3 spp. each of *Rhizopus* and *Mucor* and 4 of the other *Phycomycetes;* 2 spp. of *Chaetomium* and 2 of remaining *Ascomycetes;* 14 spp. *Aspergillus,* 6 of *Penicillium,* 3 each of *Cladosporium* and *Curvularia,* 5 of *Fusarium* and 15 of other *Deuteromycetes;* 1 species of *Mycelia sterilia* and 6 spp. of sterile colonies comprised the total mycoflora (Table 1). In the harvested set, 3, 1 and 2 spp. of *Rhizopus, Mucor* and other *Phycomycetes* respectively; 3 spp. of *Chaetomium;* 11, 6, 3, 3, 5 and 8 spp. of *Aspergillus, Penicillium, Cladosporium, Curvularia, Fusarium* and other *Deuteromycetes* respectively, and 1 and 5 spp. of *Rhizoctonia* and sterile colonies respectively

Table 1

Distribution of fungi of different taxonomic groups in nonrhizosphere /NR/, rhizosphere /RS/ and rhizoplane /RPC- cortical and RPS- steler/ regions of standing harvested plants of P. typhoides

Species	RC				RM				RD			
	NR	RS	RPC	RPS	NR	RS	RPC	RPS	NR	RS	RPC	RPS
PHYCOMYCETES												
Rhizopus	2/1	2/1	1/2	1/1	2/1	1/-	1/1	1/1	1/1	1/2	1/1	1/1
Mucor	2/2	1/2	1/2	1/1	2/2	2/2	1/2	1/1	3/4	1/2	1/2	1/1
Others	2/1	2/2	1/-	-/-	2/-	2/1	-/-	-/-	1/-	1/1	1/-	-/-
ASCOMYCETES												
Chaetomium	2/2	1/1	1/-	1/-	2/1	1/2	1/1	1/-	2/1	-/1	1/-	-/-
Others	1/-	1/-	-/-	-/-	1/-	-/-	-/-	-/-	2/-	-/-	-/-	-/-
DEUTEROMYCETES												
Aspergillus	9/9	9/6	6/3	6/3	9/9	8/6	8/1	6/5	10/8	9/7	8/2	4/3
Penicillium	5/2	4/4	2/1	1/-	4/2	4/2	1/2	2/2	5/2	4/4	2/2	2/2
Cladosporium	3/2	3/2	2/2	2/2	3/2	2/2	2/2	2/2	2/3	3/3	2/2	2/2
Culvularia	3/1	2/-	-/-	1/-	3/1	-/-	1/-	-/-	3/3	3/1	1/1	1/-
Fusarium	3/3	4/2	5/2	3/2	3/4	3/4	4/3	4/4	3/2	4/2	3/1	3/1
Others	10/5	8/1	4/1	3/2	8/3	6/1	4/3	3/1	10/5	8/3	4/-	4/1
MYCELIA STERILIA												
Rhizoctonia solani	1/-1	-/-	1/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	1/-	1/-
STERILE COLONIES	4/3	3/2	4/11	4/2	4/3	4/3	3/1	2/1	4/3	3/3	3/3	3/1
Total No. of spp.	47/32	40/23	30/14	25/12	42/26	34/23	26/19	22/15	46/32	37/29	28/14	22/12

- Indicates absence.

were cultured from different regions of roots and nonrhizosphere soils. The number of fungi was generally greater in the standing set as compared to the harvested one in all the horizontal and vertical regions. *Aspergilli* were in majority in both of the sets (Table 1).

Horizontal distribution of mycoflora

Data in Table 2 indicated that 58, 49, 33 and 30 fungal species were isolated from NR, RS, RPC and RPS regions of root, respectively, from the standing set and 46, 36, 24 and 20 fungi from above mentioned regions of the harvested set were recorded. 14, 5, 1 and 0 species were restricted to NR, RS, RPC and RPS regions, respectively, in the standing set and 15, 5, 1 and 2 species were restricted to the above four regions of the harvested set. Thus the patterns of succession of fungal species in NR→RS, RS→RPC and RPC→RPS regions was 58→44, 49→32, 33→30 and 46→31, 36→23, 24→18 in standing and harvested sets respectively. Thus, the four above-mentioned regions of root in the standing set supported more species than the corresponding regions of the harvested set.

The distribution of the number of fungal species in crown, middle and distal zones irrespective to horizontal root regions exhibited the following pattern. As compared to the harvested set, in the standing one *Phycomycetes* were present in the greatest numbers in RC and RM zones whereas the reverse was true for the RD zone. *Ascomycetes* were represented by similar numbers in RC and RM zones, while in the RD zone of the standing set, their number was higher than that in the harvested set. *Deuteromycetes* and *Mycelia sterilia* in all the zones were present in greater numbers in the standing set than in the harvested one and sterile colonies were exhibited in more numbers in RC and RM of the standing set and in equal numbers in RD zone of two sets. In both the sets the crown, followed by distal and middle regions harboured the highest number of fungal species (Table 2).

The trend of distribution of fungal species in different root regions on different sampling periods in the two sets varied. At the partial decomposition stage (January), the number of fungal species in all the 3 horizontal root regions and in nonrhizospere was either greater in the standing set than in the harvested one or it was nearly equal. At the semi-decomposed stage (February and March) in most of the regions of different depths the fungi were present in greater numbers in the harvested set than that in the standing set. At an advanced stage of decomposition (April-June) no regular pattern of fungal distribution was observed. The roots in the harvested set were completely decomposed

Table 2

Distribution of fungal species of different taxonomic groups in horizontal /NR, RS, RPC and RPS/ and vertical/RC, RM, RD/ regions of standing / harvested P. typhoides roots

Species	Horizontal regions				Vertical regions			Total
	NR	RS	RPC	RPS	RC	RM	RD	
PHYCOMYCETES								
Rhizophus	2/1	3/2	1/2	1/1	3/2	2/1	1/2	3/3
Mucor	3/1	2/3	1/3	1/1	2/3	2/3	3/4	3/4
Others	3/1	2/2	1/-	-/-	3/2	3/1	1/1	4/2
ASCOMYCETES								
Chaetomium	2/2	1/2	1/1	1/-	2/3	2/2	2/1	2/3
Others	2/-	1/-	-/-	-/-	1/-	1/-	2/-	2/-
DEUTEROMYCETES								
Aspergillus	11/12	11/7	8/1	7/6	11/9	10/10	11/9	14/14
Penicillium	6/3	4/5	3/2	2/-	6/5	5/3	5/5	6/6
Cladosporium	3/3	3/3	2/2	2/2	3/2	3/2	3/3	3/3
Curvularia	3/3	3/1	1/1	1/-	3/1	3/1	3/3	3/3
Fusarium	4/4	4/4	5/3	5/4	5/3	5/5	5/3	5/5
Others	14/7	11/5	4/3	4/2	12/6	9/6	11/6	15/8
MYCELIA STERILIA	1/1	-/-	1/-	1/-	1/1	-/-	1/-	1/1
Sterile colonies	4/5	4/4	5/3	5/2	6/5	6/3	4/4	6/5
Total	58/46	40/36	33/24	30/20	57/42	51/37	52/41	67/57

- Indicates the absence

before sampling in June and the data, therefore, could not be obtained for this month. In the standing set total decomposition was noticed in June after sampling. In the case of rhizoplane regions the average number of colonies per plate varied in different regions (Table 3).

The fungal population per g dry soil in the nonrhizosphere region of three different zones in the two sets exhibited a similar trend. It was every time lower than the population at the root surface. Being highest in the beginning it gradually decreased in the subsequent months till the end of the experiment. The population generally decreased from RC to RD regions. Population at the root surface in standing set during January to March was highest in the crown region and second highest in the distal region. A low population was noticed in all the three depths of this set during March and April (Fig. 1). There was a uniform

Table 3

Horizontal distribution /NR - nonrhizosphere, RS - rhizosphere, RPC - rhizoplane cortical and RPS rhizoplane steller region/ of number of fungal species in the crown, middle and distal zones of standing /S/ and harvested /H/ plants of P. typhoides

Root regions		January		February		March		April		May		June	
		S	H	S	H	S	H	S	H	S	H	S	H
Crown region	NR	15	10	9	11	16	17	17	17	13	10	8	
	RS	13	9	8	9	10	10	8	11	10	10	5	
	RPC	11/18	6/17	4/12	5/9	9/10	5/13	8/9	5/3	8/10	5/9	5/7	
	RPS	3/12	3/8	5/9	6/9	6/9	5/8	4/4	3/3	5/3	3/4	2/4	
Middle region	NR	10	12	8	9	12	16	10	10	17	10	10	
	RS	13	10	10	8	6	8	8	8	6	8	4	
	RPC	10/15	10/17	5/9	5/7	7/9	6/8	7/8	5/3	6/8	6/8	4/4	
	RPS	4/7	6/9	2/4	5/5	8/6	4/6	3/3	4/3	6/8	3/3	4/4	
Distal region	NR	17	17	5	15	22	19	7	15	9	9	11	
	RS	19	13	13	11	7	9	8	14	9	5	8	
	RPC	6/16	6/8	7/19	6/6	7/12	5/8	5/6	6/3	7/4	4/10	5/6	
	RPS	4/10	4/3	4/6	3/6	4/9	7/6	3/2	3/3	6/4	4/9	2/4	

Denominators in RPC and RPS indicate the average eds/plate in rhizoplane regions

lowering of population in the three vertical regions of the root from January to May when total decomposition of the roots was obtained in the harvested set. At root surface the fungal population in the standing set was comparatively higher in all the vertical regions than the corresponding regions of harvested set from January to March whereas in April and May the pattern was reversed. (Fig. 1). There existed no regular trend of distribution of number of fungal species in cortical and steler portions of rhizoplane in the two sets at different sampling periods (Table 3).

Amino acids in the root extracts of standing and harvested *P. typhoides*

Seventeen amino acids were chromatogrammed from the root extracts of standing and harvested plants. Thirteen and 15 amino acids (Table 4) were recorded from root extracts of 3 zones of standing and harvested sets, respectively. Cysteine and lysine recorded in the standing set were not found in the harvested one whereas aspartic acid, histidine, tryptophane and un unidentified acid (R_f 0.46) were present only in the root extract of the harvested set. The amount of several individual amino acids was generally higher in the harvested set than in the standing set whereas the amount of total free amino acids was every

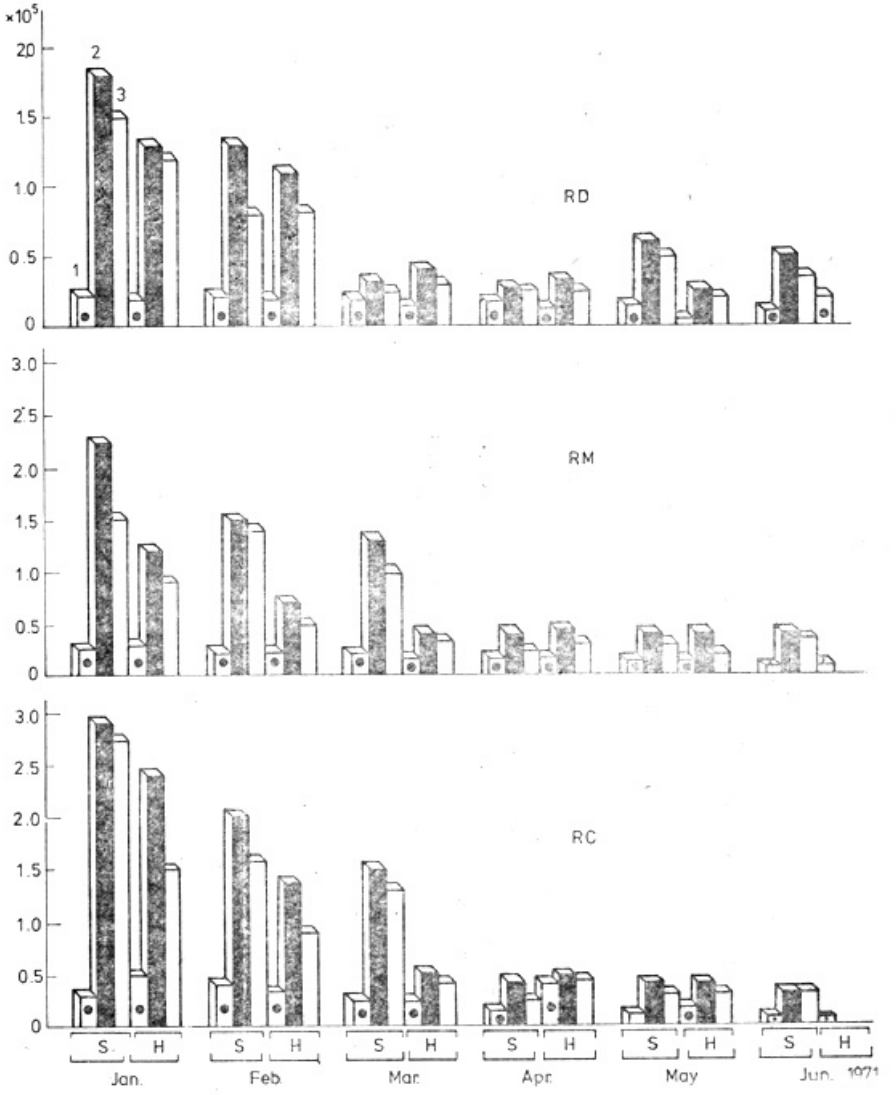

Fig. 1. Fungal population (×10⁵) of different regions of decomposing standing (S) and harvested (H) *P. typhoides* roots

RC — crown regions, *RM* — middle regions, RD distal regions; 1 — nonrhizosphere g/dry soil, 2 — rhizosphere g/dry soil; 3 — rhizosphere g/dry root

Table 4

Amino acids in the root extracts of crown /RC/, middle /RM/ and distal /RD/ regions of standing/harvested P. typhoides

Amino acids	January			February			March			April			May			June			Total No.
	RC	RM	RD	RC	RM	RD	RC	RM	RD	RC	RM	RD	RC	RM	RD	RC	RM	RD	
Alanine	50		-/100	45	-/75	88	30/50		50										
Arginine	-/170	-/125	30/140	75/150	70/	T/50	-/40												
Aspartic acid								-/T											
Cystein							60		25										
Glutamic acid	-/90			T/50	T/70	-/40			-/25										
Glycine	-/100	100	100	Nil		-/50	T		25										
Histidine				-/50	-/30	-/70	-/30												
Iso-leucine	90/70	50	90/69	50/50	35/50	60/25				25/25	T/40	40	25	T	T	T	T	T	
Leucine	87	70/T	110							T		-/35							
Methionine	80			50	T	25/65	40/40	T/70	T/40	25/40	T/25	T/30	25/T	T/T	T/T				
Proline	-/70	25/T		-/60															
Serine	-/50	40	60				T/30	T/40	T										
Theonine	35/85	-/100	45/110			-/45			-/30	25/25	30/25	40/25	25		25	25	T	T	
Tryptophane		-/80	-/90																
Valine	70/60	T/75	-/90	-/45	T/40	25/25	T/30	T/40	T/25	25/30	T/25	T/25	-/25	-/25	-/T				
Lysine		60	100	T	45	50	T	25	40										
Unknown /Rf 0.46/							-/T												
No. of amino acids	6/8	7/6	8/6	6/6	6/5	6/8	7/7	5/4	7/4	5/4	4/4	4/4	3/2	2/2	3/2	2	2	3	
Amount of amino acids /mg/2g fresh root/	412/695	355/450	565/590	220/404	150/265	248/370	130/245	25/170	140/140	100/120	30/115	80/115	75/80	T/35	25/35	25/-	T/-	T/-	

T - represents presence in traces

time greater in the standing set (except during May in extracts of all the 3 depths). The free amino acid content in both standing and harvested sets decreased gradually from predecomposition to total decomposition stages of the root. In May, in most of the cases, only trace amounts were present. (Table 4).

Sugars in the root extracts of standing and harvested plants

Ten and six (including one unidentified) sugars were detected from the root extracts of standing and harvested sets respectively. Glucose and xylose were commonly present in all the regions of the sets up to April and in RC and RD in the standing set during May and June. Arabinose, lactose, ribose and an unknown (R_g 21.1) sugar present in different regions of the standing set were not detected in the harvested one. The number of sugars in extracts of various regions of two sets differed marginally whereas the quality showed a wide difference (Table 5).

Table 5

Sugars in the root extract of crown /RC/, middle /RM/ and distal /RD/ regions of standing /S//harvested /H/ P. typhoides

Sugars		1		2		3		4		5		6		7		8		9		10		No. of sugars		Total sugars ug/2g fresh roots	
		S	H	S	H	S	H	S	H	S	H	S	H	S	H	S	H	S	H	S	H	S	H	S	H
January																									
	RC				+	+	+			+		+	+					+	+			4	4	650	670
	RM					+	+			+		+						+	+			4	2	450	425
	RD					+	+			+		+	+					+	+	+		5	3	732	720
February																									
	RC					+	+					+	+	+			+	+	+			4	4	350	385
	RM					+	+											+	+			2	2	225	275
	RD			+	+	+	+					+	+			+		+	+			5	4	600	625
March																									
	RC					+	+	+	+					+		+	+	+	+			5	4	320	350
	RM					+	+							+				+	+			3	2	125	130
	RD			+		+	+		+							+		+	+			4	3	340	360
April																									
	RC					+	+					+	+				+	+	+			3	4	210	225
	RM					+	+					+						+	+			3	2	100	150
	RD	+				+	+					+	+					+	+			4	3	300	200
May																									
	RC					+												+				2	-	180	-
	RM					+												+				1	-	45	-
	RD					+												+				2	-	270	-
June																									
	RC					+												+				2	-	56	-
	RM					+																1	-	T	-
	RD					+												+				2	-	75	-

+ present. 1 - arabinose, 2 - deoxyribose, 3 - glucose, 4 - lactose, 6 - rhamnose, 7 - ribose, 8 - sucrose, 9 - xylose, 10 - unidentified sugar

Moisture content and pH of grown, middle and distal regions of standing and harvested plot

The moisture content and pH of the soils of two sets differed to a little extent. pH in standing and harvested sets ranged in between 6.9 to 7.5 and 6.8 to 7.3 respectively. In RC and RM zones of the standing

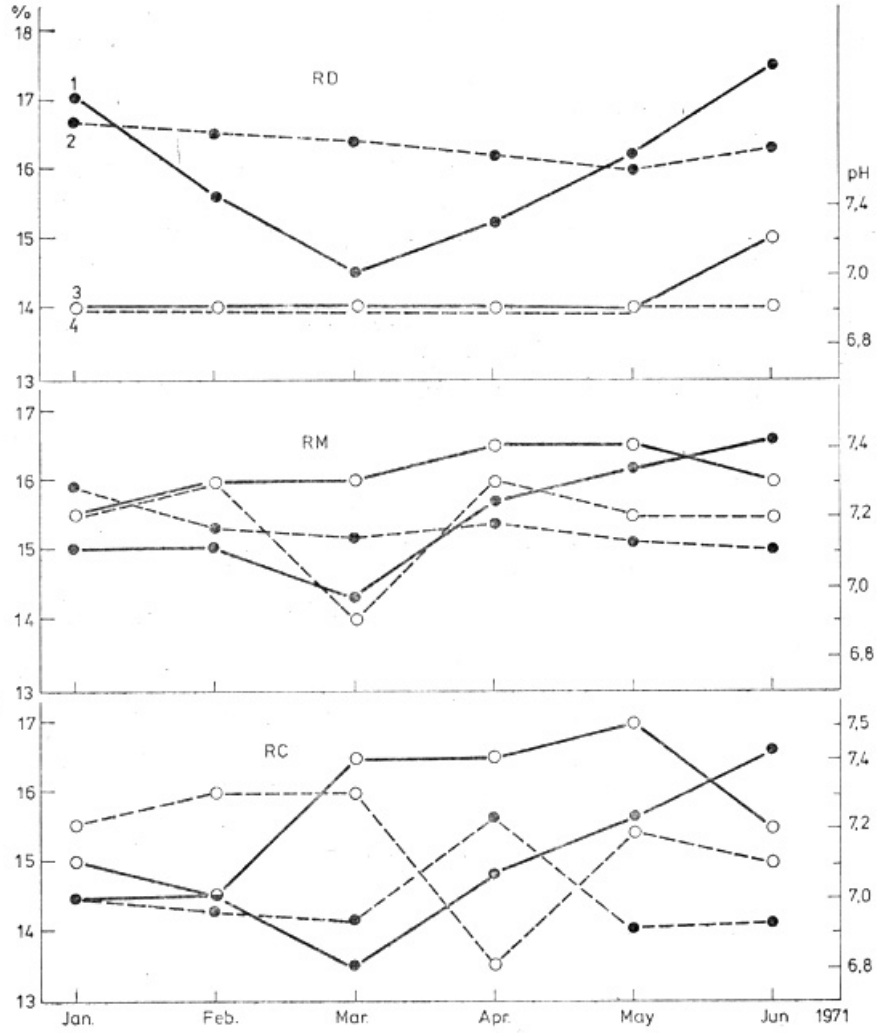

Fig. 2. Moisture content (1-2) and ph (3-4; an average of 3 replicates) of non-rhizosphere soil of different regions of *P. typhoides* plot
Standing (1, 3) and harvested (2, 4) plots soil; *RC*, *RM*, *RD* — see Fig. 1

set it always was slightly alkaline and in the RD region it was constant at 6.9. In the harvested set it was the same in the RD region in all the months but in RC and RM it showed a narrow variation (Fig. 2).

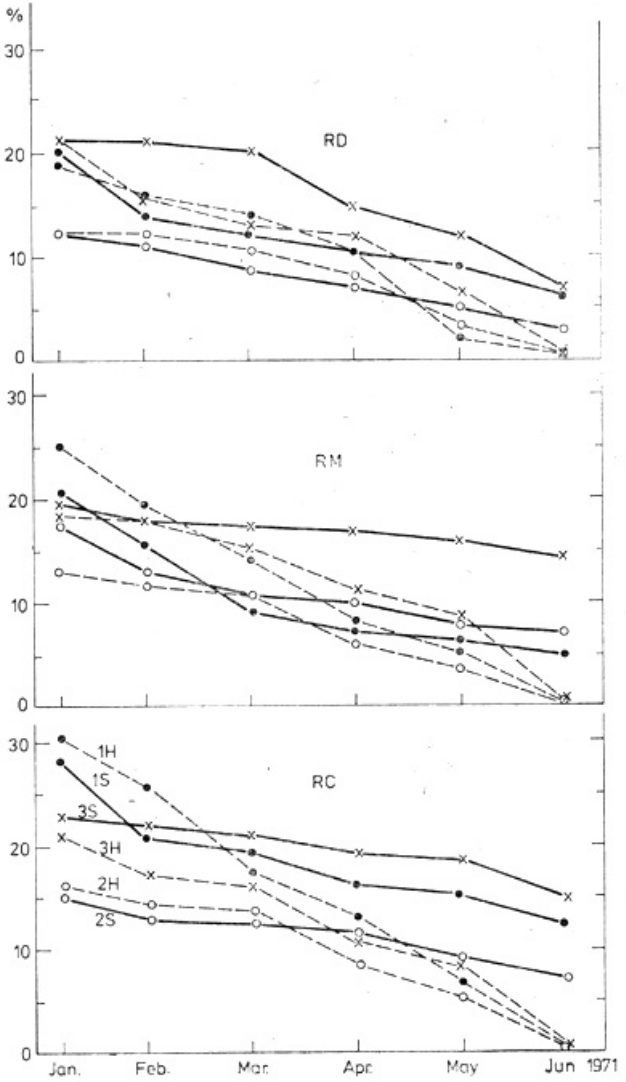

Fig. 3. Cellulose, hemicellulose and lignin (expressed as percentage of initial dry weight of roots) of different region roots of standing (S) and harvested (H). *P. typhoides*

1 — celulose, *2* — hemicelulose, *3* — lignin; *RD, RM, RC* — see Fig. 1

Celluloses, hemicelluloses and lignins of roots of standing and harvested *P. typhoides*

The amount of the three above components of the roots in the two sets was highest in January. In the subsequent months it decreased gradually. No considerable variation in the two sets of celluloses, hemicelluloses and lignins was noted except the decomposition of hemicelluloses and lignins in the standing set during March and April was greater than in harvested set (Fig. 3).

Detection of phytotoxins in the root-washings

It was noticed that phytotoxins were only detected whe aerial parts of the plants were left intact. Two phytotoxins, viz., vanillic acid and 3-4 dihydroxy benzoic acid were produced by decomposing roots during March and April. The former was liberated by roots of RM and RD regions in both the above mentioned months whereas the latter was only liberated by RM and RD regions during March and April respectively (Fig. 4).

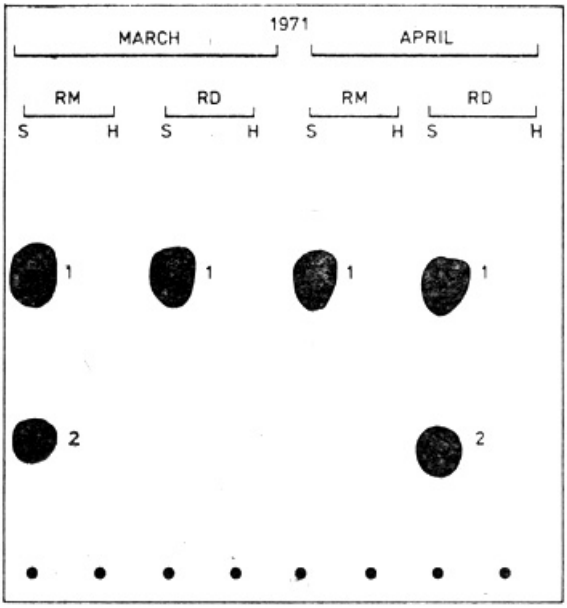

Fig. 4. Production of phytotoxins (*1* — vanilic acid and *2* — 3-4 dihydroxy benzoic acid) by decomposing roots of middle and distal regions of standing (S) and harvested sets (H)
RM, RD — see Fig. 1

DISCUSSION

Number of fungal species isolated from nonrhizosphere of the two sets was greater than in the corresponding rhizosphere regions whereas the fungi per g dry soil were every time higher at the root surface of two sets than in their respective NR regions (Tables 1-3; Fig. 1).

Soil is an unique assemblage of various micro- and macroliving and non-living entities and provides a suitable habitat for the survival of large number of fungi. Fungi requiring a wide range of nutrition thrive better in soil than in any other natural environment (Garrett 1963) and the presence of large number of fungi in soil is expected.

Various types of nutrients are regularly leached from the root surface in the living condition and sloughed-off materials, decomposition products and the root components themselves in dead stage of root. The competition for nutrition and space, the selective behaviour of nutrients, the soil fungistasis, the antagonism among microbes and some phyto- and mycotoxins liberated by substrate and fungi respectively (Garrett 1951, 1963; Hudson, Webster 1958; Waid 1957; Carter 1958; Das 1963; Saito 1960; Loockwood 1964; Park 1967 a, b; Kanaujia 1974; Siu 1951; Brian 1960; Brian et al. 1948) cause the arrivals from NR→RS regions, of fungal isolates in considerably lower numbers and this caused the lower number of fungi in RS than NR in both standing and harvested sets.

The greater number of fungal species in NR as compared to RS has been noted by many workers (Rovira 1956, 1959; Kanaujia 1973; Mishra, Kanaujia 1972, 1973, 1974; Rao 1962; Katznelson 1946; Srivastava 1969). Generally the number of fungal species was marginally higher in the standing crop than in the harvested one whereas the fungal population per g dry soil was every time higher in all the 3 vertical regions of harvested set as compared to the standing set. The higher nutritional status of harvested regions (Tables 4, 5) may be ascribed to the higher fungal population in this region. The advanced degradation of the roots in the harvested set possibly raised the nutritional status of the harvested region which ultimately supported the higher fungal population. In March and April, the fungal population in the standing set was considerably decreased as compared set (Fig. 1). The production of phytotoxic substances by decomposing roots in the standing set (Fig. 4) reduced the fungal population in the region which consequently caused higher fungal population in the harvested region as compared to the standing one.

The production of phytotoxins by decomposing plant residues in soil and their effect on soil fungi and plants has been reported by several

workers (Bonner 1950, 1960; Loehwing 1937; Cochrane 1948; Becker et al. 1950; Kanaujia 1973, 1978; Patrick 1971; Patrick, Tonssoun 1965; Tonssoun, Patrick 1962; Snyder et al. 1959).

The physico-chemical characters of the soil in the two sets differed to a slight extent (Fig. 2). They also do not seem to play a significant role in the succession of fungi in this study.

The fungal population was generally highest and lowest in the crown region during January to March respectively of both the years in the two sets. Most of the roots in crown at this stage being quite old provide sloughed off materials for microbial colonization which accounted for a higher fungal population. During later months sufficient degradation of roots in the distal region due to their soft nature as compared to the roots of the other two regions occurred (Fig. 4) which consequently increased the fungal population in this part of the root.

In the course of normal fungal colonization on a virgin substrate the fungi having greater affinity for sugars appear first. These constitute most of the species of *Phycomycetes*. Sugar fungi are followed by those capable of utilizing hemicelluloses, celluloses, *Ascomycetes* and several *Deuteromycetes* fall in this category and in the last the substrate which is chiefly composed of lignin, suberin like complex substances is colonized by *Basidiomycetes* (Garrett 1951, 1963). In the present study *Phycomycetes* along with variety of *Deuteromycetes* appeared in the first phase. Then came the majority of *Deuteromycetes* and few species of *Ascomycetes*. The last phase constituted the species of *Deuteromycetes* and some *Mycelia sterilia*. The simultaneous appearance of *Deuteromycetes* along with *Phycomycetes* in the beginning, their dominance in the second and third phased may possibly be due to the wide range of nutritional requirement of these fungi (Cochrane 1958).

I am deeply thankful to Dr R. R. Mishra, Reader, Dep. of Botany, N.-East. Hill. Univ., Shillong (Meghalaya) for his valuable guidance and encouragement; to the Head, Dep. of Botany, Univ. of Gorakhpur, for laboratory and library facilities and to my brother, Dr. R. V. Verma for financial assistance.

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