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Observations on soil fungistasis. V. Fungistasis in relation to rhizosphere effect*

R.S. KANAUJIA**

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

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This paper presents the results of study the effect of rhizosphere of *Pennisetum typhoides* on the soil fungistasis. The fungistasis of three different regions of the root has been investigated in relation to rhizosphere effect.

INTRODUCTION

The fungistasis property of crown, middle and distal regions of rhizosphere and nonrhizosphere soil of *Pennisetum typhoides* using 10 fungal isolates obtained from the rhizosphere has been investigated. It exhibited the regular pattern in both the regions. It was generally higher in seedling stage(July and August) decreased till flowering and fruiting and increased again in the month of June in nonrhizosphere. In rhizosphere also the pattern of fungistasis was somewhat similar to that obteined in nonrhizosphere except during the decomposition stage of roots (March and April) when it was very high. The fungistasis in crown, middle and distal regions also varied. It was greater in crown and was followed by middle and distal regions. Various determinants of rhizosphere phenomenon, viz., root exudates, root extracts, celluloze, hemicellulose and lignin components and their degradation products possibly play a considerable role in the phenomenon of fungistasis in rhizosphere. pH and moisture variations obtained in the present study seem to exert negligible effect on soil fungistasis in rhizosphere.

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

A great attention has been paid to fungistasis in last twenty years. The fungistasis property of different soil types for different fungal isolates has been studied. Fungistasis in natural and sterilized soils and in soils amended with various substances has extensively been studied (Dobbs, Hinson 1953; Griffin 1962; Hora, Baker 1972, 1972; Hsu, Lockwood 1973; Jackson 1957, 1958a, 1958b; Ko, Lockwood 1967; Lingappa, Lookwood 1961; Lookwood 1959, 1960, 1964; Lookwood, Lingappa 1963; Mishra, Kanaujia 1972a, 1972b, 1972c). Very little is known about the effect of rhizosphere of crop plants on the soil fungistasis (Jackson 1957; Kanaujia 1973). In the present communication the fungistasis of three different regions of the root has been studied in relation to rhizosphere effect.

EXPERIMENTAL

To study the effect of rhizosphere on the soil fungistasis, *Pennisetum typhoides* (Burm f.) Staph et Hubb., raised in the experimental plot situated in the campus of University of Gorakhpur was selected. The soils from three root regions viz., crown (RC), middle (RM) and distal (RD) were separately collected as described by Kanaujia (1973) during August 1970 to July 1971. Simultane ously the nonrhizosphere samples of soils were also collected on the same days. The first sampling was done on August 25, 1970 and subsequent samples were taken at an interval of one month till July, 1971 when the crop of the next season aged 25 days. The soils from three depths each for nonrhizosphere and rhizosphere were assayed separately for fungistasis as described by Jackson (1958a) *Rhizopuus nigricans, Mucor hiemalis, Aspergillus fumigatus, A. flavus, A. niger, A. aculeatus, Penicillium chrysogenum, Cladosporium herbarum, Curvularia lunata* and *Fusarium nivale* were used as test fungi.

The nonrhizosphere and rhizosphere soil samples were also assayed for fungal population which was calculated on the basis of one g dry soil. The root exudates (from August to October 1970 and July 1971) and root extracts from three root regions stated above were separately prepared (Kanaujia 1973). The root exudates and extracts were separately analysed for the free amino acids and total sugars with the help of colorimeter (Peach et Tracey 1955), and the quality of the two components was detected by paper chromatography (Smith 1960a, 1960b). The roots collected from crown, middle and distal regions were used to estimate the cellulose, hemicellulose and lignin by method suggested by Wise and coworkers (1945). The amount was expressed as percentage of initial dry weight of the root. The root exudates collected

were used to study their effect on Jackson (1958) rhizosphere fungi (*Rhizophus 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 tenuis* and *Fusarium nivale*). The germination of above stated test fungi was studied in cavity slides by hanging drop method. The moisture content and pH of the nonrhizosphere and rhizosphere soil samples from three regions were also determined by methods suggested by Piper (1966).

The washings of the roots from three regions were collected (Kanaujia 1973) every time and were assayed for the presence of phytotoxic substances by unidirectional paper chromatography using iso-propanol: ammonia: water (200:10:20) as solvent (Smith 1960) and ferric chloride ($FeCl_3$ — 2% in water containing 1 ml 2N HCl) as location reagent (Hatway 1960). The spots appearing on unknown chromatograms were compared with those of standard running side by side.

RESULTS

The fungistatic property of crown, middle and distal regions of nonrhizosphere and rhizosphere of *Pennisetum typhoides* varied for different test fungi. For *Rhizopus nigricans*, it was lesser in the beginning (August 1970) in all the three regions of nonrhizosphere. It increased gradually till November and December in RC and RD regions respectively. Thereafter a gradual decrease in fungistasis was observed up to the end of the experiment (July, 1971). The fungistasis was always lesser in the rhizosphere than that in the nonrhizosphere region except in the months of April (RC, RM, RD) and March (RM and RD) in the regions indicated in the brackets (Fig. 1a, b). For *Mucor hiemalis* and *Curvularia lunata*, the pattern of fungistasis was generally similar to that obtained for *Rhizopus nigricans* (Fig. 1a, b).

For *Aspergillus niger*, the trend of fungistasis in the three depths was nearly alike. In NR region in the month of August, it was low, increased gradually in the succeeding months resulting in the highest fungistasis in the month of October (RM) and November (RD) in the regions indicated in the brackets. In RD region, it was nearly the same up to December, increased subsequently till January and was constant later on till the end of the experiment. The fungistasis in the rhizosphere region was always lesser than in the NR except during March-May (RC) and February-May (RD) in the depths indicated in the brackets. Lowest fungistasis was reported in the end (July, 1971) in all the three depths of NR and RS regions for this organism (Fig. 1a).

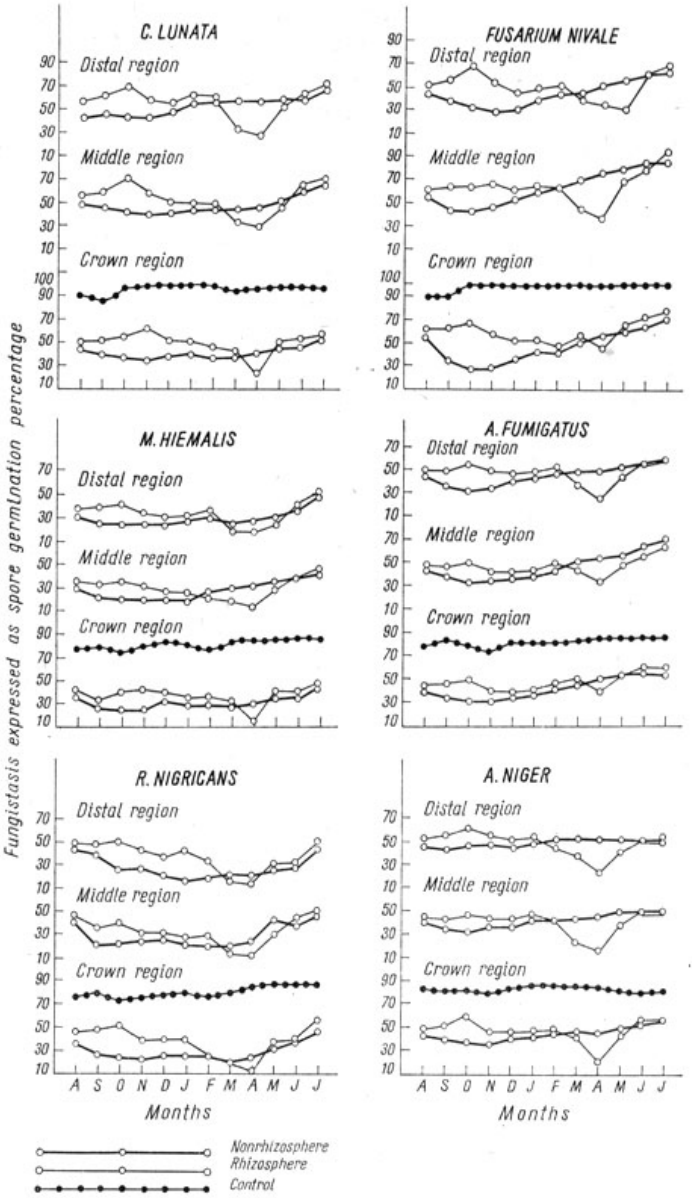

Fig. 1a

Fig. 1. a, b. Fungistasis of nonrhizosphere and rhizosphere soil samples of crown middle and distal regions of roots of *P. typhoides* and control

A-J — August-July

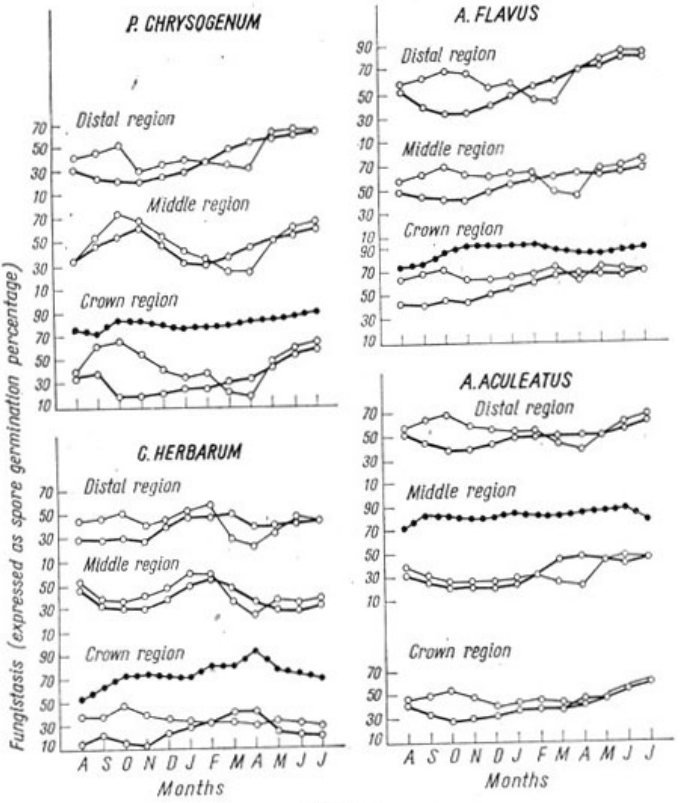

Fig. 1b

In the case of *Aspergillus fumigatus, A. aculeatus, A. flavus,* and *Fusarium nivale* the fungistasis property in nonrhizosphere region exhibited the similar pattern. It was lesser in the month of August, increased gradually in the succeeding months resulting in the highest values in the month of October, November, and December (RD region for *Aspergillus flavus*). Thereafter a gradual decrease was observed till July, 1971. In rhizosphere, the fungistasis for above test fungi differed in different months. It was the highest in the month of April for *A. fumigatus, A. flavus* (RC region) and in the same month for all the test fungi in the RD regions. Comparatively, higher fungistasis was also recorded in the RM and RD regions in the months of March for all the aforesaid fungi. The fungistasis in the RS region was generally lower than NR except during March and April for majority of test fungi. For *Aspergillus aculeatus,* however, it was always lesser in RS than in NR. For *Cladosporium herbarum* and *Penicillium chrysogenum* a regular pattern could not be

obtained both in RS and NR regions except that during greater part of the year the fungistasis was leser in RS than in NR. In NR region it being low in the beginning, increased gradually in the succeeding months resulting in the highest values in the month of November. The above pattern was, however, not maintained for *Cladosporium herbarum* in RC and *Pennisetum chrysogenum* in RM regions where fungistasis gradually

Table 1

Analysis of variance for fungistasis of various test rungi (a-j) in rhizosphere (RS) and nonrhizosphere (NR) regions of *P. typhoides*

Factors	SS	df	Variance	F		
				calculated	tabulated	
	a — *Rhizopus nigricans*					
RS	1149	11	104.4	4.03**	2.82	4.46
NR	2524	11	229.4	8.8 **	2.82	4.46
Control	285	11	25.9			
	b — *Mucor hiemalis*					
RS	919	11	83.5	0.94	2.82	4.46
NR	2248	11	204.3	2.3	2.82	4.46
Control	978	11	88.9			
	c — *Curvularia lunata*					
RS	2370	11	215.4	2.32	2.82	4.46
NR	2993	11	272.0	2.93*	2.82	4.46
Control	1018.66	11	92.6			
	d — *Fusarium nivale*					
RS	2466	11	224.2	33.9**	2.82	4.46
NR	3983	11	362.1	54.8**	2.82	4.46
Control	73	11	6.6			
	e — *Aspergillus niger*					
RS	2191	11	199.1	1.48	2.82	4.46
NR	3220	11	292.7	2.18	2.82	4.46
Control	1473	11	133.9			
	f — *Aspergillus fumigatus*					
RS	1459	11	132.6	1.54	2.82	4.46
NR	2257	11	205.1	2.18	2.82	4.46
Control	948	11	86.1			
	g — *Aspergillus aculeatus*					
RS	504	11	45.8	0.802	2.82	4.46
NR	1041	11	45.8	0.802	2.82	4.46
Control	628	11	57.0			
	h — *Aspergillus flavus*					
RS	1686	11	153.3	5.1**	2.82	4.46
NR	1424	11	129.4	4.3*	2.82	4.46
Control	329	11	29.9			
	i — *Penicillium chrysogenum*					
RS	625	11	56.8	0.41	2.82	4.46
NR	942	11	85.6	0.566	2.82	4.46
Control	1235	11	112.2			
	j — *Cladosporium herbarum*					
RS	852	11	77.4	0.64	2.82	4.46
NR	548	11	49.8	0.41	2.82	4.46
Control	1326	11	120.5			

* Values significant at 5% level.
** Values significant at 1% level.

decreased after September whereas in *P. chrysogenum* it decreased till November (Fig. 1a, b).

The statistical analysis of the data gave the following results:

(i) In the case of *Rhizopus nigricans, Fusarium nivale* and *Aspergillus flavus* the variation in the fungistasis caused due to samplig date differemt root regions were found to be significant (Table 1a, d, h).

(ii) In the case on *Curvularia lunata* variations caused due to sampling period were only significant (Table 1c).

(iii) In *Mucor hiemalis, Aspergillus niger, A. fumigatus, A. aculeatus, Pennisetum chrysogenum* and *Curvularia herbarum* the results were insignificant (Table 1b, e, f, g, i, j).

Fungal population of crown, middle and distal regions of nonrhizosphere and rhizosphere of *Pennisetum typhoides*

The variation in the fungal population (expressed on the basis of per g dry soil) exhibited a regular pattern. It was always lesser in the nonrhizosphere than in rhizosphere (Fig. 2). In NR region, the higher population was recorded during August. It generally decreased in the subsequent months till June. In July it was highest. The population in the RS region being low in the beginning (August, 1970) showed an increasing tendency till October when highest population was recorded in all the three depths. It decreased in November and December and increased again in January and thereafter it dropped off till June (Fig. 2). A definite trend of fungal population in crown, middle and distal regions was also obtained. It increased with increasing depth (crown to distal regions) in NR region. In RS, the population being highest in crown was closely followed by middle and distal regions during August, 1970 and July, 1971. During September to November the highest and the lowest population was recorded from distal and middle regions respectively. During December to April was the maximum again in crown, and thereafter it exceeded in distal region. Considerably low population was noticed during March (RM and RD) and April (RG, RM and RD) in the region indicated in the brackets (Fig. 2).

Amino acids and sugars in the root-exudataes and extracts

The amino acids and sugars exuded from the roots were lesser in August. They increased both in quality and quantity in succeeding months. The amount of amino acids and sugars (expressed as μg/2g

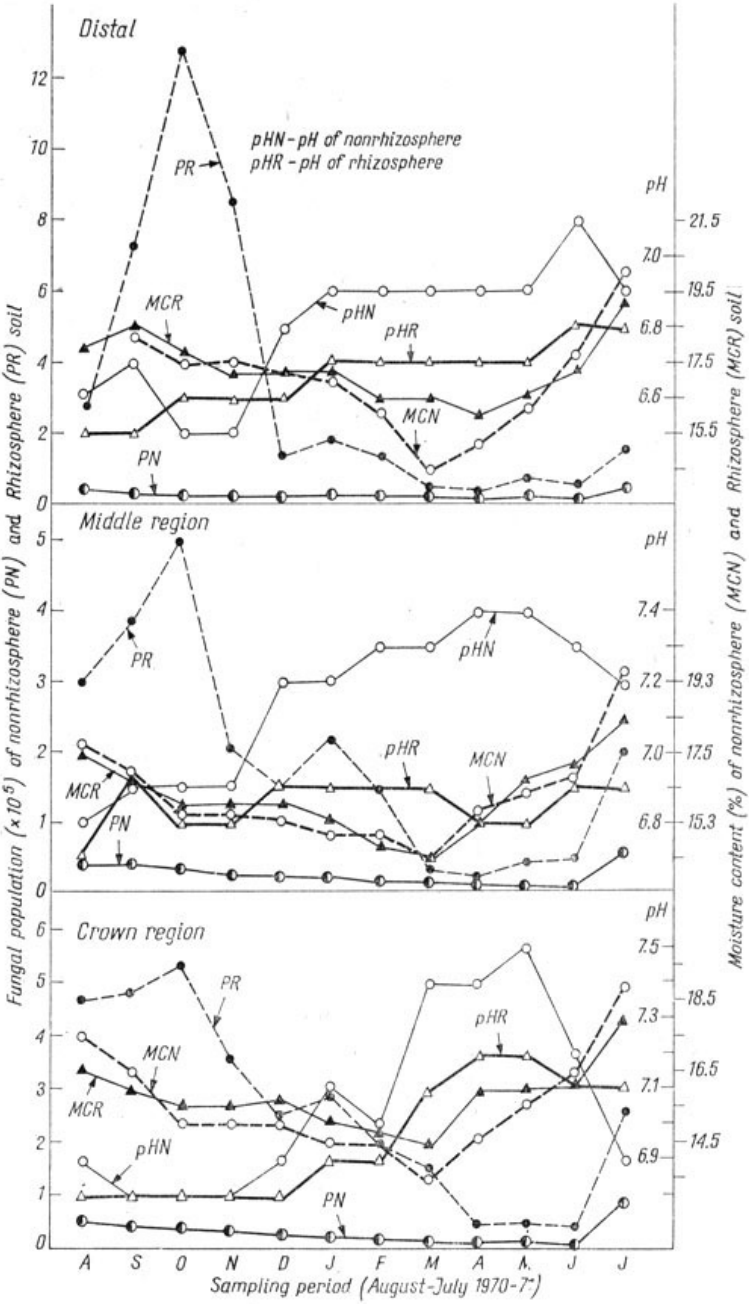

Fig. 2. Fungal population moisture content and pH of nonrhizosphere and rhizosphere soil samples of crown, middle and distal regions of *P. typhoides*

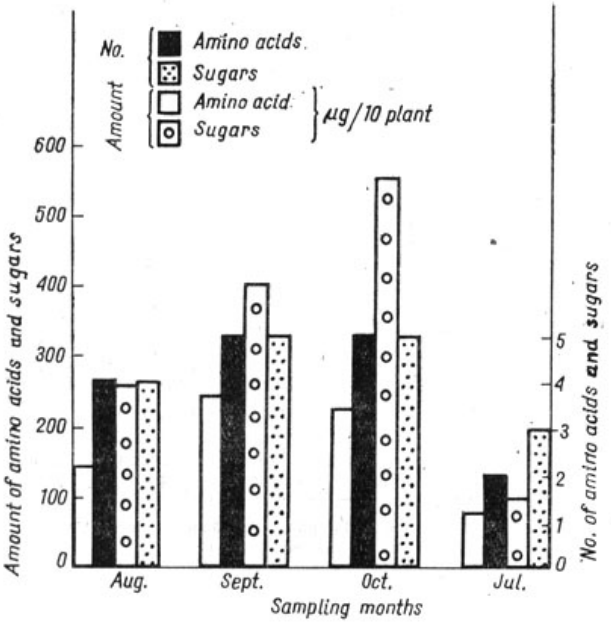

Fig. 3. Amino acids and sugar contents in the root exudates of *P. typhoides*

fresh root) were the highest in September and October respectively (Fig. 3). The amount of free amino acids was low in August. It increased with ageing of the rots till October when the plants were at fruiting stage. The number of amino acids was also highest at this stage. The amount of free amino acids decreased thereafter till June. In July 1971 (new years crop) it was found to be lesser than August, 1970, Except for May and June when the maximum and minimum amount of amino acids were obtained in RC and RM regions of root respectively, the quantity of amino acids was always highest in distal region which was followed by crown and middle ones (Table 2). The pattern found for the amount and quality of sugars in crown, middle and distal regions of root in different months was somewhat identical to that obtained for amino acids (Table 2).

Cellulose, hemicellulose and lignin components of roots

The three components of the root increased with the ageing of the roots from August to November and thereafter a gradual decrease was noticed till June. In July 1971, when the roots of the next crop were

Table 2

Amino acids and sugars in the root extracts from crown, middle and distal regions of *P. typhoides* in different months

Months		Crown region		Middle region		Distal region	
		amino acid	sugar	amino acid	sugar	amino acid	sugar
1970	August	8/933	4/1340	6/847	3/850	8/1030	5/1500
	September	8/1020	6/2000	8/1015	5/1100	8/1340	7/2300
	October	11/1920	5/1400	9/1295	5/750	12/2270	7/1900
	November	9/760	4/900	4/660	4/640	8/995	5/1500
	December	9/705	3/875	7/600	3/530	10/860	4/970
1971	January	6/412	5/650	7/353	4/450	8/565	5/732
	February	6/220	4/500	6/150	2/275	6/248	5/670
	March	7/130	5/320	5/25	3/125	7/140	4/440
	April	5/100	3/210	4/30	3/100	4/80	4/300
	May	3/75	2/180	2/Traces	1/45	3/25	2/270
	June	2/25	2/50	2/Traces	1/Traces	3/Traces	2/75
	July	5/420	4/780	4/375	3/540	6/560	5/680

Numerator — The member of amino acids and sugars. Denominator — The amount (Ekspressed as mg/2 g fresh root) of amino acids and sugars.

analysed for above three components, very low amount of these was obtained. The amounts of cellulose, hemicellulose and lignin generally being highest in crown were followed by those of middle and distal region. After January till June it was noted that the decomposition of cellulose and bemicellulose was wore rapid than of lignin (Table 3).

Effect of root-exudates on certain rhizosphere fungi

The germination of majority of test fungi used in this experiment was enhanced by root-exudates of different stages (July to October). The promotory effect was, however, not uniform. Few fungi like *Aspergillus sydowi* and *A. tamarii* adversely affected by exudates of early stages were slightly promoted by aged exudates (Fig. 4).

pH and moisture content of crown, middle and distal regions of nonrhizosphere and rhizosphere of *Pennisetum typhoides*

The pH and moisture content of the different depths of nonrhizosphere and rhizosphere regions varied to a little extent in different months (Fig. 2).

Table 3

Cellulose*, hemicellulose* and lignin* in the root of crown, middle and distal regions of *P. typhoides*

Sampling time		Crown region			Middle region			Distal region		
		cellulose	hemice-llulose	lignin	cellulose	hemice-llulose	lignin	cellulose	hemice-llulose	lignin
1970	August	19.6	8.5	10.0	18.2	7.2	6.2	15.7	6.5	5.9
	September	29.6	16.0	18.3	25.5	11.3	14.5	23.0	10.2	13.9
	October	35.0	17.5	20.5	29.7	14.3	18.9	27.6	14.2	18.0
	November	42.7	18.9	20.8	35.3	14.5	20.3	25.4	15.2	19.5
	December	26.9	21.1	25.0	28.7	17.5	21.0	20.5	15.7	21.5
1971	January	28.2	21.5	22.6	20.7	17.0	20.9	20.0	12.5	20.3
	February	20.5	13.2	20.2	15.5	13.2	18.5	14.1	11.5	20.0
	March	19.5	12.9	20.0	9.8	11.0	18.0	12.0	7.0	20.0
	April	16.3	12.0	19.6	7.3	10.3	17.5	8.5	6.2	15.0
	May	15.2	9.5	19.2	7.0	8.0	16.6	7.3	5.2	12.0
	June	12.0	8.0	17.5	6.9	5.3	16.0	6.5	3.1	10.0
	July	9.0	3.4	3.9	7.6	3.0	2.0	5.3	2.0	1.0

* Amount expressed as percentage of the initial dry weight of the root.

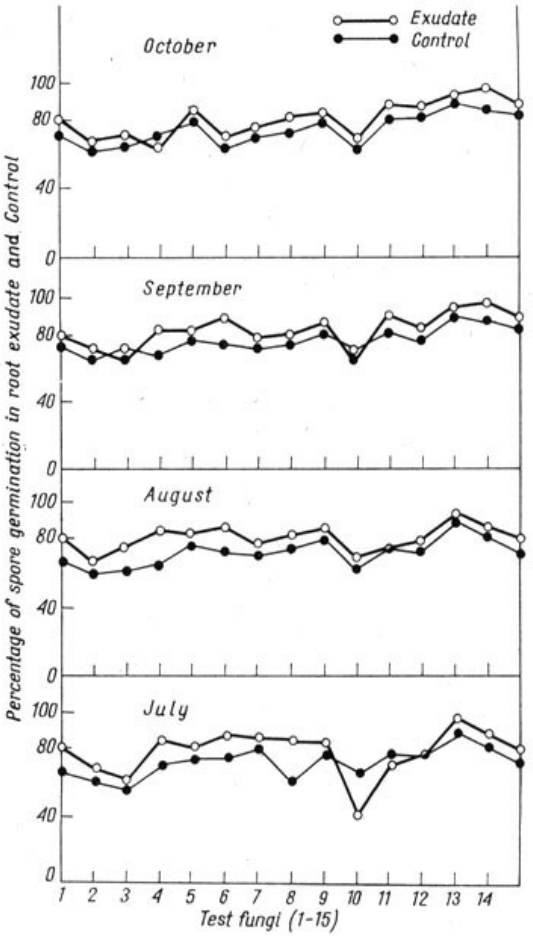

Fig. 4. Percentage germination of spores of rhizosphere fungi (1-15) in the root exudates and in control (Sterilized distilled water)

Fungi: *1 — Rhizopus nigricans, 2 — Mucor hiemalis, 3 — Cunninghamella bertholletiae, 4 — Trichoderma viride, 5 — Aspergillus fumigatus, 6 — A. flavus, 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*

Release of phytotoxic substances by the roots of crown, middle and distal regions

The root-washings from RC, RM and RD regions were tested every time for the presence of phytotoxic substances. It was observed that the washings collected during March (RC and RD) and April (RG, RM and

RD) possessed vanillic acid and 3-4 dihydroxy benzoic acid. The former from RM and RD in March and April, and latter from RM (March) and RC in April respectively were detected (Fig. 5). These were found to be toxic to many fungi and succeeding crop seeds (described elsewhere).

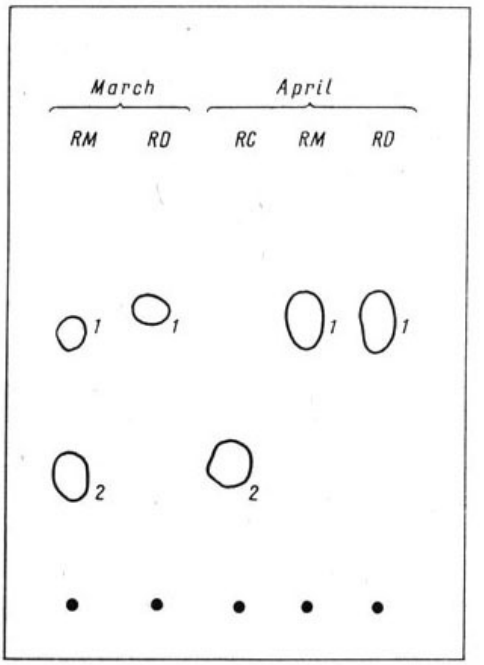

Fig. 5. Detection of phytotoxins by decomposing roots of crown (RC), middle (RM) and distal (RD) regions of *P. typhoides*
Phytotoxins: *1* — vanillic acid; *2* — 3-4 dihydroxy benzoic acid

DISCUSSION

Soil samples corresponding to crown, middle and distal regions of rhizosphere and nonrhizosphere root regions exhibited varying degree of fungistasis against 10 test fungi (Figs 1a, b). About 116 test fungal isolates were reported to be used in fungistasis by Lockwood (1964). Varying levels of fungistasis for different fungi has widely been reported by many workers (Chinn 1957; Dobbs 1953; Jackson 1958; Kanaujia 1973; Lingappa 1961; Lookwood 1960; 1964; Mishra, Kanaujia 1972a, 1972b; Park 1967).

In the present study, the crown region generally exhibited highest fungistasis and was followed by middle and distal regions (Fig. 1a, b). The crown region is rich in organic matter content resulting in higher

fungal population which is possibly responsible for increased fungistasis. Dwivedi and Dwivedi (1971) and Mishra and Kanaujia (1973c) have reported highest fungistasis in upper 6 and 2 cm layers respectively of soil. They suggested that the high organic matter content in this layer was directly responsible for high fungal population and thereby high fungistasis.

The fungistasis in present case was higher in August in nonrhizosphere soils of all the depths. It increased up to the month of November and after that it went on decreasing until July 1971 (Fig. 1a, b). In August higher fungistasis in NR is possibly due to the higher fungal population of soil (Fig. 2). A decreasing tendency in the succeeding months may by due to the decreasing fungal population in this region (Fig. 2). The biological origin of fungistasis has been emphasised by many workers. (Jackson 1957, 1958; Lingappa and Lookwood 1961; Mishra, Kanaujia 1972a, 1972b; Yoder, Lookwood 1973. Griffin (1962) studied the fungistasis of sterilized soils and noticed the complete absence of phenomenon in autoclaved soils. The direct effect of nutrition on fungistasis may also not be overlooked (Brian et al. 1948; Hsu, Lookwood 1973; Kanaujia 1973; Ko, Lookwood 1967; Lookwood 1960; Steiner and Lookwood 1969). All the nutritional substances generally contain soluble nitrogen, carbon or both. Deficiencies for these nutrients are known for the spores of many fungi (Cochrane 1960). Recently, Hessayon (1953), Hora, Baker (1970, 1972a, 1972b) and Romine, Hora (1973) emphaised the importance of water soluble volatile substances produced by soil microorganisms, on soil fungistasis. The substances inhibited the germination of spore of many fungi.

The fungistasis was generally lesser in rhizosphere soil than that of corresponding nonrhizosphere regions (Fig. 1a, b). In rhizosphere region sufficient naturients available due to the exudation and the degradation of cellulose, hemicellulose and lignin (Fig. 3 and Table 3) promoted the spore germination and this accounted for lesser fungistasis in this region. The promotive effect of root exudate on many fungi is further confirmed by spore germination of 15 rhizosphere fungi in the root exudate (Fig. 4). Kanaujia (1972) while working on succession of fungi on root regions of *Pennisetum typhoides* also reported the promotive effect of root washing on several rhizosphere fungi at different stages of the plant growth except at severe decomposition stage of the roots. This partly supports the nutritional theory advanced by Lookwood (1964).

Comparatively higher fungistasis was reported in the rhizosphere soil in the month of March and April (Fig. 1a, b) for nearly all the test fungi. The production of certain phytotoxins by decomposing roots of *P. typ-*

hoides (Fig. 5) accounted for increased soil fungistasis in these months. Earlier author Kanaujia (1973) had also studied the decomposition of sterlized P. *typhoides* roots in sterilized soils separately amended with certain rhizosphere fungal isolates in laboratory and in plot soil maintained ot different moisture levels and noticed the release of vanillic acid, 3-4 dihydroxy benzoic acid and Hydro-cinnamic acid which proved harmful to many rhizosphere fungi and succeeding crop seeds. The liberation of phytotoxins from decomposing plant residues and their antifungal property has also been reported by many workers (King et al. 1934; Patrick 1955; Patrick, Toussoun 1965; Toussoun et al. 1968; Welbank 1963) in field and laboratory conditions.

The moisture content and pH of rhizosphere and nonrhizosphere soils with narrow variations (Fig. 2) do not seem to play any direct role in the determination of fungistasis in the present study. Thus, the rhizosphere environment displays the positive correlation with soil fungistasis. The nutritional level in the rhizosphere in the form of exudates and decomposition products seem to be largely responsible for the phenomenton of soil fungistasis in the rhizosphere region. The phenomenon, however, seems to be a resultant of dynamic interactions of fungal spores and various other factors operating individually and/or in combination in the soil.

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Badania właściwości fungistatycznych gleby. V.

STRESZCZENIE

Badano właściwości fungistatyczne gleby przy różnych strefach korzeni *Pennisetum typhoides* przy użyciu 10 szczepów grzybów wyizolowanych z tej ryzosfery.