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INVESTIGATIONS INTO RHIZOSPHERE MYCOFLORA. XII. SEASONAL VARIATION IN THE MYCOFLORA OF CERTAIN GYMNOSPERNS

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Rhizosphere mycoflora of 7 gymnosperms, *viz.*, *Ginkgo biloba, Cycas revoluta, C. rumphii, Zamia* species, *Araucaria* species, *Pinus longifolia, Cupressus sampravirens* and nonrhizosphere region in relation to seasonal changes has been investigated. In all 71 fungal species, majority of them belonging to Deuteromycetes, were isolated. Aspergilli outnumbered throughout the course of present investigation. The fungal population in different gymnospermous plants differed. Higher number of amino acids was detected from *Araucaria* roots in quantity and quality both, in which the maximum fungi were also obtained. The spores of different fungi, *viz.*, *C. bertholletiæ, M. hiemalis, A. flavus, A. sydowi, A. ustus, P. chrysogenum, P. notatum, Curvularia lunata, C. tetramera, A. tenuis* and *F. nivale* exhibited different percentage-germination in the aqueous root extract of the different gymnosperms studied.

INTRODUCTION

Rhizosphere fungi of different plants have extensively been worked out in relation to their different ages. Most of the earlier studies have been confined to angiospermous plants particularly to the annuals where such investigations may conveniently be completed for the whole life cycle of the plant (Agnihotri, 1964 ; Mishra, 1964 ; Rangaswami and Vidyasekaran, 1963). In such plants handling of the complete root system is easier and the whole root system may be used for investigation. Studies regarding the rhizosphere fungi of gymnospermous plants are limited (Bowen, 1969 ; Harley and Waid, 1955 ; Hodges, 1962 ; Iverson and Katznelson, 1960 ; Meliszewska and Morean, 1960 ; Neal and Bolhen, 1941). This fact prompted the present investigation. The gymnospermous plants being generally long lived and the studies for the whole life cycle being difficult, the data for one year only has been obtained. The sampling of the roots for investigation has been done in three different seasons of the year (summer, rainy and winter) prevailing in this part of the country. Effort has also been made to study the effect of root extracts on the spore germination of certain frequently occuring rhizosphere fungi.

The results of the previous workers (Schroth and Hildebrand, 1956 ; Rovira, 1959, 1965) on this aspect are also not unanimous and both stimulatory (Davey and Papavizas, 1961 ; Schroth and Hildebrand, 1964 ; Tichelaar, 1961) and inhibitory (Buxton, 1957 ; Gupta, 1970 ; Timonin, 1941 ; Vrany *et al.*, 1962, 1965) properties of root extracts/exudates have been reported.

Materials and Methods

Seven gymnosperms, viz., *Ginkgo biloba* (GB), *Cycas revoluta* (CR_1), *C. rumphii* (CR_2), *Zamia* sp. (Z), *Araucaria* sp. (AR), *Pinus longifolia* (PL), *Cupressus sampravirens* (CS), planted in the Botanical Garden of University of Gorakhpur, Gorakhpur were selected for the present study. The sampling was completed during various seasons of the year 1970 and 1971. Nonrhizosphere soil (N) was also sampled from the vicinity of each of seven plants and was mixed together to get a single composite sample. The method of sampling and assessment of mycoflora was adopted after Mishra (1967). The fungal population was calculated on the per gm of dry weight of root and soil in rhizosphere and nonrhizosphere regions respectively. The amino acids were chromatographically detected from the extracts of root in each case. The method for amino acids detection was as described by Smith (1960). The moisture content of nonrhizosphere soil was determined by method summarized by Piper (1966), and *p*H was determined by electric *p*H meter. The spore germination of certain fungi, viz., *Cunninghamella bertholletiæ*, *Mucor hiemalis*, *Aspergillus flavus*, *A. sydowi*, *A. ustus*, *Penicillium chrysogenum*, *P. notatum*, *Curvularia lunata*, *C. tetramera*, *Alternaria tenuis* and *Fusarium nivale* in the aqueous extract (2 gm of fresh material in 10 ml of sterilized distilled water) of gymnosperms root in cavity slide by hanging drop method.

Results

Seventy-one fungul species were isolated from rhizosphere of 7 gymnosperms and nonrhizosphere region in different seasons. Phycomycetes was represented by 11 species, Ascomycetes by 7 species, Basidiomycetes by 1 species, Actinomycetes by 1 species, Deuteromycetes by 45 species and Mycelia Sterilia by 6 species. 32, 34, 29, 40, 42, 24, 22 and 52 fungal isolates were cultured from rhizosphere of GB, CR_1, CR_2, Z, AR, PL, CS and N regions respectively (Table 2), Aspergilli outnumbered throughout in this study in all the seasons. Highest and lowest number of fungal species was isolated from the rhizosphere of *Araucaria* and *Cupressus sampravirens* respectively. The number of species was low in *Pinus longifolia*, *Cycas rumphii* and *Ginkgo biloba*. In the present study 30 species of frequent occurrence and 41 infrequently distributed were recorded (Tables 1 & 2).

Lophotrichus species, *Cephalosporium acremonium* (GB), *Scolecobasidium constrictum* (CR_1), *Piptocephalis* species (CR_2), a member of Sphæriales, *Acrophialophora*, *Thielavia terricola* and *Rhizoctonia solani* (AR), *Gliocladium fimbriatum* (PL) were specialized fungi and could only be isolated from the rhizosphere regions of gymnosperms indicated in the brackets (Table 1).

Botryodiplodia theobromæ which was isolated from nonrhizosphere region, could not be detected from rhizosphere region of the plants (Table 1).

Table 1. *Distribution of fungi in the rhizosphere of different gymnosperns and in nonrhizosphere region.*

Fungal species	GB	CR_1	CR_2	Z	AR	PL	CS	N
Absidia spinosa	—	+	+	+	+	—	—	+
Rhizopus spp.	+	—	+	+	+	+	+	+
Mucor spp.	+	+	+	+	+	+	+	+
Cunninghamella spp.	+	+	+	+	+	+	+	+
Choanephora cucurbitarum	+	+	+	+	—	+	—	+
Aspergillus nidulans	—	+	+	—	+	—	+	+
Chætomium spp.	+	+	—	+	—	+	—	+
Thielavia terricola	—	—	—	—	+	—	—	—
Gelasinospora cerealis	—	+	+	—	—	—	—	—
Sphæriales	—	—	—	—	+	—	—	—
Lophotrichus spp.	+	—	—	—	—	—	—	+
Phoma humicola	—	+	+	+	—	+	—	+
Botryodiplodia theobromæ	—	—	—	—	—	—	—	+
Trichoderma viride	+	+	+	+	+	—	+	+
Monilia sitophila	—	—	—	—	—	+	+	+
Cephalosporium acremonium	+	—	—	—	—	—	—	+
Aspergillus spp.	+	+	+	+	+	+	+	+
Penicillium spp.	+	+	+	+	+	+	+	+
Pæcilomyces fusisporus	+	+	+	+	+	+	—	+
Gliocladium fimbriatum	+	—	—	—	—	+	—	+
Acrophialophora spp.	—	—	—	—	+	—	—	—
Torula spp.	—	+	—	+	+	—	+	+
Cladosporium spp.	—	+	—	+	+	—	—	+
Pestalotia spp.	—	—	—	+	+	—	—	—
Scolecobasidium constrictum	—	+	—	—	—	—	—	—
Curvalaria spp.	+	+	+	+	+	+	+	+
Alternaria tenuis	—	+	—	+	+	+	+	+
Fusarium spp.	—	—	+	+	+	—	—	+
Piptocephalis spp.	—	—	+	—	—	—	—	—
Rhizoctonia solani	—	—	—	—	+	—	—	+
Actinomycetes	+	+	+	+	+	+	+	—
Mycelia sterilia	+	+	+	+	+	+	+	+
Total No. of fungal species	15	19	17	19	21	15	13	23

'+' indicates presence ; '—' absence.

Different fungal species were dominant in different sets in various seasons ; on 10.10.1970, *Mucor hiemalis* (CR_1, CR_2 and N), *Cunninghamella echinulata* (GB),

Aspergillus flavus (AR, PL, CS), and *A. aculeatus* (Z) ; on 10 2.1971, *Rhizopus nigricans* (AR, PL), *Aspergillus niger* (GB, Z, CS & N), *A. aculeatus* (CR_2) and *Fusarium moniliforme* (CR_1) ; on 10.6.1971, *R. nigricans* (PL), *A. niger* (CR_2, CS), *A. aculeatus* (GB, CR_1, N), *Penicillium chrysogenum* (AR), white sterile colonies (Z) and on 10.8.1971, *R. nigricans* (CR_2), *A. flavus* (GB, CR_1, Z, AR, PL, CS, N) were dominat species in the sets given in the brackets.

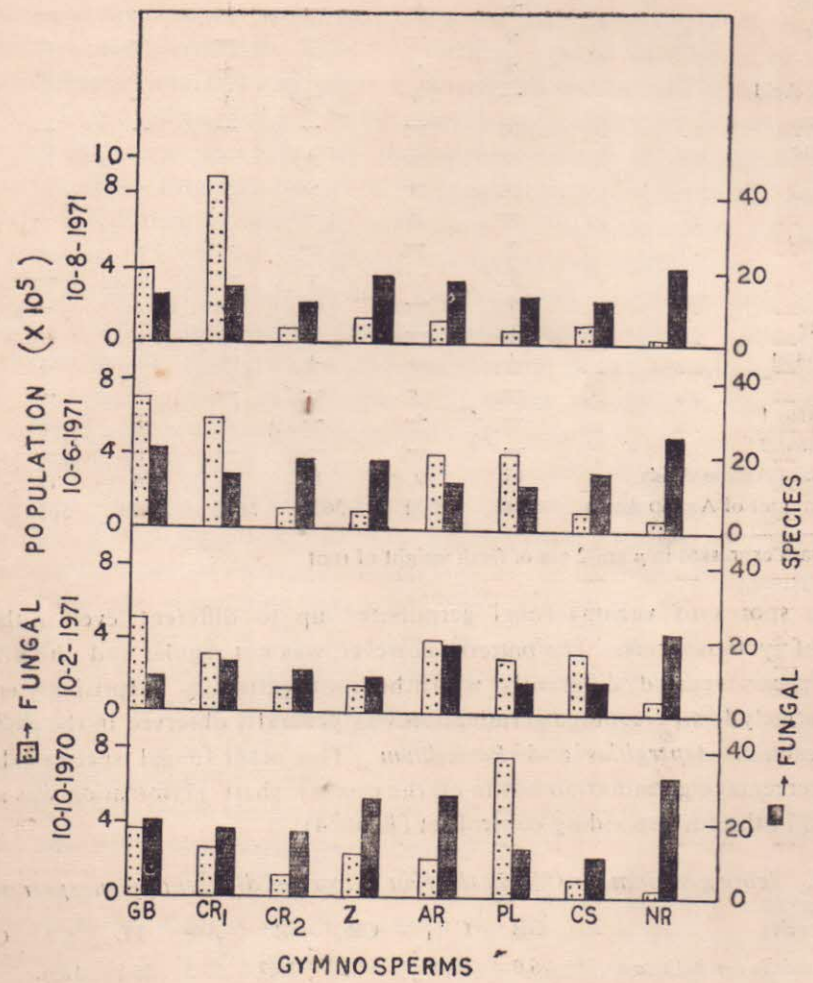
Table 2. *Number of species of different fungi in the rhizosphere of certain gymnosperms.*

Fungal species	GB	CR_1	CR_2	Z	AR	PL	CS	N
Rhizopus spp.	3	—	1	2	1	1	1	2
Mucor spp.	2	1	1	2	2	1	1	2
Cunninghamella spp.	2	2	2	2	1	1	1	2
Other Phycomycetes	1	2	2	2	1	1	—	2
Ascomycetes	2	4	2	1	3	1	1	4
Aspergillus spp.	8	8	8	10	10	7	7	11
Penicillium spp.	1	2	1	1	4	2	1	4
Cladosporium spp.	—	2	—	2	2	—	—	2
Fusarium spp.	2	1	1	1	3	—	—	4
Curvularia spp.	3	2	3	4	3	2	2	3
Other Deuteromycetes	4	6	4	7	6	5	4	9
Basidiomycetes	—	1	—	—	1	—	—	1
Actinomycetes	1	1	1	1	1	1	1	—
Mycelia sterilia	3	2	3	5	4	2	3	6
Total No. of fungi	32	34	29	40	42	24	22	52

No regular pattern of distribution of fungal population in rhizosphere region of plants was observed except in CR_1 where population gradually increased throughout the course of this investigation. In GB and AR, however, highest population was observed in summer months and lowest during early winter in former case and in rainy season in the latter. In Z and PL maxima was obtained during rainy season and early winter respectively whereas lowest values in above sets were observed during winter season. In CR_2 and CS sets, the maximum population was found during winter while population in N set was highest during winter and lowest during early winter and rainy season (Text-fig. 1). The population was always lower and number of fungal species was higher in N set as compared to other sets.

Thirteen, 9, 8, 9, 16, 11 and 9 amino acids were detected chromatographically from GB, CR_1, CR_2, Z, AR, PL and CS sets respectively. The total amount of amino acids, measured calorimetrically as μ gm/2 gm of fresh root in corres-

ponding sets was 9890, 8531, 3632, 5616, 9980, 5050 and 6722 respectively. The amount was the maximum in both quantity and quality in AR root. The



Text-Fig. 1. Graph showing seasonal variation in the distribution of fungal population ($\times 10^5$) and number of fungal species in the rhizosphere region of certain Gymnospermous plants and nonrhizosphere soil.

minimum amount of amino acid was found in *Zamia* species both qualitatively and quantitatively. Alanine, iso-leucine, leucine, tryptophane, glycine were detected in all the roots (Table 3).

Table 3. *Chromatographic detection of amino acids in the root extract of certain gymnosperms.*

Amino acids	GB	CR	CR	Z	AR	PL	HR
Alanine	+	+	+	+	+	+	+
Aspertic Acid	+	—	—	—	+	—	—
Asparagine	+	+	—	+	—	—	—
Citrulline	+	—	—	—	+	+	+
Glycine	+	+	+	+	+	+	+
Glutamic Acid	—	+	—	+	—	+	—
Histidine	+	—	—	—	+	—	—
Iso-leucine	+	+	+	+	+	+	+
Leucine	+	+	+	+	+	+	+
Lysine	—	+	+	+	+	+	+
Methionine	+	—	—	—	+	+	—
Proline	+	—	+	—	+	—	+
Serine	+	—	—	—	+	+	—
Threonine	+	—	+	—	+	—	—
Tryptophane	+	+	+	+	+	+	+
Valine	—	+	—	—	+	—	—
Unidentified 1	—	—	—	+	+	+	+
Unidentified 2	—	—	—	—	+	—	—
Total No. of Amino Acids	13	9	8	9	16	11	9
*Total Amount of Amino Acids	9890	8531	3632	5616	9980	5050	6722

* Data expressed in μ gm/2 gm of fresh weight of root.

The spores of various fungi germinated up to different levels in the root extract of gymnosperms. The pattern, however, was not regular and the different fungal species reacted differently with the root extracts. Surprisingly enough, comparatively low percentage germination was generally observed in the case of 2 species each of *Aspergillus* and *Penicillium* The other fungal species exhibited higher percentage germination but in all the cases highest germination was always observed in the corresponding control set (Table 4).

Table 4. *Spore germination (%) in the root extract of different gymnosperms.*

Fungal species	GB	CR_1	CR_2	Z	AR	PL	CS	Control
Cunninghamella bertholletiæ	76.0	56.1	49.2	38.7	60.5	26.8	18.6	80.0
Mucor hiemalis	28.6	58.5	60.0	36.9	42.3	38.9	46.0	69.5
Aspergillus flavus	39.5	48.6	42.6	21.5	59.3	63.5	69.5	86.3
A. sydowi	16.3	21.2	47.2	27.9	31.5	44.3	19.2	68.3
A. ustus	21.3	30.9	21.9	46.5	36.6	42.2	19.3	80.0
Penicillium chrysogenum	31.5	26.3	22 9	20.2	36 2	52.1	27.1	63.7
P. notatum	18.3	21.6	9.6	11.5	21.3	37.5	14.9	58.2
Curvularia tetramera	61.5	26.3	13.5	49.6	79.5	70.5	38.4	94.0
C. lunata	56.6	42.3	50.7	65.8	26.9	76.3	46.6	100.0
Alternaria tenuis	41.5	76.6	52.9	86.3	61.5	48 6	62.3	92.0
Fusarium nivale	46.0	61.2	72.2	82.5	63.9	28.2	80.0	100.0

Discussion

Variation in the rhizosphere microflora with various plant species and age has been extensively reported (England and Rice, 1957 ; Schroth and Hildebrand, 1964 ; Rovira, 1956. '59, '65). In most of the cases the maximum population has been noticed at the time of maximum vegetative growth of the plant (Gujarati, 1965 ; Mishra and Srivastava, 1970 ; Srivastava, 1969). In the cases of the gymnospermous plants, however such a correlation with plant age is not so easy due to their long lived character. In the present study where the investigation has been limited to one calender year only, the conclusion may not be definitely drawn with respect to age From the results of the present study (Table 1 and 2), however, it is clearly noted that variation in mycoflora of the rhizosphere of different gymnosperms exists both with respect to plant species and sampling time. Besides a number of species which are frequently and commonly associated with the roots of the different plants few were of specific occurrence (Table 1). The common distribution of certain species in the rhizosphere may be explained due to some uniformity in soil character and amino acids of the roots. Amongst 18 amino acids assayed, 5 were of common occurrence. The specificity in the distribution of other forms may be explained in the light of variation in plant age, height and amino acids in the root. The higher amount of amino acids expressed, in terms of μ gm/2 gm of fresh weight of root, was obtained from AR, GB and CR_1 where the rhizosphere population was also much higher In CR_2 where the amino acid content was low, the population was also much lesser. The exception to this generalization was noted in PL set where in summer and winter months the population was much higher though the amount of amino acid was not so high. In addition to amino acids which, of course, are one of the important constituents, other substances (sugars, organ c acids, vitamins, hormones etc.), present in the root, influence the rhizosphere fungi. Because no other substances besides amino acids have been assayed in the present study, the higher population in PL may be expected to be regulated by some other determinants.

The variation in fungal population in different seasons was also not uniform for different plants. Except in GB and CR_1 the population was generally low in rainy season. Even in dry summer months the population was comparatively higher in most of the sets. This is somewhat different from the normal pattern, obtained in the case of nonrhizosphere soil samples studied earlier (Mishra, 1966). The dry summer soil with low organic matter and moisture content generally favours poor fungal population. The micro environment of the rhizosphere region, however, is different from nonrhizosphere region due to continuous supply of the food materials and moisture to the environment from the roots. This probably accounted for this anamolous behaviour of the distribution of the fungi in the rhizosphere in summer season. In winter the population in most of the cases is considerably higher. The variation, however, is pronounced on the two

sampling dates i.e., 10th of December and February. In most of the cases the fungi per gm of root was higher in rhizosphere regions than N region, whereas the case was just the reverse for the number of fungal species. This is in accordance with the views of the earlier workers (Chesters and Perkinson, 1959 ; England and Rice, 1957 ; Gujarati, 1965 ; Mishra, 1967 ; Srivastava, 1969) who have suggested various reasons for this behaviour. Higher nutritional level due to root exudation and addition of dead and living root fragments in the rhizosphere region was suggested to be the main factors for higher population. Specificity in the nutrients, however, restricted the number of species occuring in this region (Mishra, 1970 ; Gupta, 1970 ; Rovira, 1969).

The fungal spores reacted differently for the root extracts of different gymnosperms. In most of the cases Aspergilli and Penicilia were most adversely affected. *Curvularia lunata*, *Alternaria tenuis* and *Fusarium nivale* were lesser affected and *C. bertholletiæ*, *Mucor hiemalis* and *C. tetramera* were in between the two extremes. In no case, however, the root extract proved stimulatory and germination was always low as compared to corresponding control sets. This is surprising for Aspergilli and Penicillia which are one of the most frequently occuring fungi of the rhizosphere region of the different plants under investigation. The condition, however, in the rhizosphere region is different due to various heterogenous factors working together and the results obtained from the slide method may not always be compared to that of the rhizosphere region.

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