

ESTABLISHMENT OF MICROFUNGI IN THE ROOT REGION OF *ANDROGRAPHIS PANICULATA* NEES

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Rhizosphere and root surface microfungi of *Andrographis paniculata* Nees., a plant of some medicinal importance, were studied in detail from early seedling stage onward to the late fruiting stage of the plant in relation to the pattern of their establishment. The qualitative analysis of fungi revealed certain notable results. The increase or decrease in percentage occurrence of some fungi was related to the age of the plants. Some fungi perpetuated throughout while certain others were confined to the particular age of the plants. The fungi were recognized as primary, secondary, late and non-colonizers of the root surface.

INTRODUCTION

Rhizosphere is a biologically active zone around the root system of the plants, where micro-organisms are influenced by organic and inorganic substances excreted by the root system. Soon after germination of seed, the excretions of young roots of seedlings stimulate the fungal spores to germinate by removing the effect of natural fungistasis (Jackson 1958). Initial colonization of root surface by soil mycoflora and the pattern of their development were recently discussed (Parkinson *et al.* 1963; Parkinson and Pearson 1967; Parkinson and Thomas 1969). Parkinson *et al.* (1963) reported that colonization of fungi on root might be by a wide range of soil fungi and that their mixed population rapidly gave way to a stable and typical root surface mycoflora. Parkinson and Pearson (1967) studied the fungal flora of barley roots in relation to their colonization with special reference to the occurrence and competitive ability of sterile dark fungi. Parkinson and Thomas (1969) reported that there was small but consistent difference in the rhizosphere and non-rhizosphere mycoflora. They also demonstrated a slight qualitative change in the rhizoplane mycoflora accompanying increasing plant age.

The present study aims at the nature of establishment of mycoflora with emphasis on the qualitative difference in the rhizosphere and rhizoplane of *Andrographis paniculata*. Observation on the actively colonizing fungi and their percentage occurrence in relation to age of the plant has been emphasized.

MATERIALS AND METHODS

The plants were grown in the glass-house by sowing surface-sterilized seeds (with 0.1 per cent $HgCl_2$) of the test plant. The rhizosphere study was done at different ages starting from emergence of seedling to the fruiting stage viz., after 1, 2, 3, 4, 5, 10, 20, 30, 60, 100 and 140 days. The pots were watered with equal amount of water at weekly intervals.

Rhizosphere samples were collected by uprooting the plants carefully with the help of a sterile spatula and the excess soil adhering to the root surface was removed by a gentle tapping. Roots were cut off and placed in 250 ml flask containing 100 ml sterile distilled water. The flask was shaken vigorously until the rhizosphere soil particles had been dislodged from the root system and a soil water suspension was thus prepared. One ml of the solution was inoculated in each petridish and a large number of such dishes were inoculated.

Non-rhizosphere soil samples were collected from the region away from root system. Soil suspension of 1:100, 1:1000 and 1:10,000 dilutions were prepared. One ml soil suspension for each dilution was inoculated in each plate and 15 plates for each dilution were inoculated. Melted and cooled 10 ml nutrient agar was poured.

For the rhizoplane fungi, roots were thoroughly washed with sterile distilled water for 10-15 times and cut into small pieces. The latter were plated onto agar plates. The nutrient agar medium for the rhizosphere, non-rhizosphere and rhizoplane fungi was of the following composition : dextrose—10g, peptone—5g, KH_2PO_4 —1g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ —0.5g, agar-agar—15g; distilled water—1 litre and rose-bengal, 1 : 15,000.

Plates were incubated at 25°C for a week. Fungi were identified and their number was counted to calculate the percentage occurrence. Non-rhizosphere samples were taken five times, first on the first sampling on the first day, 2nd on 30th day, and the rest corresponded to dates of sampling of rhizosphere.

RESULTS AND DISCUSSION

The relative percentage incidence of fungi, isolated from non-rhizosphere, rhizosphere and rhizoplane of the test plant has been given in Tables I, II and III respectively. The percentage incidence of only small number of fungi was influenced by the rhizosphere effect. Microfungi on the rhizoplane were strikingly smaller in number than those of the rhizosphere and the non-rhizosphere.

TABLE I

Percentage occurrence of fungal species in the non-rhizosphere soil of Andrographis paniculata at different times corresponding to the age of plants

Fungi	Sampling time* (in days)						
	1	30	60	100	140		
<i>Rhizopus nigricans</i>	4	4	2	7	9
<i>R. oryzae</i>	—	1	—	2	—
<i>Mucor luteus</i>	—	3	—	1	6
<i>Cunninghamella bertholletiae</i>	—	—	—	—	1
<i>Aspergillus nidulans</i>	4	—	—	3	—
<i>Chaetomium spirale</i>	—	—	—	1	1
<i>Neocosmospora vasinfecta</i>	2	1	1	2	—
<i>Phoma hibernica</i>	—	—	—	1	—

<i>P. glomerata</i>	2	1	1	2	-
<i>Coniothyrium fuckelii</i>	-	-	-	1	-
<i>Botryodiplodia theobromae</i>	1	2	-	2	-
<i>Amerosporium applanatum</i>	-	-	-	2	-
<i>Cephalosporium</i> sp.	1	-	-	-	-
<i>Trichoderma lignorum</i>	2	-	-	2	3
<i>Aspergillus flavus</i>	6	9	4	9	10
<i>A. terreus</i>	6	8	16	9	16
<i>A. luchuensis</i>	2	1	1	2	1
<i>A. niger</i>	12	15	7	20	16
<i>A. alliaceus</i>	-	-	-	-	1
<i>A. candidus</i>	4	-	1	1	1
<i>Penicillium javanicum</i>	2	3	-	-	-
<i>P. fellutanum</i>	2	-	-	-	-
<i>P. citrinum</i>	6	4	3	4	7
<i>P. chrysogenum</i>	2	-	-	-	-
<i>P. nigricans</i>	2	-	-	-	-
<i>P. rubrum</i>	-	3	-	2	-
<i>Sporotrichum</i> sp.	-	-	1	-	-
<i>Sepedonium</i> sp.	-	-	-	-	1
<i>Paecilomyces fusisporus</i>	7	3	8	3	6
<i>Hormiscium</i> sp.	-	2	-	1	-
<i>Stachybotrys atra</i>	1	-	-	-	-
<i>Humicola grisea</i>	-	2	3	-	2
<i>H. nigriscens</i>	-	2	2	-	-
<i>Cladosporium lignicolum</i>	-	-	4	-	-
<i>C. herbarum</i>	-	14	18	4	2
<i>Curvularia tetramera</i>	1	-	-	-	-
<i>C. geniculata</i>	2	-	2	-	-
<i>C. lunata</i>	2	-	2	2	-
<i>Alternaria tenuis</i>	1	-	-	-	-
<i>Fusarium poae</i>	4	1	3	4	3
<i>F. chlamydosporum</i>	-	3	-	3	3
<i>F. elegans</i>	-	-	4	-	-
<i>F. oxysporum</i>	3	3	-	3	-
<i>F. roseum</i>	1	-	-	2	1
<i>F. culmorum</i>	4	-	3	2	3
<i>Myrothecium verrucaria</i>	-	1	-	-	-
<i>M. roridum</i>	2	4	-	2	-
<i>Sclerotium</i> sp.	1	-	-	2	1
<i>Papulaspora</i> sp.	1	-	-	2	-
White sterile mycelium	-	1	5	3	3
Brown sterile mycelium	3	-	-	2	-
Dark sterile mycelium	7	6	6	3	2

*Corresponding to those for rhizosphere soil

TABLE II

Establishment of fungal species in the rhizosphere of Andrographis paniculata at different ages of the plant (percentage occurrence)

Fungi	Age of the plant (in days)										
	1	2	3	4	5	10	20	30	60	100	140
<i>Rhizopus nigricans</i>	2	—	2	1	—	—	—	4	2	7	30
<i>Mucor luteus</i>	—	1	—	—	1	—	2	3	—	—	2
<i>Cunninghamella bertholletiae</i>	1	—	—	—	—	—	—	—	—	2	—
<i>Aspergillus nidulans</i>	—	—	1	—	—	—	—	2	2	3	—
<i>Chaetomium spirale</i>	—	1	—	—	—	—	2	—	—	—	—
<i>Neocosmospora vasinfecta</i>	—	—	1	1	—	1	—	—	—	—	—
<i>Phoma glomerata</i>	—	—	—	—	—	—	—	11	5	3	—
<i>Botryodiplodia theobromae</i>	—	3	3	—	—	—	—	—	2	—	—
<i>Amerosporium applanatum</i>	—	—	—	—	—	—	—	4	—	—	—
<i>Cephalosporium</i> sp	—	—	—	—	—	—	—	—	2	—	—
<i>Trichoderma lignorum</i>	7	1	1	4	1	2	1	2	10	6	3
<i>Aspergillus sydowi</i>	—	2	—	—	—	—	—	—	5	—	—
<i>A. flavus</i>	3	—	3	4	4	4	4	7	3	5	21
<i>A. terreus</i>	3	1	—	4	5	4	2	—	2	—	—
<i>A. luchuensis</i>	—	—	—	—	—	—	—	—	2	—	—
<i>A. niger</i>	16	14	23	19	22	26	25	19	20	25	24
<i>A. candidus</i>	—	2	—	—	—	1	—	—	—	—	—
<i>Penicillium javanicum</i>	—	1	—	2	—	1	—	2	2	—	—
<i>P. fellutanum</i>	—	—	2	—	—	—	—	—	—	2	—
<i>P. citrinum</i>	—	2	2	4	—	1	8	4	3	4	6
<i>P. nigricans</i>	—	1	—	—	—	—	—	—	—	—	—
<i>P. rubrum</i>	—	—	—	2	—	—	—	2	—	—	—
<i>Paecilomyces fusisporus</i>	3	—	1	—	—	—	2	9	7	4	—
<i>Hormiscium</i> sp.	—	—	1	—	—	—	1	—	2	—	—
<i>Stachybotrys atra</i>	—	—	—	—	—	1	—	—	—	—	—
<i>Humicola grisea</i>	—	3	3	2	—	—	—	2	2	3	—
<i>H. nigriscens</i>	—	1	—	2	—	—	—	—	—	—	—
<i>Mammoniella echinata</i>	—	3	—	—	1	2	—	—	—	—	—
<i>Cladosporium herbarum</i>	—	—	—	—	—	—	—	7	2	15	—
<i>Curvularia geniculata</i>	—	—	1	—	—	—	—	—	—	—	—
<i>C. lunata</i>	2	3	—	3	3	2	—	—	2	—	2
<i>Alternaria tenuis</i>	—	1	—	1	1	—	—	—	—	—	—
<i>Fusarium poae</i>	3	—	—	5	4	1	5	13	12	5	3
<i>F. chlamyosporum</i>	3	2	2	2	—	—	—	3	2	2	—
<i>F. oxysporum</i>	3	2	2	—	2	—	—	—	—	—	—
<i>F. roseum</i>	1	—	2	2	—	—	2	—	—	—	—
<i>F. culmorum</i>	3	3	2	6	6	5	5	—	—	5	—

<i>Myrothecium roridum</i>	—	5	4	—	3	2	2	—	—	—	—
<i>Sclerotium</i> sp	—	2	—	—	3	—	—	—	2	—	3
<i>Papulaspora</i> sp.	—	—	2	—	—	3	—	—	—	—	—
White sterile mycelium	21	22	20	20	18	20	22	6	2	5	3
Brown sterile mycelium	5	3	—	2	2	—	—	2	—	—	—
Dark sterile mycelium	23	20	19	15	22	21	17	8	5	3	3

(1) *Rhizopus nigricans*, *Aspergillus flavus*, *A. terreus*, *A. luchuensis*, *A. niger*, *Penicillium citrinum*, *Paecilomyces fusisporus* and *Fusarium poae* were frequently isolated from the non-rhizosphere at different days of sampling. In the rhizosphere *A. niger*, *A. flavus*, *Trichoderma lignorum* and *P. citrinum* were of frequent occurrence. *Rhizopus nigricans* appeared late on the rhizoplane and its percentage incidence in non-rhizosphere, rhizosphere and rhizoplane was higher during the later stage of growth of plants. Amongst aspergilli, only *A. flavus* and *A. niger* colonized the rhizoplane and others were confined only to non-rhizosphere and rhizosphere. The percentage occurrence of this species fluctuated at different stages of plants' growth in non-rhizosphere, rhizosphere and rhizoplane. The percentage occurrence of *A. flavus* in the rhizosphere was more or less similar to the non-rhizosphere.

With regard to frequently occurring fungi in the rhizosphere, three types of groupings have been recognized :

- I. Fungi isolated with high frequency fall under two categories :
 - (a) There are certain fungi viz., *R. nigricans*, *A. niger* and *A. flavus* which showed an increase in percentage incidence with increase in age of the plants.
 - (b) White and black sterile mycelial forms were isolated with high percentage occurrence but percentage decreased with increase in age of the plants.
- II. Certain fungi viz., *C. lunata*, *F. chlamydosporum* and *M. roridum* were frequently isolated from rhizosphere but without definite pattern in their percentage occurrence.
- III. Certain fungi viz., *A. terreus*, *Penicillium fellutanum*, *Memnoniella echinata*, *Alternaria tenuis* and *F. oxysporum* were of decreased percentage occurrence.
- IV. Certain forms viz., *A. luchuensis*, *Amerosporium applanatum*, *Cephalosporium* sp., *P. nigricans*, *S. atra* and *Curvularia geniculata* appeared only once.

(2) Several penicillia were isolated without any regular pattern except *P. citrinum* which occurred frequently both in non-rhizosphere and rhizosphere. No species of *Penicillium* appeared on the rhizoplane. *P. citrinum* had no definite trend of either increase or decrease with the age of plants.

(3) Several species of *Fusarium* were isolated from the rhizosphere, non-rhizosphere and rhizoplane. *F. poae*, *F. chlamydosporum* and *F. culmorum* were of almost frequent occurrence in the non-rhizosphere and rhizosphere, while on rhizoplane only *F. poae* and *F. culmorum* appeared at certain stages of plants' growth.

(4) White and dark sterile mycelial forms were frequently isolated from the rhizosphere, non-rhizosphere and rhizoplane. Their frequency and percentage

TABLE III

Establishment of fungal species on the rhizoplane of *Andrographis paniculata* at different ages of the plant (percentage occurrence)

Fungi	Age of the plant (in days)										
	1	2	3	4	5	10	20	30	60	100	140
<i>Rhizopus nigricans</i>	-	-	-	-	-	-	-	3	-	8	14
<i>Mucor luteus</i>	-	-	-	-	-	-	-	3	-	4	8
<i>Neocosmospora vasinfecta</i>	-	-	-	7	4	13	4	-	-	3	-
<i>Botryodiplodia theobromae</i>	-	-	-	4	5	5	12	-	-	4	-
<i>Trichoderma lignorum</i>	7	8	4	4	-	18	8	3	6	9	14
<i>Aspergillus flavus</i>	-	-	4	-	-	-	-	-	-	-	-
<i>A. niger</i>	11	10	9	15	15	26	16	30	36	32	14
<i>Curvularia lunata</i>	-	-	-	4	4	5	5	-	-	-	-
<i>Alternaria tenuis</i>	-	-	-	-	4	-	-	-	-	-	-
<i>Fusarium poae</i>	-	-	-	-	-	-	-	2	-	5	-
<i>F. culmorum</i>	-	-	-	6	4	3	-	-	4	4	6
<i>Sclerotium</i> sp.	-	-	17	-	4	-	4	6	8	10	15
White sterile mycelium	54	52	37	23	23	14	22	20	18	11	7
Dark sterile mycelium	24	28	26	30	31	15	16	20	8	-	7

incidence were higher on the rhizoplane than in the non-rhizosphere and rhizosphere. Occurrence of *Sclerotium* sp. was more frequent on rhizoplane than in rhizosphere and non-rhizosphere.

From the Tables it is evident that only a small number of fungi was frequently isolated from rhizoplane at different age of the plants. On the basis of their colonization on root surface they may be designated as primary, secondary and late colonizers.

Peterson (1958) reported that the frequency of Mucorales increased in the rhizosphere of wheat in the early stage of plant growth and decreased with increasing plant age. Chesters and Parkinson (1959) found similar trend while studying the rhizosphere of oat plant. Parkinson and Thomas (1969) observed that Mucorales were isolated with high frequency at the late maturity stage of the plant. They have attributed this fact to the effect of root exudates. The present finding is similar to that of Parkinson and Thomas (1969). They again reported that *T. viride* was isolated with high frequency from the rhizosphere of two-leaf stage of dwarf bean plant and decreased with increasing age of the plant. In the present study, *T. lignorum* was isolated always with high frequency from rhizosphere and rhizoplane than from non-rhizosphere. No definite trend of its percentage incidence was found with increasing age of the plant. The aspergilli such as *A. terreus* and *A. luchuensis* and *Penicillium* spp. were less frequently isolated from rhizosphere and never from rhizoplane which may be attributed to perhaps unfavourable effect of root exudate.

Parkinson and Thomas (1969) found that species of *Fusarium* changed with the age of the plant of dwarf bean. They were isolated with low frequency at the seedling stage but their incidence increased at the late flowering and early pod formation stage. The behaviour of white sterile mycelium and dark sterile mycelium with high frequency in rhizosphere and rhizoplane in the present study is in accord with the findings of Taylor (1965), Parkinson and Thomas (1969) and Parkinson and Pearson (1967 a).

A possible explanation for the primary colonizers may be given by the fact that these forms may not be sensitive to soil mycostasis in comparison to other fungi and even if they are sensitive, the effect might be reduced due to the presence of root system in the soil which secretes energy rich compounds. White sterile mycelium and dark sterile mycelium are present in the form of mycelium in the soil and may colonize the root surface at the earliest possible stage in comparison to spore forming ones which take time for establishment of hyphae after spore germination. The strong antagonist may increase in the percentage incidence while the fungi susceptible to its effect may decrease. The increasing percentage incidence of *A. niger* and *B. theobromae* may be attributed to their strong antagonistic effect which has been studied by authors in laboratory conditions in culture plates, while white sterile mycelium and dark sterile mycelium decreased in their percentage occurrence possibly by the antagonistic effect of *A. niger*, *F. culmorum* and *B. theobromae*.

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