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Mycoflora Associated with the Seeds of Forest Trees and Their Effect on Germination

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Fungi belonging to forty-two species of genera *Mucor, Rhizopus, Aspergillus, Penicillium, Epicoccum, Pithomyces, Cladosporium, Paecilomyces, Fusarium, Gliocladium, Trichothecium, Trichoderma, Cephalosporium, Alternaria, Ulocladium,* and *Curvularia* were isolated from the forest seeds of *Quercus, Cupressus, Sapium, Pyrus, Melia, Casuarina* and *Thuja.*

Frequency of occurrence of moulds in the fallen-off seeds was generally, more in comparison to that in the plucked seeds. Amongst the fungi isolated *Pithomyces cynodontis* and *Penicillium granulatum* were the new findings from the seeds of forest trees.

Association of most of the fungi with the seeds of *Quercus* inhibited their germination. However, *Aspergillus fumigatus, Penicillium nigricans* and *P. islandicum* stimulated the growth of plumule. The culture filtrates (5 and 15 days old) of *A. fumigatus* and *P. nigricans* stimulated the growth of plumule while 25 days old culture filtrates of both the fungi suppressed the growth of plumule.

Key Words: Seed mycoflora, Seed germination, Seeds of forest trees

Introduction

For regeneration of a forest proper germination of seeds and subsequent establishment of the trees is essential. Seeds of forest-trees like others, carry numerous fungi which are known to cause considerable damage to sprouting seeds and seedlings (Grawatt 1931, Gibsson 1957, Urosevic 1969). In the present investigation, an attempt has been made to study the mycoflora of the seeds of forest trees (both fallen-off and directly plucked from the tree) and its effect on the germination of *Quercus* seeds.

Materials and Methods

Mycoflora studies

The seeds of *Quercus, Cupressus, Sapium, Pyrus, Melia, Casuarina* and *Thuja* were directly plucked from the tree as well as the ones fallen off from the tree were

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collected in polythene bags and the seed-borne mycoflora was detected within 24 hr of collection.

Ten gram of seeds was *aseptically* weighed and transferred to 250 ml Erlenmeyer flask containing 100 ml of sterilized water and 10 g of sterilized sand (a dispersing agent) (James et al. 1946). The flask was then shaken for 30 min. After the foam developed during the shaking had subsided, serial dilutions were prepared from the original dilution. One ml of this solution was poured in 10 Petridishes (5 containing Czapek's medium and the other 5 PDA) and the plates were incubated for 4–5 days at 28°C. The colonies of the particular fungus were counted and the differently appearing colonies were isolated for identification.

The isolation of the colonies was continued for 10–12 days until appearance of new colonies ceased. Percentage frequency and relative abundance of each fungus was calculated as follows:

$$\text{Percentage frequency} = \frac{\text{Total number of plates in which a particular fungus appeared}}{\text{Total number of plates studied}} \times 100$$

$$\text{Relative abundance} = \frac{\text{Total number of colonies of a particular fungus in all the plates}}{\text{Total number of colonies of all fungi in all the plates}} \times 100$$

Germination Studies

(a) Effect of Seed Mycoflora on Germination of Quercus Seed:

The fungi isolated both from plucked and fallen-off seeds of *Quercus* were grown in the culture tubes containing Czapek's solution agar medium for three days at 28°C. Then 10 ml of sterilized water was added to each tube containing the fresh culture of the respective fungus. The tubes were shaken and the spore suspension was transferred to a sterilized 250 ml conical flask.

The seeds of the *Quercus* were surface-sterilized with 2% sodium hypochlorite solution and washed thoroughly with sterilized water. The sterilized-seeds were dipped in the suspension of the respective fungi with the help of sterilized forceps and placed in the sterilized Petridishes for germination. Control plates were also made. All the plates were kept in an incubator at 28°C for 6 days. The germination of the seeds was observed and the results were obtained by measuring the length of the plumule.

(b) Role of Culture Filtrate of Two Fungi on Seed Germination

A. fumigatus and *P. nigricans* were grown at 28°C in Czapek's liquid media (Ichinoe et al. 1968). Three replicates of each culture with different incubation periods 5, 15 and 25 days were studied. At the end of the incubation period the medium was filtered through a Seitz bacterial filter No. 3G5F. Seeds were surface-sterilized with 0.2% NaClO (Sodium hypochlorite) for one min. (Christenesen 1964), washed five times and kept in sterilized water for an hr to soften the hard seed coat. The seeds were then soaked in the culture filtrates for 24 hr at room temperature (Armolik & Dickson 1956) and transferred to sterilized dishes containing moist sand. The germinability of seeds was determined by placing 50 to 100 kernels on sterilized sand-bed and allowing them to germinate at room temperature. The observations were taken after 6 days.

To study the effect of various fungi on

germinated *Quercus* seeds, one-day-old seedlings were soaked in the culture filtrates for 24 hr, at 28°C and kept on the sand beds at room temperature. The seeds in which plumule developed into a shoot were taken to as sprouted.

For control, seeds steeped in Czapek's liquid medium were kept side by side with the experimental ones.

Results and Discussion

Mycoflora Associated with the Seeds of Forest Trees

In the case of the seeds of each forest tree, plucked from the tree, the total population of fungi was more in *Cupressus* followed by *Quercus incana*, *Melia azedaroch*, *Thuja orientalis*, *Casuarina* spp., *Pyrus pashia* and *Sapium sebiferum* while in the seeds fallen off from the tree, the total population was more in *Casuarina* followed by *Melia azedaroch*, *Quercus incana*, *Cupressus torulosa*, *Thuja orientalis*, *Sapium sebiferum* and *Pyrus pashia* (table 1).

Of the 42 fungi isolated, 3 fungi (*Mucor pusillus*, *M. racemosus* and *Rhizopus nigricans*) were members of Zygomycetes, 11 were different species of *Aspergillus* and 12 were different species of *Penicillium*. Among the other 16 Hyphomycetes there were 2 species each of *Cephalosporium*, *Cladosporium*, and *Fusarium*. Besides, one species each of *Alternaria*, *Curvularia*, *Gliocladium*, *Drechslera*, *Epicoccum*, *Paecilomyces*, *Pithomyces*, *Trichoderma*, *Trichothecium* and *Ulocladium* was isolated.

It was observed that although a number of fungi were exclusively obtained from the seeds of a particular forest tree and some were common to more than one tree seeds, their frequency varied with different seeds. Just to give an idea of the frequency of occurrence of fungi in each seed, the fungi isolated were classified into 2 categories (arbitrarily) one with frequency below 10% and the other with above 10.

It was observed that among the moulds isolated from plucked seeds of various forest trees, majority showed percentage frequency below 10%. The ones above 10% were 2 in case of *Quercus*, 5 in *Cupressus*, 4 in *Sapium*, 5 in *Pyrus*, 3 in *Melia* and 4 in *Casuarina* and 3 in *Thuja* (table 1).

In case of fallen-off seeds of various forest trees the percentage frequency of majority of moulds was found to be below 10%. The ones above 10% were 4 in *Quercus*, 4 in *Cupressus*, 3 in *Sapium*, 4 in *Thuja* and *Pyrus* and *Casuarina* and 5 in *Melia* (table 1).

It was important to note that the frequency of occurrence of moulds in the fallen-off seeds of majority of forest trees was generally more in comparison to that in the plucked seeds. This may be due to contamination from the environment, soil or the dust round the seeds. However, a high percentage of *Penicillium* spp. was found to be associated with majority of the seeds. The predominance of Penicillia over Aspergilli may be due to the low temperature and high humidity in the Kumaun hills. The former are known to require minimum relative humidity of 79% and the latter 65% for their spore germination (Armolik & Dickson 1956).

Effect of the Seed-Mycoflora on the Germination of Quercus Seeds

It was observed that association of fungi with seeds generally, inhibited the germination of seeds in both cases (i.e. seeds plucked from tree and fallen off seeds). Contrarily, *Aspergillus fumigatus*, *Penicillium nigricans* and *P. islandicum* promoted the growth of the plu-

Table 1 *Total population and frequency of occurrence of fungi from seeds of forest trees*

Sl No.	Name of fungi	Total population counts (Average of plates each of the two media of each seeds)													
		Quercus		*Cupressus*		*Sapium*		*Pyrus*		*Melia*		*Casurina*		*Thuja*	
		Tree 124	Ground 147	Tree 129	Ground 112	Tree 58	Ground 73	Tree 65	Ground 63	Tree 124	Ground 172	Tree 76	Ground 202	Tree 111	Ground 101
1.	*Mucor pusillus*	—	—	13.3	16.2	—	2.0	—	—	—	—	—	—	—	—
2.	*M. racemosus*	—	5.3	—	—	—	—	5.7	15.5	—	—	4.7	3.0	—	—
3.	*Rhizopus nigricans*	—	—	—	—	—	—	—	—	—	—	—	4.0	—	—
4.	*Aspergillus clavatus*	—	3.4	—	—	—	5.3	—	—	—	6.0	—	—	3.0	6.0
5.	*A. fumigatus*	8.2	8.0	—	18.0	8.7	7.1	5.8	—	—	14.0	22.0	2.0	—	—
6.	*A. ochraceous*	—	—	—	—	—	—	—	—	—	—	—	8.0	—	—
7.	*A. niger*	—	—	—	8.0	—	5.0	—	—	—	—	3.7	—	—	—
8.	*A. flavus*	2.3	—	3.3	—	—	—	4.9	—	—	20.0	—	—	—	17.0
9.	*A. nidulans* var. *echinulatus*	—	—	—	—	—	—	2.8	—	—	—	—	3.7	—	—
10.	*A. variecolor*	—	—	—	4.5	—	—	—	—	—	—	—	7.1	8.1	3.2
11.	*A. ustus*	—	—	—	—	8.7	—	—	—	—	—	—	—	—	—
12.	*A. flavipes*	—	—	6.7	—	—	—	—	—	—	5.0	—	—	—	—
13.	*A. carneus*	—	—	—	—	4.3	—	—	8.2	—	—	—	—	—	—
14.	*A. terreus*	6.1	10.0	—	—	—	—	—	—	4.0	—	8.2	3.0	4.1	—
15.	*Penicillium frequentans*	—	—	—	23.1	—	—	—	—	—	26.0	21.6	—	—	—
16.	*P. multicolor*	—	—	—	—	—	—	—	8.0	—	—	—	15.1	10.1	—
17.	*P. implicatum*	—	7.0	—	—	—	15.1	11.4	4.0	—	—	—	—	—	—
18.	*P. nigricans*	15.2	20.0	—	—	—	24.1	—	20.0	—	—	—	18.1	—	30.2
19.	*P. citrinum*	—	—	—	—	—	—	20.8	—	22.0	—	—	—	14.1	18.1
20.	*P. steckii*	4.1	—	—	—	17.3	21.0	—	—	—	—	—	10.1	—	—
21.	*P. chrysogenum*	—	—	—	—	14.0	—	—	—	10.0	—	—	—	—	8.1
22.	*P. cyclopium*	28.0	8.0	—	—	—	—	—	20.0	—	—	13.5	—	—	—
23.	*P. martensii*	—	—	—	7.2	—	—	—	—	—	4.0	—	—	8.2	—

24.	*P. urticae*	—	—	28.0	—	—	—	2.8	—	—	—	—	—	—	—
25.	*P. granulatum*	—	6.1	16.0	—	21.1	—	—	—	—	—	—	—	—	—
26.	*P. islandicum*	7.0	3.0	—	—	—	—	—	—	—	—	—	—	—	—
27.	*Epicoccum purpurascens*	—	—	—	—	—	—	—	—	—	—	—	2.0	—	11.2
28.	*Pithomyces cynodontis*	—	—	—	—	—	—	—	—	—	—	—	12.1	—	3.1
29.	*Cladosporium cladosporioides*	9.0	—	20.2	7.0	—	—	13.2	—	5.0	15.0	3.7	—	24.0	—
30.	*C. sphaerospermum*	5.0	—	—	—	—	3.1	14.2	—	—	—	16.2	—	—	—
31.	*Paecilomyces varioti*	—	—	—	—	—	—	—	8.8	—	—	—	—	—	—
32.	*Fusarium semitectum*	—	—	—	—	—	—	—	—	4.0	8.0	—	4.0	—	—
33.	*F. moniliforme*	—	12.0	—	—	—	—	—	—	10.0	—	—	—	—	—
34.	*Gliocladium deliquescens*	—	—	—	—	26.0	8.6	—	—	—	—	—	—	—	—
35.	*Trichothecium roseum*	—	2.1	—	—	—	—	—	—	—	—	—	—	4.0	—
36.	*Trichoderma viride*	8.1	—	—	—	—	—	—	—	—	—	—	—	—	3.1
37.	*Cephalosporium curtipes*	—	—	—	—	—	—	—	—	—	14.0	—	—	—	—
38.	*C. acremonium*	—	—	—	—	—	4.3	—	—	—	8.0	—	—	—	—
39.	*Alternaria alternata*	3.0	12.1	—	—	—	—	5.6	—	—	—	—	—	3.0	—
40.	*Ulocladium botrytis*	—	—	—	—	—	—	12.8	—	—	—	6.4	—	—	—
41.	*Curvularia lunata*	—	—	—	—	—	—	—	16.0	—	—	—	—	5.0	—
42.	*Drechslera australiensis*	—	3.0	12.5	17.0	—	—	—	—	—	—	—	—	—	—
43.	*Mycelia sterilia*	4.0	—	—	—	—	6.0	—	—	—	—	—	—	—	—

mule. Earlier, it has been reported by Nielsen in 1928, that the agar after growth of *Rhizopus suinus* and *Absidia ramosa* contained a substance which promoted the growth of *Avena* coleoptiles.

In these three instances, the increase in length of plumule may possibly be due to the production of some growth promoting substances by these three fungi associated with the seeds.

Association of other fungi namely *Rhizopus nigricans*, *Aspergillus terreus*, *A. niger*, *Penicillium implicatum*, *P. steckii*, *P. granulatum*, *Cladosporium cladosporioides*, *C. sphaerospermum*, *Trichoderma viride*, *Trichothecium roseum*, *Alternaria alternata*, *Drechslera australiensis* inhibited the growth of plumule in both plucked and fallen off seeds of *Quercus*. This inhibition observed during germination of seeds may be due to the production of some growth inhibiting substances by these fungi (table 2).

Table 2 *Effect of different moulds on the germination of* Quercus *seeds*

Sl. No.	Names of fungi	Length of plumule in cm (Average of 5 seeds in each case)			
		Plucked seeds		Fallen-off seeds	
		Control	Treated	Control	Treated
1	*Rhizopus nigricans*	—	—	1.2	0.6
2	*Aspergillus fumigatus*	1.8	2.3	2.2	2.3
3	*A. terreus*	2.5	0.5	2.1	1.2
4	*A. niger*	2.1	1.3	—	—
5	*Penicillium islandicum*	2.0	2.4	1.8	2.0
6	*P. implicatum*	—	—	—	—
7	*P. steckii*	8.5	5.9	1.6	1.1
8	*P. granulatum*	—	—	—	—
9	*P. nigricans*	—	—	1.0	1.0
10	*Cladosporium cladosporioides*	7.9	2.4	—	—
11	*C. sphaerospermum*	5.7	2.2	—	—
12	*Trichoderma viride*	1.5	1.1	—	—
13	*Trichothecium roseum*	—	—	1.7	1.5
14	*Alternaria alternata*	2.6	2.5	5.4	2.8
15	*Drechslera australiensis*	—	—	6.6	2.6

— indicates length of plumule less than 0.3 mm

3. *Role of Culture Filtrates of Two Fungi on Seed Germination*

In the present investigation it was found that both the species *A. fumigatus*, *p. nigricans* tested showed detrimental or enhancing effect on the germination of the seeds (table 3). It was interesting to note that the length of the seedlings increased when treated with the 5 and 15 days old culture filtrates of *Aspergillus fumigatus* and *Penicillium nigricans*, while 25 days old culture filtrates of both the species were found to be effective in suppression of the growth of plumule. This may be attributed to the maximum production of toxins in 25 days old culture. The *p*H of the medium also changed with incubation period (table 4). It is also clear that the filtrate with toxic effect could be both alkaline or acidic for example, *Aspergillus fumigatus* produced alkalinity in the medium and *Penicillium nigricans* turned the medium acidic but both equally inhibited the germination of seeds.

Armolick and Dickson (1956) has observed that the culture filtrates from different species had different inhibitory effects on germination of barley grains and they need varying periods for the production of toxins. Also most of the Aspergilli and Penicillia tested for their germination and sprouting of paddy seeds needed 20–25 days for the production of maximum toxic metabolites (Dwivedi 1978). It is true that certain species and their strains produce toxin and not the others. It is also possible that the toxin production can increase or decrease with change of substrate and the environment (Mehrotra 1976).

Table 4 *Influence of incubation period on the* pH *of the culture filtrates (initial* pH–6.7)

Isolates	Incubation period in days		
	5	15	25
Aspergillus fumigatus	6.7	7.0	7.5
Penicillium nigricans	6.5	6.0	5.5

Table 3 *Influence of culture filtrates on the shoot elongation of* Quercus incana *seeds*

Isolates		Age of the culture filtrates in days		
		5	15	25
Aspergillus fumigatus	Control	4.4*	4.0	5.3
	Treated	5.3	5.3	4.5
Penicillium nigricans	Control	4.6	.5.3	5.5
	Treated	6.4	6.5	3.5

* Values represent length of plumule in cm

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