FUNGI INHABITING ALDER PHYLLOPLANE

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Abstract : A total of 37 fungal species belonging to 26 genera of fungi were isolated from the phylloplane of alder (*Alnus nepalensis* D. Don) at different growth stages of the leaves in open and closed alder forests. The fungi increased gradually from folded to senescent stage and the peak was obtained at the maturity of the leaves. The least population was obtained at the bud or folded stages soon after flushing. *Penicillium fumiculosum*, *Alternaria alternata* and *Aspergillus nidulens* were dominant species immediately after flushing. When the leaves were undergoing maturation and entered into the senescent stage *Cladosporium herbarum*, *Trichoderma viride* and *Fusarium oxysporium* became dominant. The leaf spot disease caused by *Septoria alnifolia* of deuteromycetous pathogen was also observed during the study period.

INTRODUCTION

Alder (*Alnus nepalensis* D. Don) is an important tree in North-Eastern region and as a pioneer species in the prolific colonisation of degraded landslide prone or affected habitats. It is fast growing, highly efficient in biological nitrogen fixation and colonizes denuded habitats, freshly exposed soils and rocky and eroded slopes in the sub-tropical to temperate belts of the Himalaya (Sharma, 1988). There is no record available on the fungi inhabiting alder phylloplane. Therefore, the study was undertaken to analyse the fungi inhabiting phylloplane in relation to the age, climate and the forest canopy of alder stand which developed due to timber extraction.

MATERIALS AND METHODS

The study was conducted at Upper Shillong, 5 km away from Shillong , the capital of Meghalaya, North-East India, located at an altitude of 1600 m (MSL) between 25° 34' N lat. and 91° 57' E long. Two forest stands dominated by alder trees (*Alnus nepalensis* D. Don) were selected at the same latitude. Both faced the eastern side of the hill with undulated slope and were about 600 m apart from each other. Each of the two sites chosen was subdivided into closed and open forest stands depending upon the biotic disturbance in term of tree falling. The sites are closely comparable, the functional differences are attributed to their tree density. The closed forest comprised of 42 years old alder plantation being the original undisturbed stand and the other one the young open cleared forest stand of 23 years old along with a few tree stumps of *Pinus kesiya* and *Myrica esculenta* exposed to disturbances such as cutting twigs and collection of wood for fuel by the local inhabitants. Tree densities per hectare were 1180 and 383 in closed and open stands respectively.

The predominant understorey vegetation of closed forest stand was dominated by *Rubus ellipticus, Osbeckia crinata*, *Cassia mimosoides*, *Hedychium aurentiacum* and *Eupatorium adenophorum*. While the open forest stand was dominated by herbaceous weedy species *Ageratum conizoides*, *Lantana camara*, *Artimesia nilagirica* and *Eupatorium riperum*.

The study area is red loamy with fine silt and gravel constituting the major fraction (Sand 54%, Silt 25.9%) and acidic in reaction. The climate of the area is sub-tropical monsoonic type largely controlled and influenced by the Himalayan hill ranges and seasonal winds, like the south-west monsoonic wind and the north-east winter ones. On the basis of meteorological data, four distinct seasons, i.e., spring season (March to April),

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summer-rainy (May to Sept.), autumn (October to Nov.) and winter (Dec. to Feb.) are recognised. The maximum temperature recorded during 1990 and 1991was 24.3°C and 24.6°C respectively. The average minimum temperature was 15.5°C in 1990-1991. The monthly rainfall during 1990 and 1991 ranged from 2.1 mm to 421.2 mm and 0.1 mm to 574 mm respectively. The average humidity ranged from 69% to 89% in 1990 and 64.5% to 90% in 1991. Winter months are cold and dry and the temperature range was from 7.1 to 7.7°C. Low temperature of the winter results into frost during December and January months.

EXPERIMENTAL DESIGN

The survey extended from March 1990 when the leaves bud unfolded to January 1991 when they were senescent and falling. Collection of leaves was carried out at 15 days interval from different stages of leaf maturity (bud to abscission). Five alder trees of approximately equal age and height were selected for collecting leaves throughout the study period. From each tree five leaves were collected in aseptic condition at random from approximately equal height of the trees (3-5 m). Five replicate samples were comprised separately from the leaves of different degree of maturity after flushing as folding (bud), unfolding (buds opened) and young expanding green leaves up to the leaves which entered into the litter stage. During each collection the sample were collected in previously sterilized polythene bags using sterilized scissors and forceps. The bags were properly sealed and transported to the laboratory and the leaves were used on the same day for the isolation of fungi. Additional samples were collected for the determination of moisture content and pH.

The leaf surface fungi were examined using various techniques; direct cellophane impression (Edward and Hartman, 1951), nail polish impression (Masurovsky and Jordan, 1960), moist chamber (Keyworth, 1951), impression plate (Potter, 1910), dilution plate (Dickinson, 1971) and washed leaves plating. Several media such as potato dextrorse agar, Czapex's dox agar (Raper and Thom, 1949); cellulose agar (Eggins and Pugh, 1962) were used for the isolation of fungi. The plates were incubated at 25°C and colony forming unit of fungi were counted after 5 days. Identification of fungal species was done (Barnett, 1955; Gregory, 1973; Subramanian, 1971).

Physical analysis of the leaves : 10 g of leaves were collected and crushed in 25 ml of double distilled water and filtered. The filtrate was then used for determination of pH by electronic digital pH meter (Systronics, India). Moisture content of leaf was assessed by oven dry method at 105°C.

Quantitative estimation of fungi : Frequency of each fungus isolated by different techniques was determined using the formula of Tresner *et al.* (1954).

$$\text{Frequency of occurrence} = \frac{\text{No. of samples of occurrence in each culture plate}}{\text{Total No.of samples of occurrence in culture plate}} \times 100$$

Total population of fungi was determined using the formula :

$$\text{Fungal population /g} = \frac{\text{Colony forming unit of fungi x dilution (10)} \times \text{inoculum}}{\text{Dry weight of leaves (g)}}$$

Relative abundance of fungal species was obtained by the following formula:

$$\text{Relative abundance (\%)} = \frac{\text{Total No. of individual species of fungus}}{\text{Total No. of individuals of all species}} \times 100$$

Table-1: Percentage frequency of occurrence of phylloplane fungi at two forest stands of *Alnus nepalensis*

FUNGAL SPECIES	ISOLATING TECHNIQUES									
	D.O		I. P		W. L		M. C		D. P	
	O	C	O	C	O	C	O	C	O	C
Mucor hiemalis Wehmer	-	-	-	-	22	23	41	52	-	-
Rhizopus nigricans Ehrenberg	-	-	-	27	21	29	46	58	23	41
Pythium sp.	-	-	13	17	23	31	-	-	11	15
Chaetomium bostrychodes Zopf	-	-	-	25	-	27	-	42	-	24
C. globosum Kunze	-	-	-	-	-	-	5	6	36	11
Colletrichum capsicum (Syd) Butler & Bisby	-	-	-	-	-	-	-	5	-	-
Melanospora zamiae Corda	-	-	-	31	-	11	-	7	-	12
Phoma glomerata (Corda) Wollenweber & Hochapfel	-	-	26	14	31	37	43	51	36	16
Rhizoctonia solani Kuhn	-	-	-	8	-	-	-	32	-	-
Alternaria alternata (Fr.) Keissler	81	89	91	98	-	-	84	93	88	95
A. solani (Ellis & Mert.) Sorauer	-	-	64	69	85	87	-	-	3	6
Arthrinium sp.	-	-	-	61	70	68	-	11	18	-
Aspergillus clavatus Desm	-	-	-	-	-	-	-	-	-	32
A. flavus Link. ex Fries	-	-	66	71	50	55	87	88	43	41
A. fumigatus Fresenius	-	-	-	-	-	-	-	-	-	10
A. nidulans (Eidam) Winter	-	-	-	76	84	88	91	94	78	42
A. niger V. Tiegh	51	78	77	80	41	50	65	68	41	18
Cladosporium cladosporioides (Fres) de Vries	-	-	67	70	22	30	71	80	70	10
C. herbarum Link ex Fries	90	98	92	97	87	88	89	96	81	94
Curvularia lunata (Wakker) Boedijn	47	70	44	49	23	30	51	60	42	-
C. pallescens Boedijn	-	-	-	-	2	3	1	4	-	-
Fusarium oxysporium Schl. ex Fries	-	61	-	-	-	-	-	-	-	-
F. moniliforme Schldon	-	-	81	86	46	50	60	74	81	90
Gliocladium penicilloides Corda	-	-	-	8	-	10	-	-	-	12
Humicola grisea Traaen	-	-	-	10	-	16	-	-	-	21
Monilia sitophylla (Mont) Sacc.	-	-	-	-	-	21		-	-	-
Nigrospora oryzae (Berk. & Br. Petch)	-	30	-	10	-	11	-	-	-	37
Paecilomyces vertioti Bainier	-	-	-	-	-	-	-	-	44	49
Penicillium funiculosum Thom.	82	88	87	96	92	99	88	89	81	88
P. chrysogenum Thom.	-	-	44	48	3	6	23	31	45	24
Torula herbarum (Pers.) Link. ex Fries	-	-	-	1	-	-	-	3	-	7
Trichoderma viride (Pers.) Gray	31	47	45	48	70	76	90	94	80	82
T. roseum Pers.	-	-	70	73	-	24	-	21	-	18
Verticillium alboatrum	-	-	-	56	-	31	64	61	46	30
White sterile mycelia	-	23	-	43	-	42	-	45	41	50
Orange sterile mycelia	-	-	30	24	-	-	3	2	-	-

D.O = Direct observation technique, W. L. = Washed leaves plating technique, I. P. = Impression plate technique,
M. C. = Moist chamber technique, D. P. = Dilution plate technique, O = open, C = closed.

Based on the frequency, fungi are grouped as dominant, 80 – 100%; common, 61 – 80%; frequent, 41 – 60.5%; occasional, 21 – 40% and rare, 1 – 20% (Vittal, 1976).

RESULTS

A total of 37 and 26 fungal species were isolated from the phylloplane of alder at different growth stages of the leaves in closed and open forest stands respectively (Table-1). The results obtained from different techniques of isolation although gives some picture about the colonization of different fungi on the phylloplane region but they may entice misleading speculations about successional patterns. As there is no single method which can selectively remove the mycelia directly from the leaf surface for culture purpose. The information gained from all the different techniques used simultaneously was taken as a measure of fungal activity *in vivo* (Dickinson, 1971). The moisture content of leaves at different stages of maturity varied. It was higher in rainy months (55% to 65%) and lower in winter and during senescent stage of the leaf (20% to 30%) in both the sites . Initially the leaves were less acidic (5.0 to 5.5) in both the sites which subsequently became more acidic (3.5 to 4.7) in winter and at the senescent stages. Direct observation methods (Table-1) yielded *Cladosporium herbarum*, *Penicillium funiculosum* and *Alternaria alternata* as dominant species on the phylloplane while impression plate method also allowed *P. funiculosum*, *A. alternata*, *C. herbarum* and *Fusarium moniliforme* as a major dominant species and *Aspergillus niger* and *Trichoderma viride* were common in occurrence in both the stands. By plating washed leaves some idea about the actual activity of fungi which had penetrated the leaves could be obtained that fungi like *A. solani* and *C. herbarum* were the dominant colonizers in addition to fungi like *A. nidulans* and *P. funiculosum*. Moreover, fungi like *Arthrinium* sp. and *T. viride* were of common occurrence and fungi like *Mucor hiemalis*, *Rhizopus nigricans* and *Pythium* sp. were isolated occasionally in both the forest stands. The fungi recorded on leaf surface of both the forest stands by moist chamber technique exhibited that Aspergilli, Mucorales and a few hyphomycetes and ascomycetes were frequent on matured leaves and later on as the leaves started senescing, few species like *A. alternata, A. flavus, A. nidulans, A. niger, C. cladosporioides, C. herbarum, Curvularia lunata, F. moniliforme, P. chrysogenum, P. funiculosum, T. viride* and *Verticillium alboatrum* appeared. These species seem to be foremost and frequent colonizers on senescent leaves with high frequency of occurrence.

Relative abundance of fungal species varied in open and closed canopy forests. The composition of the phylloplane colonizers was also influenced by the age of the leaves. Dilution plate technique exhibited that *A. alternata, C. herbarum, F. moniliforme, P. funiculosum* and *T. viride* dominated the entire phylloplane region at different stages of the leaves in both the forest stands. Higher count of fungal propagules was during late summer rainy period (August and September) on matured leaf of both the forest stands (Fig. 1). *P. funiculosum, A. alternata* and *C. herbarum* were the dominant species immediately after flushing in both the stands. When the leaves were under going maturation and entered into the senescent stage where *C. herbarum, T. viride* and *F. moniliforme* were dominant colonizers. In addition, *R. nigricans, Phoma glomerata, A. flavus, Paecilomyces verioti, V. alboatrum* and white sterile mycelia occurred with low frequency in both the forest stands (Table-1). Fungi like *Chaetomium bostrychodes, Collectrichum capsicum, M. zamae, Rhizoctonia solani, A. clavatus, A. fumigatus, Gliocladium penicilloides, Humicola grisea, Monilia sitophylla, Torula herbarum* and *Nigrospora oryzae* were of rare occurrence and present only in the closed forest stand (Table-1).

The leaf spot disease of *Alnus nepalensis* D. Don was detected by all the isolation techniques caused by *Septoria alnifolia* a deuteromycetous pathogen. The disease first appear on the young green leaves in the month of April-May of the study period forming a small round yellow spots on the leaves which gradually turn reddish brown on mature green leaves up to the stage of senescent (June to December).

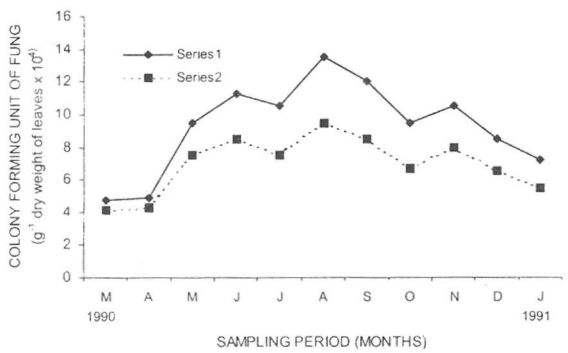

Fig. 1: Colony forming unit of fungi inhabiting alder phylloplane during differrent ages of the leaves at two forest stands (Series 1= closed, Series 2= open).

The variation in the fungal population of the young stage of alder leaves between the two stands (closed and open) was non significant but statistically significant ($p<0.01$) between different seasons and matured and senescent leaves ($p<0.05$). Phylloplane fungi showed a positive correlation ($p < 0.05$) with moisture contents and relative humidity of atmosphere.

DISCUSSION

The composition of fungi during different stages of the leaves was similar in both the alder forest stands which could be attributed to the similarity of the resource quality. The representatives of phycomycetes and ascomycetes fungi were weak colonizers, whereas deuteromycetes were strong colonizers, showing better adaptability and higher competitive saprophytic ability (Garg and Sharma, 1985). Population of fungi increased gradually from folded to senescent stage and the peak was attained at the maturity of the leaves. Low population of the folded leaves to young green leaves was due to the presence of thick waxy cuticle on the leaves. Although young leaves appear delicate and fragile, they are less susceptible to leaching than are the older leaves (Tukey and Morgan, 1963). However, low population during the months of December and January on senescent leaves was due to extreme desiccation temperature and low relative humidity in these months. An increase in number of fungi with the age of leaf due to increased availability of nutrients on older leaves (Sinha and Dayal, 1983; Adhikari,1990). The change in succession of fungi on leaf was related to the qualitative changes in nutrients exudated from aerial plant parts (Tukey, 1971 & Dick, 1992). An increase in moisture content of the leaves towards maturity encouraged the uniform spread of leached nutrients on the surface which favoured the growth of fungi vigorously in the month of August and September in both the alder stands when the temperature and relative humidity were also favourable. The increasing trend of fungal communities may also be due to weathering of surface waxes (Forester, 1977), decreased amount of phyto alexin (Bailey, 1971) and the expanding leaf provide more surface areas to be occupied by the fungi. The higher number of phylloplane fungi of matured green leaves than their corresponding senescent leaves was possibly due to lower relative humidity and moisture content during winter (Nov.-Feb.). However, at senescent stage in the month of November onward there was an increase in percentage frequency of saprophytic fungi like *F. moniliforme*, *T. viride*, *P. funiculosum* , *V. alboatrum* and white sterile mycelia which indicate their preparatory role for the ensuing senescent stage. Their increase at senescence can be explained on the basis of increased dead tissue which provides niches to saprophytes for their multiplication and growth (Dickinson, 1976 & Mishra and Dickinson, 1981). pH of the leaves which varied from folded to senescent stage did not affect much the pattern of colonization of alder leaves by different fungi whereas some have noticed a change in the distribution of fungi both quantitatively and qualitatively (Dickinson, 1981 & Rao and Manoharachary, 1981).

Meteorological factors such as atmospheric temperature, humidity and rain were important factors to regulate the fungi significantly on the leaf surface (Hayes, 1982 & Adhikari, 1990) whereas microclimatic changes did not affect the fungal community on leaf surfaces of the two forest stands. It was clear that if host species, height of tree, age of leaf and macroclimatic factors are constant, the microclimatic factors have little role in altering the colonization and succession of fungal community on

leaf surfaces. The number of fungi and their specific composition was affected by the weather conditions. Some being present throughout the growing period whereas others were exclusively associated with a particular set of climate factors indicating their ecological amplitude (Hayes, 1982 & McCartney, 1994). In addition prevalence of significant superficial colonist of fungi on different age of the leaves reflected the abundance of the species in the air during different sampling periods (Andrews, 1992 & Kayang, 1993). The leaf spot disease primarily, is seed borne, which however may be disseminated by other factors such as wind, soil and insects. Thus the distribution of fungi on leaf surface was dependent mainly on leaf maturity and weather changes. In the present investigation distinct successional groups of fungi could not be obtained in the alder forest stands.

REFERENCES

Adhikari, R.S. (1990). Phylloplane mycoflora of three grasses : A mycological study. *Trop. Ecol.*, **31**(1): 64-68.

Andrews, J.H. (1992). Biological control in the phyllosphere. *Annual review of Phytopathology*, **30**: 603-635.

Bailey, J.A. (1971). Phyto alexins and the ability of leaf tissues to inhibit fungal growth. In: T.F. Preece and C.H. Dickinson (eds.) *Ecology of leaf surface microorganisms*. Academic Press, London. 519-528 pp.

Barnett, H.L. (1955). *Illustrated genera of imperfect fungi*. Minneapolis : Burgess Publishing Co. 218 pp.

Dickinson, C.H. (1971). Cultural studies of leaf saprophytes. In: T.F. Preece and C.H. Dickinson (eds.) *Ecology of leaf surface microorganisms*. Academic Press, London. 122-137 pp.

Dickinson, C.H. (1976). Fungi on the aerial surfaces of higher plants. In: C.H. Dickinson and J.F. Preece (eds.) *Microbiology of aerial plant surfaces*. Academic Press, London. 293-324 pp.

Dickinson, C.H. (1981). Biology of *Alternaria alternata, Cladosporium cladosporioides* and *C. herbarum* in respect of their activity on green plant. In: J.P. Blackman (eds.) *Microbial Ecology of the phylloplane*. Academic Press, London.

Dik, A.J. (1992). Interactions among fungicides, pathogens, yeasts, and nutrients in the phyllosphere. In: J.H. Andrews and S.S. Hirano (eds.) *Microbial ecology of leaves*, Springer, New York, 412-429 pp.

Edward, R.W. and Hartman, E. (1951). A simple technique for collecting fungus specimens from infected surface. *Llyodia*, **15**: 39.

Eggins, H.O.W. and Pugh, G.J.F. (1962). The isolation of cellulose decomposing fungi from soil. *Nature*, **193:** 94-95.

Forester, G.F. (1977). Effect of leaf surface wax on the decomposition of air borne propagules. *Trans. Br. Mycol. Soc.*, **68:** 245-250.

Garg, A.P. and Sharma, P.D. (1985). Phylloplane mycoflora of healthy and white blister infected mustard. *Indian Phytopath.*, **38:** 527-529.

Gregory, P.H. (1973). *Microbiology of the atmosphere*. Leonard Hill Publication, London. 377 pp.

Hayes, A.J. (1982). Phylloplane microorganisms of *Rosa* cv picadilly following infection by *Diplocarpon rosae*. *Trans. Br. Mycol. Soc.*, **79**: 311-319.

Kayang, H. (1993). Ecological studies on biodegradation of leaf litter of alder (*Alnus nepalensis* D. Don) with special reference to the role of microbes and isopods. *Ph.D.Thesis*, North Eastern Hill University, Shillong, India.

Keyworth, W.G. (1951). A petridish moist chamber. *Trans. Br. Mycol. Soc.*, **34**: 291-292.

Masurovsky, E.B. and Jordan, W.K. (1960). A plastic replica embedding and staining technique for studying the behaviour of microorganisms on food contact surfaces. *J. Dairy. Sci.*, **43**: 1000 pp.

Mc Cartney, H.A. (1994). Spore Dispersal : Environmental and Biological factors. In: J.P. Blakeman and B. Williamson (eds.) *Ecology of plant Pathogens*. CAB International, UK. 171-185 pp.

Mishra, R.R. and Dickinson, C.H. (1981). Phylloplane and litter fungi of *Ilex aquifolium*. *Trans. Br. Mycol. Soc.*, **77:** 329-337.

Potter, M.C. (1910). Bacteria in relation to plant pathology. *Trans. Br. Mycol. Soc.*, **3**: 150-168.

Rao, M.M. and Manoharachary, C. (1981). Studies on phylloplane fungi of two medicinal plants. *Proc. Nat. Acad. Sci. India*, **51** (B) IV: 361-366.

Raper, K.B. and Thom, C. (1949). *Manual of the Penicillia*. Baltimore, md., The Williams and Wilkins Co.

Sharma, E. (1988). Altitudinal variation in nitrogenase activity of the Himalayan alder naturally regenerating on landslide affected sites. *New Phytologist*, **108**: 4111-4116.

Sinha, A. and Dayal, R. (1983). Fungal decomposition of teak leaf litter. *Indian Phytopath.*, **36**: 54-57.

Subramanian, C.V. (1971). In: *Hypomycetes- an account of Indian species, except Cercospora*. ICAR Publication.

Tresner, H.D.; Backus, M.P. and Curtis, J. T. (1954). Soil micro-fungi in relation to hardwood forest continuum in southern Wisconsin. *Mycologia*, **46**: 314-333.

Tukey, Jr. H.B. (1971). Leaching of substances from plants. In: T.F. Preece and C.H. Dickinson (eds.) *Ecology of leaf surface microorganisms*. Academic Press, London.

Tukey, Jr. H.B. and Morgan, J.V. (1963). *Physiol. Plant*, **16**: 557-565.

Vittal, B.P. (1976). Studies on litter fungi. 1. Mycoflora of *Atlantia* and *Gymnosporia* litter. *Proc. Indian Acad. Sci.*, **83**(B): 133-138.