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Pollution influenced mycofloral load residing on the phyllosphere of Birbhum district of West Bengal

KONINEEKA SEN[1], CHANDAN SENGUPTA[1] AND SUKANTA KUMAR SEN[2]

[1]Mycrobilogy Laboratory, Department of Botany, University of Kalyani, Kalyani, Nadia 741235, West Bengal, and [2]Microbiology Laboratory, Department of Botany, Visva Bharati, Santiniketan, Birbhum 731 235, West Bengal

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The phyllospheric fungal diversity and load of Bolpur and Santiniketan areas of Birbhum district were studied by *in vitro* growth of roadside leaf discs on suitable fungal media. Largest diversity and highest population density of the different species was noted during the monsoon, followed by higher numerical statistics during summer with the prevalence of high humidity and sporadic rains but contrastingly a significant low variation and load was noted during the winter months. The load was highest in case of *Hibiscus* leaf discs of both the areas in all seasons. The pollution dominated flora of the urban area showed predominance of *Alternaria* spp under the influence of O_3, SO_2, smoke, and *Trichoderma* under smoke and dust respectively, These had a positive allelophathic influence on *Aspergillus* proliferation and survival. Other species were adversely inhibited probably by SO_2, NO_x. Ascomycetes are capable of survival in methane and ammonia rich environment. A relatively pollution free environment is required for the growth of *Geotrichum* and *Helminthosporium.*

Key words : Air pollution, fungal flora, phyllosphere, rural, urban

INTRODUCTION

The aerial surfaces of plants are colonized by diverse groups of microorganisms including bacteria, yeast, fungi, less frequently by nematodes and protozoa. Microbial communities residing on the leaf surface and its vicinity have been studied by several workers since the beginning of last century. This zone of the leaf densely populated by microorganisms that exert their influence on the physiology and growth of plants (Last, 1955, Ruinen, 1956). Bacteria being the most abundant inhabitants of the phyllospere, have been extensively studied and documented by researchers for many years. (Ercolani, 1991, Thompson *et al.,* 1993, Andrews *et al.,* 2000). Due to the increase in the pollution level, fungal spore in the air as well as on the plant surface are increasing day by day and their population sizes differ sharply with the seasonal variation depending on temperature and humidity, physical and nutritional requirements. The major factors affecting the growth and survival of the fungal populations on leaf surfaces depend on the availability of the fungal inocula that are available from the atmosphere. Rain plays a significant role in dispersal of its viable propagules. Epiphytic fungal population fluctuation depending on the nature of the leaf colonized including macro and micro morphology, surface chemistry of higher plant organs have been studied and Cutter (1969). Ruinen (1961) has reported that long lived, broad leaved trees in the tropics support dense and complex populations in contrast to short lived, narrow leaves of the temperate region which show simple microbial population. Long lived tropical leaves are more preferred by a wide range of fungal populations, especially the slow growing lichenised species dwelling on the leaf surfaces due to a combination of factors, chiefly the chemical nature of leaf surfaces (Godfrey, 1976). Substantial amounts of sugars and amino acids are deposited on the leaf surfaces by the process of gutation (Goatley and Lewis, 1966) along with the chemicals produced by the surface microbes undoubtedly influence the leaf surface environment. Various pollutants such as solid non-viable inorganic particles encourage or inhibit surface microbial growth and activity (Babich and Stozky, 1974; Saunders, 1971, 1973). Under conditions of low air pollution level, the microbes residing in the phyllosphere serve as source and sink for air contaminants. Under such conditions, microbes acting as a source of air pollution exert allelopathic influence on the ecosystem. Microbes significantly contribute as a source of air pollution that can be evidenced from the levels of emissions of carbon monoxide, hydrogen sulphide, ammonia, oxides of nitrogen and hydrocarbons derived from natural sources that remarkably exceeds that from anthropogenic sources (Robinson and Robbins, 1968), Plant surface micro-organisms also release trace contaminants to the atmosphere as *Puccinia graminis tritici* release n-normal (French and Weintraub, 1957). Carbonmonoxide by several fungi including *Aspergillus niger, Fusarium* sp. and *Cephalosporium* sp (Westlake *et al.* 1961). Babich *et al.* (1974) have reported microbial volatiles released by plant surface microbes impose allelopathic influence that extends to include enhancement or suppression of growth, infectivity, reproduction, alteration of morphology on plant surface ecosystem. Fungal volatiles either accelerate (Dick and Hutchinson, 1966) or inhibit (Robinson *et al.*1968) spore germination of other fungi on plant surface niche. The phylloplane microbes also serve as a major sink for the naturally occurring organic volatiles in the air below the plant canopy of tropical forests (Rasmussen and Hutton, 1972). The fact that various ascomycetes and plant surface bacteria are capable of growing in ammonia, nitrogen and methane rich atmosphere (Siegel and Giumarro, 1965) and the ability of tropical forest leaf litter microbes to degrade automobile exhaust hydrocarbons positively indicates the role of microbes in removal of atmospheric pollutants at plant surface-air interface. Critical concentrations of air pollutants, viz sulphur dioxide in urban atmosphere cause acute diseases of air-borne and leaf surface micro-organisms eventually leading to seroius increase in mortality of such organisms like *Serratia mercescens* (Lighthart *et al.* 1971); growth of lichen and mosses, their reproduction, chlorohhyll content, structures are restricted by toxicity of HF, SO_2 like contaminats in the air (Nylander, 1866, Pyatt, 1970). Differential influence of air pollutants exerted on plant surface saprophytes and parasites have profound consequences. Till date very little work has been done on phyllosphere microbes in Indian context and considering that an attempt has been made to study the variation in phyllospheric fungal flora of selected, polluted and less polluted areas along with their seasonal fluctuations of Birbhum district of West Bengal.

MATERIALS AND METHODS

Isolation of fungi from the roadside leaves and enumeration of fungi

Fungi were isolated from the phyllosphere of healthy leaves collected from the polluted roadside low-height trees and shrubs, commonly growing in six selected spots of highly polluted Bolpur town and five spots of outskirts (Santiniketan), susceptible to low pollution levels of Birbhum district of West Bengal. The study was conducted during the period of December 2009 to August 2010. The collection was done in three different phases depending on seasonal variation during midday and late in the evening when the pollution level is high in separate sterile plastic bags measuring 3 x 2", with the help of sterile hand gloves and brought into the laboratory, Leaf discs of 1 cm^2 area were placed on malt extract agar medium within 2-3 hrs of collection, with their dorsal and vental surfaces in contact with the medium in separate plates respectively, maintaining aseptic condition for isolation of fungal organisms following leaf impression method. The plates were incubated at 28 ± 1^0C in a incubator for 24 hrs. Similar leaf discs of 1 cm^2 diameter were added to sterile 100 ml distilled water and shaken well with a magnetic stirrer, serially diluted and plated on Potato Dextrose agar with aliquots of 10^{-2}, and 10^{-3} dilutions, maintained at 28 ± 1^0C for total plate count.

Identification of fungal isolates

The colony morphology of the different isolates obtained from different phyllospheres were recorded and microscopic evaluation of the same were carried out after staining the isolates with cotton blue and lactopenol for genus identification. Morphological variations of the isolates of genus from different localities were compared.

Meteorological parameters and pollution status on fungal load fluctuations and vice versa

Data on average rainfall and temperature during the experimentation period and previous years were obtained from Agriculture faculty of the same

University and inferences on the nature and concentration of the major atmospheric pollutants in the two areas of our interest were drawn from the correlation between existing fungal diversity and the anthropogenic activities of the local people based on previous literature studied above. The subsequent role of the fungal community in contribution of the gaseous pollutants to the atmosphere and subsequent removal were also studied from their distribution pattern and the probable predominant pollutants in the locality.

RESULTS AND DISCUSSION

In present study, the fungal populations residing in the different phyllospheres along with their colony load were tabulated for three consecutive seasons, viz. winter, summer and monsoon respectively (Tables 1 and 2). The effects of pollution on fungal population as well as the contributions of fungal counterparts to such pollution is shown in Tables 3 and 4.

Phyllosheric microfungi were numerous and diverse as revealed from our present study. Their diversity and population fluctuations were significant in different seasons and varied with the pollution level and nature of pollutants, in the environment more precisely atmospheric pollutants. Of the ten genera isolated most of them belonged to Deuteromycetes group and a few Ascomycetes. From Tables 1 and 2, the colony load and fungal types were hightest in *Hibicus* phyllosphere in both the areas and in all seasons, followed by *Mangifera, Adhatoda Tabernaemontana, Alstonia, and Nerium,* respectively indicating that broad leaved phyllosphere were preferentially inhabited by phylloplane dwelling microfungi (Ruinen, 1969). Though a contrasting fluctuation was noted in *Nerium,* showing relative abundance of species and number during summer, perhaps being due to the cuticular interaction with the water of gulation meeting congenial growth requirements of many such fungi (Goatley, 1966). Colony forming units were relatively high during the monsoon in all the six phyllospheres involved in our

Table 1 : Fluctuations in phyllospheric fungal load during the different seasons in Bolpur area

Time of collection[a]	Fungal load of the different phyllospheres of Bolpur (urban) area[b]											
	Adhatoda		Alstonia		Hibicus		Mangifera		Nerium		Tabernaemontana	
	Ad[c]	Ab[c]	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab
December-February	4.3	4.6	2.8	3.0	5.0	4.9	4.1	4.0	2.3	1.9	3.8	3.5
March-May	4.7	5.0	3.2	3.4	5.1	5.4	4.5	4.2	3.0	2.9	4.1	3.9
June-August	4.9	5.1	3.5	3.8	5.9	5.5	5.1	4.9	2.7	2.5	4.7	4.2

[a]Leaf samples were collected thrice during period at an interval of 30 days.
[b]Fungal load expressed as mean of 10 leaf discs as number of colonies/cm2 of leaf tissue isolated by washed leaf disc method.
[c]Ad, Ab represents the adaxial (upper) and abaxial (lower) surfaces of leaves respectively.

Table 2 : Fluctuations in phyllospheric fungal load during the different seasons in Santiniketan

Time of collection[a]	Fungal load of the different phyllospheres of Bolpur (urban) area[b]											
	Adhatoda		Alstonia		Hibicus		Mangifera		Nerium		Tabernaemontana	
	Ad[c]	Ab[c]	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab
December-February	2.7	2.8	2.3	2.4	3.9	3.7	3.1	2.9	1.1	1.0	2.7	2.7
March-May	2.9	3.1	2.5	2.6	4.1	3.8	3.4	3.0	1.9	1.5	3.0	2.8
June-August	3.1	3.3	2.6	2.7	4.5	4.0	3.6	3.3	1.6	1.4	3.1	2.9

[a]Leaf samples were collected thrice during period at an interval of 30 days.
[b]Fungal load expressed as mean of three during each period
[c]Ad, Ab represents the adaxial (upper) and abaxial (lower) surfaces of leaves respectively.

Table 3 : Effect of pollution on the distribution of fungal flora of Bolpur

Cololection spot[a]	Pollution status[b]	Sources of pollution[c]	Probable pollutants	Ecological indication[d]	Fungal flora	Effect on fungal community
Bhubandanga	Moderate	Vehicles burning of fossil fuels, fungal community	CO, CO_2, SO_2 N_2O, O_3, poly-cyclic aromatic hydrocarbons	Interveinal and veinal chlorosis of leaves, necrosis, low species diversity	*Alternaria Aspergillus,*	Carbon Particles, N_2O inhibits growth of several fungi
Bolpur Raiway Station	High	Diesel engines automobiles burning of fossil fuels, urbanization	CO, CO_2, hydro-carbons, NO_x, respirable particulate matters	Brown necrosis from tip downwards, respiratory distress of humans	*Trichoderrma Penicillium*	Absence of conidia of *Penicillium*
Bolpur Road	High	Heavy traffic Petrol pump stations, hardware coal, cement shop	CO, CO_2, O_3, Pb, PAN, SO_4	Headache, eye irritation, avarage increase in annual temperature range decrease in vegetation cover	*Alternaria Trichoderrma*	Retarded growth and reproductive suppression, diversity low
Jamboni	Moderate	Heavy vehicles, small, factories	CO, NO_x, Heavy metals carbon particulates	Decrease in leaf size, solid crust on leaves, flaccidity and drooping of leaves	*Carvularia*	Growth retarded by heavy metals and particulates
Santiniketan Road	Low	Swampy areas ponds, methanogenic bacteria fungi.	CH_4, NH_3, CO, hydrocarbons	Cooked green appearance of leaves, subsequent browning on drying	*Penicillium Aspergillus*	NH_3 and hydrocarbons stimulate removal by microbes
Super Market	High	Heavy trafic, burning of fossil fueles, air conditioners,dyeing of textiles, earthen ware making	CO, CO_2, CFC NO_x, SO_2, particulate matters	Bleaching of chlorophyll of lichens mosses, veinal and interveinal chlorosis of leaves, increase in annual temperature	*Aspergillus, Cephalosporium, Fusarium*	Low smoke emission permits the growth of *Aspergillus*

[a]Collection spots selected on the basis of vulnerability to pollution threats, Bolpur represents pollution sensitive and Santiniketan relatively less sensitive areas respectively. Collection done randomly from ten samples of each plant taxa from every spot.
bPollution status designated on the basis of anthropogenic activity and vegetation pattern of the locality
[c]Sources of pollution arranged according to their intensity of contribution to atmospheric pollution
[d]Meteorological data on average annual temperature and rainfall have been gathered from Pally Shiksha

study due to the rains and wind facilitating the dispersal of spores and propagules and its subsequent germination and diminished during summer, lowest being recorded during winter months (Cutter, 1969). Generally ventral surface showed higher population load than dorsal surface. Dust and particulate deposition on the ventral surface of leaves had retarded fungal growth in Jamboni locality of Bolpur, whereas it had triggered proliferation in Santiniketan in association with other factors as evidenced from Tables 3 and 4 that is in conformity with the findings of Manning (1971). Only methane rich environment of Sriniketan and Ballavpur sanctuary supported large population and species range on the ventral surfaces, previously witnessed by Rasmussen and Hutton (1972). As reported by earlier workers, though primarily phyllospheric microbes served as sink for the atmospheric contaminants, seldom they may serve as source of such pollution as observed in Gurupally locality of Santiniketan (Westlake *et al.,*1961). From Tables 3 and 4, it is clear that *Alternaria* spp predominates in ozone rich environment, as observed by Treshow *et al.,* (1969), that may have positive allelopathic influence on survival and spore germination of *Aspergillus* as noted in the locality of Bhubandanga (Dick and Hutchinson, 1966). Though *Aspergillus* have been reported to be highly sensitive to smoke

Table 4 : Effect of pollution on the distribution of fungal flora of Santiniketan

Cololection spot[a]	Pollution status[b]	Sources of pollution[c]	Probale pollutants	Ecological indication[d]	Fungal flora	Effect on fungal community
Gurupally	Low	Domestic fuel, microbial sources	CO, CO_2, particulate matters N_2O	Chlorosis and basal scorching of leaves, relative abundance of fungal population	*Aspergillus, Fusarium, Geotrichum Cephal–osporium*	Nutritional stimulation for CO, H_2S, rich diversity
Purbapally	Moderate	Diesel locomotives, automobiles , gardening	CO, hydrocarbons, NO_x, pesticides fertilizers	Reduced blossom, early leaf fall incrustatation on leaves	*Geotrichum Penicillium*	High density
Ballavpur Sanctuary	Low	Deforestation human intervention	CH_4, NH_3, water vapour dust	Browning, cook ed green leaves infrequent rains	*Geotrichum Aspergillus Trichoderma*	NH_3 tolerance stimulates hydrocarbon degradation, rich species diversity, adundance
Ratanpally	Moderate	Local civic market, coal burning	CO_2, CH_4, hydrocarbons	Incrustation on leaves, prematured yellowing	*Aspergillus Colletotrichum*	Low smoke and gases allows growth of pollution sensitive genera
Sriniketan	Low	Agricutural practices, paddy fields	CH_4, NH_3, particulate residues	Lush green vegetation heavy blossom	*Helminthos-porium Collectotrichum*	Methane degraders

[a]Collection spots selected on the basis of vulnerability to pollution threats, Bolpur represents pollution sensitive and Santiniketan relatively less sensitive areas respectively. Collection done randomly from ten samples of each plant taxa from every spot.
bPollution status designated on the basis of anthropogenic activity and vegetation pattern of the locality
[c]Sources of pollution arranged according to their intensity of contribution to atmospheric pollution
[d]Meteorological data on average annual temperature and rainfall have been gathered from Pally Shiksha Bhavan, Faculty of Agriculture, Visva Bharati, Santiniketan, Ecological indications were inferred from last five years study

(Parmeter, 1975), indicating that the smoke and gas emission is tolerably low the genus to thrive. On the contrary, heavy smoke and dust have probably suppressed the growth of *Aspergillus* in the adjacent area of the railway station. Higher concentration of ozone in the atmosphere is known to have adverse effect on growth and proliferation of numerous fungi growing *in vitro* such as *Trichoderma viride, Penicillium egyptiacum* (Babich and Stotzsky, 1974), that is evident from the result of the locality of Bhubandanga.

Severely polluted localities of Bolpur showed limited mycofloral diversity, where only *Alternaria, Penicillium* and *Trichoderma* can thrive. *Curvularia* seems to be insenitive to heavy metal and particulate pollutants.

Unlike Bolpur, three new genera were found in Sriniketan (Santiniketan), viz. *Colletotrichum, Geotrichum* and *Helminthosporium* respectively, indicating that pollution free environment is required for the growth and proliferation of such genera (Gorny *et al.*,2002). Dust and particulates have encouraged the growth of *Colletotrichum* (Manning, 1971). In conclusion, this study has provided an overall information on the pollution status of Bolpur and Santiniketan and is invaluable for exploring the causes of the fast disappearance of the floristic glory of this area.

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