Phylloplane Flora of Some Novel Medicinal Plants of Family Ficaceae.

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ABSTRACT

The Present investigation was conducted during the year 2012-2013, to study the phylloplane flora from some novel plants of family Ficaceae. It was observed that in nature the plants faces several problems in terms of attack of several pathogens, which cause the diseases. By keeping this view in mind, the above titled study was conducted to observe the relative phylloplanic flora on the sacred plants of family Ficaceae. It was reported that incidence of many fungi occurs on the surface of these plants, namely *Fusarium, Aspergillus niger, Chaetomium, Aureobasidium,Cunninghemella, Curvularia , Trichoderma, Alternaria. Aspergillus sp.,Phoma,* and *Rhizopus sp.*Therefore, it was concluded that these sp. of fungus were of common occurrence within this year on plants.

Key words: Phylloplane Flora, Novel, Medicinal Plants, Ficaceae, *Ficus bengalensis, F. Religiosa, F. Glomerata, Fusarium, Aspergillus niger, Chaetomium, Aureobasidium,Cunninghemella etc.*

Introduction:

Fungi are found every where, its incidence is variable and it variously occurs in water, in soil, in air, and even in an Antarctic region too i.e. in snow also. The term aerobiology was first coined by the American plant pathologist "Fred Cambell meier" in 1930. Hence, the term aerobiology came in to existence since 1930's to denote the airborne fungal spores, pollen grains and for other airborne micro-organism. Therefore, aerobiology deals with the study of airborne fungal spores, pollen grains, and other airborne micro-organisms. The outdoor environment is never completely free from the incidence of microbial propagules, which are collectively called as "air spora".

The word "Aerobiology", is a huge word that deals with every corner's of the world, and in large parts with bio-particles present in air, it contributes a lot, in enumeration of types of bio-particles present in the air. Among all, the air borne bio-particles, spore's from algae, fungi, bryophytic, pteridophytic, gymnospermic materials, and pollen's from angiospermic flower's contributes the greatest and most important portion in air(Salvaggio, and Lars.,1981). On this basis, of the recent aerobiological investigations, it can be broadly classified into two categories as Indoor or Intramural aerobiology and Outdoor or Extramural aerobiology, Tilak, (1982).

Biodiversity is the variation of life forms in a given ecosystem. Biodiversity is to be studied to avail the knowledge and behaviour, and interaction between the living things in a particular environment or in a biological system. Study of fungi is essential for anyone who collecting or monitoring the any fungi from the study area or from a particular environment. Several workers develop the different methods to evaluate the diversity and distribution from their own way. A wealth of information, especially regarding sampling protocols, compiled by an international team of fungal biologists, make biodiversity of fungi an incredible and fundamental resource for the study of organism biodiversity.

The related term phylloplane, the surface of plant leaves is a complex terrestrial habitat that is characterized by a variety of

microorganisms including the bacteria, filamentous fungi and yeast. Phylloplane fungi are the mycota growing on the surface of leaves. There are two groups of phylloplane fungi: residents and casuals. Residents can multiply on the surface of healthy leaves without noticeably affecting the host. Whereas, casuals land on the leaf surface but cannot grow. Phylloplane fungi have been poorly studied as compared to endophytes, saprobes and pathogenic fungi.

A. H. M. El-Said.,(2001) reported the seventy three species and five varieties belonging to 36 genera were collected from leaf surfaces of banana plants on glucose and cellulose-Czapek's agar media at 28^{o}C. The results obtained from leaf surfaces (phyllosphere and phylloplane) were basically similar on the two types of media and the most common fungi were *Alternaria, Aspergillus, Chaetomium, Cladosporium, Cochliobolus, Curvularia, Gibberella, Memnoniella, Mycosphaerella, Setosphaeria* and *Stachybotrys*. The monthly counts of these fungi were irregularly fluctuated giving maxima at various months. *Chaetomium globosum* was in the top of fungi in producing both exo and endo □-1, 4-glucanases among the 34 tested isolates obtained from leaves (phylloplane) on cellulose-Czapek's agar. Maximum production of these enzymes by *C. globosum* was 6 and 8 days after incubation at 25^{o}C with culture medium containing wheat bran as a carbon source and peptone as a nitrogen source and initially adjusted to pH 6.

Cunningham, (1873) reported the changes in the weather condition, affects the air-spora both qualitatively and quantitatively, while Marchisio, and Airarudi.,(2001) reported the relative humidity, temperature and rainfall plays a key role in the occurrence of fungal spores in the indoor air of library. In addition to this, Florian, (1994) reported the fungal growth on materials is initiated by conidia from air-spora which have fallen on the surface and germinates. Aero-mycology deals with the study of air borne fungi and their spores. Fungi have both positive and negative effects on our lives from the negative point of view, they destroy our food, fabrics, leather, wooden articles, museum specimens, and other similar articles, they are also responsible for causing a large number of diseases in the plants like Rust, Smut, Blight, Mosaics etc. They can also cause diseases in humans like ringworm, athlete's foot, and several more serious diseases caused by fungi, because fungi are more chemically and genetically similar to animals than other organisms, this makes fungal diseases very difficult to treat.

Aureobasidium pullulans, *Sporobolomyces roseus*, and *Cryptococcus laurentii* var. *flavescens*, added to the inoculums, reduced the superficial mycelial growth of *Septoria nodorum*, and the infection of wheat leaves by 50% or more. The mycelial growth was affected similarly in vitro, on slides covered with water agar. The antagonistic effect on germination was slight. The concentration of the saprophytes on the leaves after inoculation was comparable to population densities occurring on field-grown wheat (N. J. Fokkema., and F. van der Meulen., 1976).

Lot of investigations have been carried out on the phylloplane flora of leaf surfaces of several plants growing in garden or cultivated in many parts of the world by several researchers (Abdel-Fattah et al., 1977; Abdel-Hafez, 1981, 1984, 1985; Abdel-Hafez et al., 1995; Eicker, 1976; Khallil and Abdel-Sater, 1993; Mazen et al., 1985; Nagaraja, 1991; Sharma, 1974). EI-Said A.H.M. (2001) reported the fungi from leaf surfaces (phyllosphere and phylloplane) and observed that they were basically similar on the two types of media and the most common fungi were *Alternaria, Aspergillus, Chaetomium, Cladosporium, Cochliobolus, Curvularia, Gibberella, Memnoniella, Mycosphaerella, Setosphaeria and Stachybotrys.* Prabakaran, M., Merinal, S. and

Panneerselvam, A., (2011) reported the occurrence of phylloplane fungi on leaves surface of three important medicinal plants such as *Ocimum sanctum, Phyllanthus amarus and Azadirachta indica.* A total number of 10 fungal species belonging to five genera. Among them *Aspergillus flavus, Penicillium expansum, Fusarium semitectum, Fusarium oxysporum* were isolated from the phylloplane of *Ocimum sanctum.Scopulariopsis sp.* was isolated from the phylloplane of *Phyllanthus amarus, Penicillium janthinellum, Aspergillus fumiculosis, Aspergillus sp., Curvularia lunata and Fusarium moniliforme* were isolated from the phylloplane of *Azadirachta indica. Aureobasidium pullulans, Sporobolomyces roseus,* and *Cryptococcus laurentii* var. *flavescens,* added to the inoculums, reduced the superficial mycelial growth of *Septoria nodorum,* and the infection of wheat leaves by 50% or more. The mycelial growth was affected similarly in vitro, on slides covered with water agar. The antagonistic effect on germination was slight. The concentration of the saprophytes on the leaves after inoculation was comparable to population densities occurring on field-grown wheat (N. J. Fokkema., and F. van der Meulen., 1976).

Many physical, chemical and biological factors bring about causative changes in composition of aeromycoflora of an area and different fungal species are restricted to that particular area with specific environmental conditions(Bajwa, R., M. H. Shah., A. Javaid and Z. Tasneem, 1997; Verma, 1990). MD. Ashaduzzaman and Rahman, M.A., (2000) isolated the fungi from heart rot affected *Melia azadirach*(L.). The dehiscence of their sporangia or cleistothecia, perithecia or apothecia or fruiting bodies spreads the spores in air and that are spread up through the air and falls on suitable substratum and again they continue their life cycle. Variations in composition of aeromycoflora of different areas has been reported by many workers (Barth, O.M,(1981), Pasanen, A.L, (1990). Shinde, P.V.,(2003) studied the grain mold fungi in relation to physical and nutritional parameters of Sorghum grains. Garud, T.B.,(1992) reported resistance sources, mechanisms and resistance screening techniques for grain moulds. Magar, Sunita, J.,(2003) reported the occurrence of mold flora at different grain development stages in Sorghum. Smut spores of *Nigrospora, Cladosporium, Alternaria, Aspergillus* from outdoor air. The human pathogenic fungal spores recorded in outdoor and indoor air are *Rhizopus, Mucor, Aspergillus , Alternaria , Cladosporium, and Diploidia.* The allergic fungal spore types recorded in both places are *Aspergillus , Alternaria , Chaetomium, Cladosporium, Curvularia, Dreshlera, Epicoccum, Helminthosporium, Mucor, and Rhizopus*(Kotwal, S.G., Gosavi, S.V., and Deore, K.D. 2010). To view the leaf surface environment i.e phyllosphere, the present study was undertaken during the period of January 2012 to December 2013.

Materials and Methods:

Sample Collection:

The fresh and healthy leaves of *Ficus religiosa (Linn), Ficus religiosa (Linn) and Ficus glomerata (Linn)* were collected from the surrounding area of College premises, and immediately brought to the laboratory. Five discs of 6mm diameter from every leaf were cut by employing the sterile cork borer. These discs were transfer to 10 ml of blank water and mixed thoroughly and serial dilution was made. From these serial dilution solution, 1 ml of each dilution concentration were taken into the sterile petri-plate and then melted and cooled potato dextrose agar media was poured allowed to solidification. These plates were then incubated at room temperature for a week. These plates then were observed after 5-7 days of incubation and number of colonies was counted.

Preparation of Potato Dextrose Agar Medium:

The potato tubers were peeled and weighed for about 250g. The tubers were chopped into small pieces with the help of sterile knife. The chopped potatoes were transferred into a conical flask containing about 1000ml of distilled water. The content was boiled for 20 min. The supernatant were decanted and filtered by muslin cloth and the filtrate was collected. Dextrose (15g) and agar (15g) were transferred into the extract and swirled to dissolve the ingredients. The medium was made up to 1 litre by addition of distilled water. The pH of the medium was adjusted to 5.6. Finally, the medium was cotton plugged and autoclaved at 121ºC for 15 minutes.

Media Preparation:

Composition of Potato Dextrose Agar Medium:

Potato (peeled) - 250gm
Dextrose - 15 gm
Agar - 15 gm
Distilled water- 1000 ml.

Several methods are employed to study the phylloplane. 1. Direct method-which includes direct observations, impression of films and scanning microscopy. 2. Culture method-which includes plating, spore fall, leaf washing and leaf impression, of these serial dilution method and leaf impression methods are the two commonly employing technique. In this experimental work leaf impression with cello-tape touching method were employed.

The air monitoring was carried out for a period of twelve months from January 2013 to December 2014 in the college garden area of Wardha. For these experiment petri-plates containing media were touched with the cello-tape containing/ receive the fungal spores from both the leaf surfaces / phylloplane. These plates were then incubated in biological incubator at 28^0C. The fungal isolates were obtained on potato-dextrose-agar and Czepedox-Agar media, and were then incubated and observed for two days, later sub cultured to obtain pure fungal colonies. The grown colonies of 2-4 days up to maturity were continuously observed. Slides were made and identified under trinocular research microscopes. For staining lacto-phenol and cotton blue stains were utilized.

Experimental Plants:

1.*Ficus bengalensis(Linn):* Family –Ficaceae.

Plant is large shade tree, supposed to be sacred tree of Hindus. The wood is quite durable under the water. The leaves are used as fodder for cattle and elephants. A fibre is obtained from the bark which is used for coarse cordage. The milky juice is applied in rheumatic pains externally. The bark of the tree are used for the treatment of dysentery and diarrhoea. The trees are grown as hedge plants by the sides of the roads. This is found throughout India.

2.*Ficus religiosa (Linn):* Family –Ficaceae.

The Hindus regard the tree very sacred and grow it near the temples. The bark is astringent. The seeds are cooling and the fruits are laxative. The leaves and young shoots are given as purgative. The bark is used for tanning. The tree is grown as an avenue.

3.*Ficus glomerata (Linn):* Family – Ficaceae.

This is a tree producing edible fruits. The leaves used as fodder and the bark is used for tanning. The unripe fruits are used as vegetable. The wood of the tree is supposed to be sacred and burnt in hawans. The root is used as a remedy for dysentery. The milky juice is used for treatment of piles and diarrhoea. Also serves as a host for lac insect.

Observation Table 1: Showing the fungal species from leaf surface of plants.

S. N.	Name of Plant and leaf taken for impression	Dilution Factor					Name of fungal species observed
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
1.	*Ficus bengalensis*	24	14	5	0	0	*Fusarium, Aspergillus niger, Chaetomium, Aureobasidium, Cunninghemella sp.*
2.	*Ficus religiosa*	132	21	6	1	1	*Curvularia , Trichoderma , Fusarium , Aureobasidium, Aspergillus, Alternaria.*
3.	*Ficus glomerata*	71	49	11	2	1	*Chaetomium, Fusarium, Aspergillus sp.,Phoma, Aureobasidium, Rhizopus.*

Observation Table 2: Showing the month wise fungal species from both leaf surfaces of plants.

S.N.	Months	Incidence of fungal species.
1.	January 2012.	*Fusarium, Curvularia, Aspergillus niger.*
2.	February 2012.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger.*
3.	March 2012.	*Chaetomium, Fusarium, Curvularia, Trichoderma, Aspergillus niger.*
4.	April 2012.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger, Trichoderma.*
5.	May 2012.	*Chaetomium, Fusarium, Phoma, Curvularia, Aspergillus niger, Trichoderma.*
6.	June 2012.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger, Aureobasidium, Trichoderma.*
7.	July 2012.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger, Phoma, Aureobasidium, Trichoderma, Cunninghemella.*
8.	August 2012.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger, Phoma, Aureobasidium.*
9.	September 2012.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger, Aureobasidium.*
10.	October 2012.	*Fusarium, Curvularia, Aspergillus niger, Aureobasidium.*
11.	November 2012.	*Fusarium, Curvularia, Aspergillus niger.*
12.	December 2012.	*Fusarium, Curvularia, Aspergillus niger.*
13.	January 2013.	*Fusarium, Curvularia, Aspergillus niger.*
14.	February 2013.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger.*
15.	March 2013.	*Chaetomium, Fusarium, Curvularia, Trichoderma, Aspergillus niger.*
16.	April 2013.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger, Trichoderma.*
17.	May 2013.	*Chaetomium, Fusarium, Phoma, Curvularia, Aspergillus niger, Trichoderma.*
18.	June 2013.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger, Aureobasidium, Trichoderma.*
19.	July 2013.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger, Phoma, Aureobasidium, Trichoderma, Cunninghemella.*
20.	August 2013.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger, Phoma, Aureobasidium, Alternaria.*
21.	September 2013.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger, Aureobasidium.*
22.	October 2013.	*Fusarium, Curvularia, Aspergillus niger, Aureobasidium.*
23.	November 2013.	*Fusarium, Curvularia, Aspergillus niger.*
24.	December 2013.	*Fusarium, Curvularia, Aspergillus niger.*

Results and Discussion:

During the present investigation a well-marked variation in phylloplane was found. The species of *Aspergillus, Curvularia, and Fusarium,* were found more prevalent in all the months during the experimental study. A well marked variations in phylloplane in different areas were also found in different months. It was reported that *Chaetomium* were reported more prevalent in the months of February 2012 to September 2013. *Trichoderma* were observed to be more precisely incidence during the months of March to July of both the years, *Aureobasidium was reported* strictly in the months of June to October of 2012 and 2013, where as *Phoma were* less reported only in the months of May, July, and August of 2012 and 2013. The fungal species

Cunninghemella was only the species which occurs only in the months of July of 2012 and 2013 in our study. These results were in conformity with the reports of Bajwa et al.,(1995). The distinct variations in aeromyco-flora of different residential environments was investigated by Pasanen in 1990, while,Verma in 1990 reported that the composition of fungal flora was different in urban and rural areas of India. The variation in composition of aeromycoflora in different areas of city probably attributes to co-existance on concentration of pollutants in the air along with the climatic variations. Presence of transportation, congested houses and decaying materials and waste are also affect the aeromycoflora. It may be concluded from present study that phylloplane flora is highly sensitive to environmental factors. Aeromycoflora with a specific area quickly responds to change in environmental conditions from locality to locality.

Conclusions:

From the present study, it may be concluded that phylloplane flora is highly sensitive to environmental factors. Phylloplane flora with a specific area quickly responds to change in environmental conditions from locality to locality. The incidence of fungi like *Fusarium, Curvularia, Aspergillus niger*, were the most common. The some fungi were restricted to the peculiar or have the habitual of a particular condition or environment.

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