Study of Phylloplane Flora of College Premises of Wardha(M.S).

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Abstract

The experiment was performed during the period of July 2010 to June 2011, to observe the phylloplane flora of garden from Wardha(M.S). The experiment was performed by cello-tape touching method to petriplate containg the media. It was observed that species of *Aspergillus, Curvularia and Fusarium,* were found more prevalent in all the months of experimental study than the other forms of genera. It was observed 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.

Key words : Phylloplane, flora, fungi, *Aspergillus, Curvularia,Fusarium, Phoma, Aureobasidium. Trichoderma.etc,* environment, Mussaenda frondosa, sensitivity.

Introduction:

Fungi are found every where, its incidence is variable and variously occurs in water, in soil, in air, and even in an Antarctic too i.e. in snow also. The term aerobiology was first coined by the American plant pathologist "Fred Cambell meier" in 1930. So the term aerobiology came in to existance since 1930 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-organism. The outdoor environment is never completely free from the incidence of microbial propagules,which are collectively called as "air spora".

Cuningham, (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.

As aerobiology deals in large parts with bio-particles present in air, it contributes a lot in enumeration of types of bio-particles present. Among all the air borne bio-particles, fungal spores constitute 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 of behaviour of living things in a particular environment or in a biological systems. Study of fungi is essential for anyone collecting or monitoring the any fungi from the study area. Several workers develop the methods to documenting 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.

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 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 indoor aeromycology includes the study of indoor aeromycoflora of libraries (Tilak and Saibaba, 1984; Tripathi, 1987; Nitrini *et al*., 1992), of

hospitals (Babu, 1983; Greene *et al*., 1962; Noble and Clayton, 1963; and Joshi *et al*.,2006), of museums (Barve and Thakre, 1992; Ary *et al*.,2001), of residential and of office environment (Reynold *et al*.,1990), of poultry shed (Chute and Gershaman, 1961; Tilak and Saibaba, 1986), of school and public institutions (Gravesen *et al*., 1983) etc. The dehiscence of their sporangia or cleistothecia, perithecia, or apothecia or fruiting body spreads the spores in an air and that spreads up through air and falls or deposited on various objects like- soil, water, on surface of leaves, flowers, barks, trunks, open roots, wooden logs of trees, iron articles, furnitures, and even they enters into the museums, and gets deposited on articals of the museums. On getting the suitable substratum, they germinate, forms mycelia and complete their life cycle or multiply themselves and causes the diseases to the objects like human skins, eyes, heads, throats, lungs etc. and causes the infections to the plants and living organisms too. Such fungi which deposited on leaf surfaces and the study of such leaf surface environment is called the phyllosphere. The term phyllosphere was first coined by Last, (1955) to denote the leaf surface environment. The fungi which are responsible for causing the diseases to the plant and their study of science is called as plant pathology. It is very important to study the plants diseases and their causing pathogen or causal organisms. These causal organisms comes from leaf surface through air by different agencies. The phylloplane, the surface of plant leaves is a complex terrestrial habitat that is characterized by a variety of microorganisms including bacteria, filamentous fungi and yeast. Phylloplane fungi are the mycota growing or 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.

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 spreads up through the air and falls on suitable substratum and again they continue their life cycle. 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 molds. Magar., Sunita, J.,(2003) reported the occurrence of mold flora at different grain development stages in Sorghum. These spores present in air and inhaled by various living beings like human beings, animals, birds, and also they settled on the plant surfaces, where they germinate and produce mycelia or they may produce toxins which

are allergic in nature, sometimes they cause diseases of incurable forms to living beings. These fungal spores act as a allergens to human beings and also the pollutant in air. They also associated with seeds, grains, fruits etc.(Sawant, 2000; and Magar, 2003). Studies conducted over past so many years indicates that *Curvularia* sp. and *Fusarium* sp. are principle fungi associated with grain mold problems. Increasing incidence reported from different safflower growing areas . Chakrabarti, D.K., and Basuchaudhary, K.C., (1978) identified the wilt of safflower caused by *Fusarium oxysporum sp*. *carthami* and its relationship with age, host, soil and environmental factors. Despande, G.D., (1991) studied the development of medium for selective expression of *Curvularia lunata (W)* Boj. in Sorghum seed health testing. Jadhav, S.K., and Lall, B.M.,(2011) reported the seasonal variation of Indoor aero-mycoflora of Dr. BhimRao Ambedkar Hospital, Raipur. More work is being done on the study of airborne fungal spores and pollen grains and its impact on human health, animal beings and plants and other flora and fauna. Many physical, chemical and biological factors brings out causative changes in composition of aero-mycoflora of an area and different fungal species are restricted to that of particular area with specific environmental conditions (Bajwa et al., 1997). The airborne fungal spores cause the respiratory disorders like asthma, allergic rhinitis, skin diseases, ear diseases etc. The airborne fungal spores and their concentration vary from place to place. To view this type of study in our area and in the city, the present investigation was undertaken. To view such causal organism deposited on leaf surfaces, the study or experimental work was undertaken to identify the fungal flora from leaf surface of a stipulated area.

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 areas 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 molds. 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 July 2010 to June 2011.

Materials and methods:

Sample Collection:

The fresh leaves of *Musaenda(), Ixora(),* and *Bougainvillea spectabillis(),* were collected from the surrounding area of Thanjavur and immediately brought to the laboratory.

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 July 2010 to June 2011 in the college garden area of Wardha. For this 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 subcultured 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 were utilized.

Experimental Plants:

1. *Mussaenda frondosa* (Linn), Family – Rubiaceae.

Description: Plant is small, shrubby habit, erect, cylindrical, branched, nodes and internodes very prominent, solid, hairy and leaves are green, opposite decussate, stipules interpetiolar, simple, sub-sessile, ovate, entire, acute, unicostate reticulate. Inflorescence dichasial cyme. Flowers bracteate, pedicillate, complete, younger flowers actinomorphic, older flowers zygomorphic, hermaphrodite, pentamerous, some are tetramerous also, and epigynous.

2. *Ixora coccinea* (Linn.), Family – Rubiaceae.

Description: Plant is small, with shrubby habit, lower portion woody, aerial, erect, cylindrical, branched, solid, smooth and green. Leaves are green, opposite decussate, stipulate, stipules interpetiolar, simple, sessile, elliptical, entire, acute, glabrous, unicostate reticulate venation. Inflorescence terminal trichotomously branched cyme. Flowers bracteate, pedicillate, complete, actinomorphic, hermaphrodite, tetramerous, and epigynous.

3. *Bougainvillea spectabillis* (Linn.), Family –Nyctaginaceae.

Description: Plant is woody weak, with shrubby habit, lower portion woody, spiny, ornamental, aerial, erect, cylindrical, branched, alternate, solid, smooth and green. Leaves are green, ovate, entire, unicostate reticulate venation. Inflorescence cymose. Flowers in a petalloid bracts, bracts becomes brightly coloured and appears like a petal in which three small flowers are enclosed

within three coloured bracts. Flowers bracteate, pedicillate or sessile, complete, actinomorphic, hermaphrodite, pentamerous, and superior.

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

S.N.	Name of Plant and leaf taken for impression	Name of fungal species observed
1.	*Mussaenda frondosa.* a) Dorsal surface. b) Ventral surface.	*Trichoderma, Phoma, Fusarium, Aspergillus niger, Curvularia.* *Chaetomium, Trichoderma, Fusarium, Aspergillus niger, Aspergillus sp.*
2.	*Ixora coccinea.* a) Dorsal surface. b) Ventral surface.	*Curvularia , Trichoderma, Phoma, Fusarium , Aureobasidium, Aspergillus niger.* *Phoma, Fusarium, Curvularia, Aureobasidium.*
3.	*Bougainvillea spectabillis.* a) Dorsal surface. b) Ventral surface.	*Fusarium, Trichoderma, Aspergillus niger, Aspergillus sp.* *Aspergillus sp., Phoma, Fusarium, Curvularia.*

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

S.N.	Months	Incidence of fungal species observed.
1.	July 2010.	*Fusarium, Curvularia, Aspergillus niger.*
2.	August 2010.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger.*
3.	September 2010.	*Chaetomium, Fusarium, Curvularia, Trichoderma,Aspergillus niger.*
4.	October 2010.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger. Trichoderma.*
5.	November 2010	*Chaetomium, Fusarium, Phoma, Curvularia, Aspergillus niger. Trichoderma.*
6.	December 2010.	*Chaetomium, Fusarium, Curvularia, Aspergillus*

		niger, Aureobasidium. Trichoderma.
7.	January 2011.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger, Phoma, Aureobasidium. Trichoderma.*
8.	February 2011.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger, Phoma, Aureobasidium.*
9.	March 2011.	*Chaetomium, Fusarium, Curvularia, Aspergillus niger, Aureobasidium.*
10.	April 2011.	*Fusarium, Curvularia, Aspergillus niger, Aureobasidium.*
11.	May 2011.	*Fusarium, Curvularia, Aspergillus niger.*
12.	June 2011.	*Fusarium, Curvularia, Aspergillus niger.*

Results and Discussion:

During the present investigation a well-marked variation in phylloplane were reported. It was reported that, the species *Fusarium*, *Trichoderma*, and *Aspergillus niger*, were observed to be common in all the three plants on dorsal surfaces. The species *Phoma*,*and curvularia*, was common on the dorsal surface of both the plants i.e.*Mussaenda frondosa* (Linn), and *Ixora coccinea* (Linn.) respectively. As far as ventral surface is concern the species of *Fusarium* were reported as a common participant in all the plants. Species *Phoma*, were common on ventral surface of two plants, namely *Ixora coccinea* (Linn.) and *Bougainvillea spectabillis*(Linn.), while species of species of *Aspergillus* were common on ventral surface of plant *Mussaenda frondosa* (Linn) and *Bougainvillea spectabillis*(Linn.). Fungal species i.e. *Chaetomium*, *and Aureobasidium* were reported on the ventral surface of plant *Mussaenda frondosa* (Linn) and *Ixora coccinea* (Linn.) respectively. It was further reported that, the species *Phoma*, *Fusarium* ,*and Aureobasidium*, were the only fungi species present on both surfaces of plant *Ixora coccinea* (Linn.). It was further reported that, the species of *Aspergillus, Curvularia and Fusarium,* were found more prevalent in all the months of a year of investigation period, (Samina, 1975; Nair et al., 1986; Nautiyal and Midha, 1978; Kumar, 1984; Ali and Salma, 1973, were also reported the findings). 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 August 2010 to March 2011. *Trichoderma* were observed to be more precisely incidence during the months of September 2010 to January 2011, *Aureobasidium was reported* strictly in the months of December 2010 to April 2011, where as *Phoma were* less reported only in the months of November 2010, January and February of 2011. Some fungi were more restricted and

reported in the particular environmental conditions. These results are in confirmioty with Bajwa et al.,1995., Pasanen, 1990., Verma, 1990. 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.

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