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STUDY OF PHYLLOPLANE MYCOFLORA OF SOME SELECTED MEDICINAL PLANTS

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ABSTRACT: The present investigations were performed in the medicinal plant nurseries during the rainy season in and around Dehradun, Uttarakhand for disease severity. In this investigation direct method which includes direct observations, impression of films and scanning microscopy were employed. During this investigation *Aspergillus niger, Trichoderma harzianum, Trichoderma piluliferum, Penicillium sublateritium, Penicillium tardum, Penicillum herquei, Penicillium frequentans, Penicillium citreo-viride and Cladosporium cladosporioides* were reported. It was reported. It was reported that *Aspergillus niger, Trichoderma harzianum, Penicillium sublateritium* and *Cladosporium cladosporioides* were the most common fungi in most of the months on the phylloplane of all the plants. While *Penicillium tardum, P.herquei, P.frequentans* and *P.citreo-viride* were identified to be the specific and restricted to the particular months.

Key words: Incidence, common, specific, restricted, months, phylloplane.

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INTRODUCTION

Among all the air borne bio-particles, fungal spores constitute the greatest and most important portion in the air (Salvaggio and Lars.,1981). Cuningham, (1873) reported that the changes in the weather condition, affects the air-spora both qualitatively and quantitavely, while Marchisio, and Airarudi., (2001) reported that, the relative humidity, temperature and rainfall plays a key role in the occurrence of fungal spores in the air.

Fungi that live on the aerial parts of plants have been defined as endophytes if they live inside the plant tissues and as epiphytes if they live on the surface of their host (De Barry, 1866; Arnold, *et al*., 2000; Inacio *et al*., 2002; Lindow and Brandl, 2003).

The surfaces of aerial plant parts provide a habitat for epiphytic micro-organisms, many of which also influence the growth of pathogens. Bacteria are generally the predominant initial inhabitants of newly expanded leaves, while yeasts and filamentous fungi dominate later in the growing season (Kinkel *et al*., 1987). The phyllosphere is the three-dimensional space on the leaf surface. The environment of the phyllosphere includes physical, chemical and the biological components occupying the surrounding space. In recent years, considerable attention has been paid to the components of the microflora present on the leaf surface, a specialized habitat commonly known as the phylloplane.

The microbial communities of phyllosphere are diverse, supporting numerous genera of bacteria, filamentous fungi, yeasts, algae, and less frequently protozoa and nematodes which may form resident populations on leaves (Morris *et al*., 2002; Lindow and Brandl 2003; Andrews and Harris 2000; Beattie and Lindow 1995; Kinkel 1997; Lindow and Leveau 2002). The non-pathogenic fungi that inhabit the phyllosphere depend on nutrients exuded from the leaf or those deposited from the atmosphere (Belanger and Avis, 2002; Inacio *et al*., 2002).

Sometimes, these fungal spores acts as allergens to human beings, and also the pollutant in the air. They are also associated with seeds, grains, fruits etc. (Sawant, 2000; and Magar, Sumita J.,2003). Studies conducted over pass so many years indicates that *Curvularia* sp. and *Fusarium* sp. are principal fungi associated with grain mold problems. Many physical, chemical and biological factors bring out causative changes in the composition of aero-mycoflora of an area and different fungal species are restricted to that of particular area with specific environmental conditions (Verma, 1990; Bajwa *et al*.,1997)

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. (Breeze and Dix, 1981; Mishra and Dickinson, 1981; Andrews *et al*., 2002, Osono, 2002, Osono *et al*., 2004). The non-pathogenic fungi that inhabit the phyllosphere depends on the nutrients exuded from the leafs or deposited from the atmosphere. (Belanger and Avis, 2002; Inacio *et al*., 2002). Spore release from many fungi inhabiting the phylloplane is passive through the action of wind or rain splash; however other spores are actively propelled into atmosphere by various mechanisms (Kinkel, 1997; Aylor, 2002; Levetin, 2002). Microscope-based observation of surface microbes can support indirect techniques, such as culturing or DNA analysis of surface washings, by illustrating microbial distribution patterns, inter-relationships and the presence of unculturable or non-recovered organisms.

Phylloplane fungi are the mycota growing on the leaf surfaces. There are two groups of 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. Lot of investigations have been carried out on the phylloplane flora of leaf surfaces of several plants growing in the 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). El-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*, *Chaetomium*, *Cladosporium*, *Cochliobolus*, *Curvularia*, *Mycosphaerella*, *Setosphaeria* and *Satchybotrytis*.

There are certain physical, chemical and biological factors which are responsible causative changes in composition of aermycoflora of an area and different fungal species are restricted to those particular areas with specific environmental conditions (Verma, 1990). Variations in composition of aeromycoflora of different areas have been reported by many workers (Pasanen, A.L., 1990). Shinde, P.V., (2003) studied the grain mold fungi in relation to physical and nutritional parameters of sorghum grains. To view the leaf surface environment and its surface fungal flora i.e. phyllosphere, the present study was undertaken during the period of July 2014 to August 2016.

MATERIALS AND METHODS

Various techniques were employed in aeromycological studies. 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, leaf washing and leaf impression, of these serial dilution method and leaf impression methods are the two commonly employing technique. Of these, Leaf impression methods (Lamb and Brown 1970; Bainbridge and Dickinson 1972) and Leaf washing methods (Dickinson, 1967; Bainbridge and Dickinson 1972; Kuthubutheen 1981; Mishra and Dickinson 1981; Aneja, 2003) are the two commonly employed techniques and were used during the present studies. This study was done during the period of July 2014 to August 2016.

Leaf impression was made by pressing leaf surfaces against agar in Petri plates to produce leaf-imprints. The leaves were then discarded and the plates were incubated at room temperature until colonies grow on the agar surface. In leaf washing method, fresh healthy leaves were collected in fresh sterile polythene bags and brought into the laboratory. Five discs each of 5mm diameter was cut from each leaf using sterile cork borer. About 50 discs were transferred to 100-ml of sterile water in a 250 ml Erlenmeyer flasks. Discs were stirred for 20 minutes using magnetic stirrer. From this initial suspension, serial dilutions were prepared. One ml of the sample aliquots were then plated onto PDA plates. The plates were then incubated at 25±1^{o}C and examined after 5-6 days for the fungal growth. Identification was done by preparing a slide and Micrometric measurements of phialospores and phialides were done by mounting 3-days-old, young culture stained with cotton blue and observed under high power of a binocular research microscope. The cultural and morphometric characters were noted separately for each isolate and identification of each fungus was done with the help of available expertise, keys and descriptions given by (Gilman, 1957; Ellis, 1971 and Rifai 1969). The fungi observed during the study was listed in the table 1.

Experimental Plants

1. ***Rauwolfia serpentina*** (L). Benth. ex Kurz.

Rauwolfia serpentina (Apocynaceae family) commonly known as *sarpagandha* is an important medicinal plant of Indian subcontinent and South East Asian countries. It is an erect, evergreen perennial undershrub and contains a number of bioactive chemicals, including ajmaline, deserpidine, rescinnamine, serpentinine, and yohimbine. Reserpine is an alkaloid first isolated from *R.serpentina* and was widely used as an antihypertensive drug (Fabricant and Fransworth, 2001; Harisaranraj *et al*., 2009; Dey and De, 2010).

b) *Spilanthes oleracea* Linn.

Spilanthes *oleracea* (Asteraceae family) is a flowering herb also known as toothache plant as the leaves and flower heads contain an analgesic agent spilanthol used to numb toothache. A decoction or infusion of the leaves and flowers is a traditional remedy for stammering, toothache, stomatitis, and throat disorders. It's properties provide relief to dry mouth by enhancing saliva production (Anon.,1976).

c) *Piper longum* Linn.

Piper longum (Piperaceae family) is commonly known as *pippali*. It possesses good medicinal properties such as carminative, stimulant, aromatic, good for constipation, gonorrhea and paralysis of the tongue. *P.longum* is most commonly used to treat respiratory infections such as bronchitis, diseases of the spleen, cough, tumors, and asthma. When applied topically, it soothes and relieves muscular pains and inflammation.

In Ayurvedic medicine, it is said to be a good rejuvenator. It also helps in stimulating the appetite and used as sedative in insomnia and epilepsy. Also as cholagogue in obstruction of bile duct and gall bladder (Dahanukar *et al*., 1984; Dhiman, 2005).

d) *Chlorophytum tuberosum* Baker.

Chlorophytum tuberosum (Liliaceae family) is a genus of about 200-220 species of evergreen perennial flowering plants native to the tropical and subtropical regions of Africa and Asia, commonly known as *safed musli*. *C.tuberosum* used as remedy for the treatment of diabetes, arthritis, rheumatism and joint pains. It also acts as diuretic, aphrosiadic agent and revitalizer. It is a rich source of over 25 alkaloids, vitamins, minerals, proteins, carbohydrates, steroids saponins and polysachharides (Sharma, 2003; Biswas and Temburnikar, 2003).

Table 1. Fungal species observed on leaf surfaces of medicinal plants

S.No.	Name of Plant	Fungal species observed
1.	*Rauwolfia serpentina*	*Aspergillus niger, Trichoderma harzianum, Penicillum tardum, Penicillium frequentans, Penicillium citreo-viride*
2.	*Spilanthes olerace*	*Penicillium sublateritium, Penicillium tardum, Trichoderma sp., Penicillium sp.*
3.	*Piper longum*	*Penicillum herquei, Aspergillus niger, Penicillium frequentans, Cladosporium cladosporioides*
4.	*Chlorophytum tuberosum*	*Trichoderma piluliferum, Trichoderma harzianum, Aspergillus sp.*

During the isolation of antagonistic fungi from the phylloplane of healthy plants some human and plant pathogenic fungi were also identified which and are presented in table 2.

Table-2. Identification of isolated human and plant pathogenic fungi

S.No.	Fungi	Host	Location
1.	*Aspergillus fumigatus*	*Piper longum*	Non Wood Forest Products Division Nursery, F.R.I., Dehradun
2.	*Alternaria alternata*	*Piper longum*	Ayurvedic Nursery Herbertpur, Uttarakhand
3.	*Botrytis cinerea*	*Spilanthes oleracea, Piper longum*	Biotech Green Herbal Nursery, Kolhupani, Dehradun
4.	*Aspergillus flavus*	*Piper longum, Spilanthes oleracea*	Non Wood Forest Products Division Nursery, F.R.I., Dehradun
5.	*Fusarium sp.*	*Spilanthes oleracea*	Non Wood Forest Products Division Nursery, F.R.I., Dehradun
6.	*Cylindrocladium sp.*	*Spilanthes oleracea*	Ayurvedic Nursery Herbertpur,Uttarakhand
7.	*Scytalidium sp.*	*Spilanthes oleracea*	Biotech Green Herbal Nursery, Kolhupani, Dehradun
8.	*Pestalotiopsis sp.*	*Spilanthes oleracea, Piper longum, Rauwolfia serpentina*	Non Wood Forest Products Division Nursery, F.R.I., Dehradun
9.	*Fusarium equiseti*	*Spilanthes oleracea*	Ayurvedic Nursery Herbertpur, Uttarakhand
10.	*Pythopthora sp.*	*Spilanthes oleracea*	Non Wood Forest Products Division Nursery, F.R.I., Dehradun

RESULTS AND DISCUSSION

Data depicted in table 1, indicates that the *Aspergillus niger*, *Trichoderma harzianum*, *Penicillium frequentans* were the common fungi in *Rauwolfia serpentina, Chlorophytum tuberosum and Piper longum* in the month of July and August. Whereas, table 2. shows the human and plant pathogenic fungi. Biological significance of phyllosphere fungi has been emphasized by some workers (Sinha, 1965; Kapooria and Sinha, 1969; Barnes, 1971; Norse, 1972; Pace and Campel, 1974; Skidmore and Dickinson, 1976). Fungi present on the phylloplane such as (*Aspergillus* spp., *Trichophyton* spp. and *Gliocladium* spp.) have been reported for their antagonistic activity against *Colletotrichum* leaf disease of *Hevea brasiliensis* (rubber plant) (Evueh *et al*., 2008). Antagonists present on the phyllosphere such as *Cladosporium cladosporioides* H39 when applied on apple seedlings were effectively found to reduce the sporulation of *Venturia inaequalis* (Kohl *et al*., 2008).

CONCLUSION

In the present study, *T.harzianum* ISO-1, *T.harzianum* ISO-2, *T.piluliferum, A.niger* and *P.sublateritium* were considered as resident antagonists because they were isolated from the phylloplane of healthy plants of the hosts.

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