

Phylloplane of Mycoflora of Medicinal Plants

Indikos Shylla¹ and Indu Sharma²

¹Department of Microbiology, Nims Institute of Allied Medical Science and Technology, NIMS University, Rajasthan, Jaipur 303121, Rajasthan (India)

²Department of Biotechnology, Nims Institute of Allied Medical Science and Technology, NIMS University, Rajasthan, Jaipur 303121, Rajasthan (India)

Corresponding author: endusharma@gmail.com

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Correspondence:

E-mail: endusharma@gmail.com

ABSTRACT

Leaves constitute the major part of exposed plant surface. They are open to infestation or sporotrophic colonization by air dispersed or splash dispersed mycoflora. The leaves provide unique environment to their surface occupants. The topography of the leaf surface, the microclimate around the leaves and the concerned. The occurrence of phylloplane fungi on leaf surface of four important medicinal plants such as *Tradescantia pallida*, *Calotropis procera*, *Withania somnifera* and Chhota Gokhru (*Tribulus terrestris*).

1. INTRODUCTION

Biodiversity is the variation of life forms within a given ecosystem. Biodiversity is often used as a measure of the health of biological systems. Biodiversity of fungi is essential for anyone collecting or monitoring any fungi. Fascinating and beautiful fungi are vital components of nearly all ecosystems and impact human health and our economy in a myriad of ways. Standardized methods for documenting diversity and distribution have been lacking. 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 organismal biodiversity.

Plants have been used for medicinal purposes since prehistoric period. They have played a critical role in maintaining human health and civilizing the quality of human life for thousands of years. Treatment with medicinal plant is considered to be very safe and there is less or no side effects because these remedies are linked with nature and they contain components of therapeutic value. Various parts of these plants are used in treatment of various diseases and disorders. Sometimes the whole plant may

be used in the treatment of certain diseases. The medicinal plants might be used either directly or after processing. Economic importance of medicinal plants are higher in countries like India and China. These countries provide two third of plants in traditional and modern system of medicine. In India large number of plants are used in primary health care and they have got various traditional uses. They include *Tradescantia pallida* and *Calotropis procera*, *Withania somnifera*, and *Tribulus terrestris* etc. These medicinal plants are destructed by various microorganisms, which infects roots, stems, leaves, flowers and fruits. Large number of microorganisms inhabit the phyllosphere of plants. While a few microbial species can be isolated from within the plant tissues and many microbes are recovered from the surface of healthy plants.

A fungus is a member of a large group of eukaryotic organisms that includes microorganisms such as yeast and molds. Fungi are classified as a kingdom that is separate from plants, animals and bacteria. One major difference is that fungal cells have cell walls that contain chitin, unlike the cell walls of plants, which contain cellulose.

Phylloplane is a natural habitat of various microorganisms including bacteria, fungi, yeasts and filamentous. phylloplane fungi are the mycota growing on the surface of leaves. However, quality and quantity of microorganisms on the leaf surface differ with age of leaf, leaf area, morphology and atmospheric factors such as temperature and humidity. The leaf surface being very rich in nutrients offer a suitable substratum for the colonization of various microorganisms both the parasites and saprophytes. In addition to nutrient level, growth and abundance of phylloplane fungi are also influenced by environmental conditions such as temperature, humidity, light intensity, wind speed, UV radiation and presence of air pollutants. The size, density of hairs and sculptures of leaf surface seem to be most reliable factors of fungal biodiversity on the studies plant species. Amongst the different microorganisms, two groups of phylloplane fungi i.e. residents and casual are generally present on leaf surface. Residents can multiply on the surface of healthy leaves without noticeably affecting the host. Whereas casuals land on the leaf surface but cannot grow.

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value. The medicinal plants have been used by humans from the pre-historical times. It has been well recognized that human health and well-being are directly dependent on biodiversity. Medicinal plants contribute substantially to health, cultural integrity and local economies, particularly among the poor, and particularly for women, children and the elderly.

1.1 ***Tradescantia pallida*** commonly known as ‘Spiderwort’ or purple heart or purple queen, is the species of perennial plant native to the Gulf Coast region of eastern Mexico and the second largest genus within the Commelinaceae family. It’s widely cultivated as an ornamental plant for its striking purple foliage and its ability to tolerate a variety of growing conditions. It’s often grown as a ground

cover or in hanging baskets for its trailing habit. Some species of the genus *Tradescantia* have been traditionally used for their presumed anti-inflammatory, antioxidant, antibacterial and antiarrhythmic properties as well as improving blood circulation and prevent sore eyes. For example, roots have been used as a drink to heal kidney diseases and digestive system ailments. Leaves have been applied to relieve stings and insect bites.

1.2 *Callotropis procera*, also known as apple of Sodom or Sodom apple, is a species of flowering plant belonging to the Apocynaceae family. It is native to North Africa, the Middle East, and the Indian subcontinent. This plant is characterized by its woody shrub or small tree form and its thick, leathery leaves. The flowers are typically white to purple and have a unique bell shape.

While it has some traditional medicinal uses in certain cultures, it's important to note that *Callotropis procera* contains toxic compounds and can be harmful if ingested. It has been used traditionally for treating ailments like skin diseases, fever, and rheumatism, diarrhea, stomach ulcer but its toxic properties make it potentially dangerous for medicinal use without proper preparation and dosage control.

1.3 *Withania somnifera*, commonly known as Ashwagandha or Indian ginseng, is an herb native to India, Africa, and the Middle East. It belongs to the family Solanaceae. Ashwagandha it's been used in traditional Indian Ayurvedic medicine for thousands of years to ease pain and inflammation, boost nutrition, and treat insomnia, along with other conditions. Ashwagandha is also considered an adaptogen. That means it helps your body better manage stress.

The plant is also famous for the anti-cancerous activity, low back pain treatment, and muscle strengthening, which may be attributed to the withanolide alkaloids. *W. somnifera* is also rich in numerous valued secondary metabolites such as steroids, alkaloids, flavonoids, phenolics, saponins, and glycosides. Alkaloids were used for calming, withanolides for their anti-tumor and satoindosides for their anti-stress activity (Elsakka et al. 1989).

1.4 *Tribulus terrestris* is also known as chhota gokhru, gokshura, bhakhdi, puncture vine, goat-head. It is an annual shrub distributed in Mediterranean, subtropical and warm climatic regions such as India, China, Southern USA, Mexico, Spain, and Bulgaria. Chhota Gokhru (*T. terrestris*) is generally known as noxious weed because of its small woody spiny fruits. The greyish brown fruit powder of *Tribulus terrestris* contains flavonoids, glycosides, alkaloids, steroids and saponin derivatives such as tigogenin, hecogenin, ruscogenin, diosgenin, chlorogenin and sarsasapogenin. Due to diuretic, analgesic, anti-diabetic, anti-urolithic, aphrodisiac, anthelm. The various plant parts act as cardiac and have circulatory stimulants, anti-tumor, anti-pyretic, anti-epileptic, anti-inflammatory, anti-ulcer, anti-spasmodic, diuretic, antihypertensive, cholesterol lowering, anti-oxidant, anti-diabetic,

hepatoprotective, anti-microbial activities, anti-hyperlipidemic, anti-nephrolithiatic, nephroprotective and aphrodisiac properties all of which are used to cure various diseases. All parts of the plants are used in preparation of medicines.

Chhota Gokhru (*Tribulus terrestris*) plant is distributed throughout India, ascending to 3300 m in Himalaya. The plants are common in hot, sandy and dry parts of the country such as Deccan, Gujarat, Andhra Pradesh, South Haryana and Rajasthan.

The plant belongs to family Zygophyllaceae, is an annual herb having weak stem, creeping, branched and up to 95 cm in length. Leaves are compound, sub-opposite, paripinnate and stipulate, similar to chickpea leaves. The leaves are oblong to linear-oblong, sub-equal, pubescent on both surfaces and mucronate type. Leaflets are oblong in 5-10 pairs with short petiole.

2 PHYLOPLANE FUNGI

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 fungi are the group of organisms having a great biodiversity and they are the largest group of microbes of typical ecosystems throughout the world (Nayak, 2015). The fungal spoilage of medicinal plant is responsible for the significant loss in their medicinal properties. 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 (Thakur 2017).

The term "phylloplane" refers to the complete leaf surface, whereas "phyllosphere" refers to the complete aerial habitat of plants. The phylloplane serves as a niche for a variety of microbial communities, making it a significant ecosystem from an ecological and monetary perspective (Susmita et al., 2021). Bacteria, filamentous fungi, yeast, and phylloplane fungi are just a few of the microorganisms that can be observed on the outer layer of plant leaves, which is a wide and varied terrestrial environment. Phylloplane fungi, unlike endophytes, saprobes, and harmful fungi, have received scanty research. The phylloplane or leaf surface, serves as a favourable environment for the development of antagonistic microbes that can outcompete the pathogen for resources and prevent pathogen growth by secreting antibiotics or toxins (Yadav et al., (2011; Blakeman 1982).

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.

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 (**Beattie and Lindow 1995; Kinkel 1997; Andrews and Harris 2000; Lindow and Leveau 2002; Morris et al., 2002; Lindow and Brandl 2003**). 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; Thakur 2017**).

2.1 Phylloplane Microflora

Phylloplane microflora refers to the microorganisms that inhabit the above-ground parts of plants, particularly on the surfaces of leaves. These microorganisms can include bacteria, fungi, yeasts, and occasionally algae. The phylloplane is a dynamic environment influenced by factors such as humidity, temperature, and plant exudates.

Phylloplane microflora play important roles in plant health and ecology. Some microorganisms on the phylloplane are beneficial to plants, aiding in nutrient cycling, pathogen suppression, and growth promotion. For example, certain bacteria and fungi can form symbiotic relationships with plants, helping them acquire nutrients from the environment or protecting them from harmful pathogens. On the other hand, some phylloplane microorganisms can be pathogens themselves, causing diseases in plants.

The phylloplane microflora influenced the plant populations and ecosystem functioning. Competition among the native microbial communities of plants and

fungal pathogen exhibited (**Brandl et al., 2013**), the fluctuating environmental, geographical, seasonal conditions largely affected the phylloplane microorganisms (**Knief et al., 2010; Wellner et al., 2011; Rastogi et al., 2012; Copeland et al., 2015; Ding and Melchner, 2016**). Various scientist has worked on the phylloplane fungi (**Prabakaran and Pannerselvam, 2011; Grbic et al., 2015; and Waill et al., 2016**).

2.2 Phylloplane fungi:

Aspergillus flavus, *Penicillium expansum*, *Fusarium semitectum*, and *Fusarium oxysporum*, *Aspergillus niger*; *Chaetomium*, *Aureobasidium*, *Cunninghamella* sp, *Trichoderma harzianum*, *Penicillium frequentans*, *Penicillium citreo-viride*, *Penicillium sublateritium*, *Penicillium tardum*, *Trichoderma* sp, *Penicillium* sp, *Penicillium herquei*, *Cladosporium cladosporioides*, *Trichoderma piluliferum*, *Aspergillus* sp, *Trichoderma viride*, *Alternaria* sp, *Verticillium* sp, *Alrernaria salani*, *Fusarium proliferatum*, *Fusarium salani*, *Hypocrea* sp, *Neonectria* sp, *Chaetomium* sp, *collectotrichum* sp, *Penicilium digitatum*, *Paecilomyces marquandii*, *Mucor*, *Rhizopus* sp, *Penicilium conidia*, *Penicilium citrinum*, *Periconia macrospinosa*.

3. To detect mycoflora associated with phylloplane of medicinal plants

The phylloplane mycoflora of *Triticum aestivum* L. The mycoflora of green and yellow leaves varied in the phyllosphere and phylloplane areas. The number of species and their proportional abundance varied according to the host plant's age (**Mishra and Srivastava 1970**).

Dilution plate technique used to isolate the leaf surface microflora of mustard (*Brassica campestris* L. cv. YS-42) from the seedling stage to the senescence stage at 30-day intervals. The microflora from washed and unwashed leaf discs of *B. campestris* had the highest number of fungal species, according to the study. *Cladosporium cladosporioides* (Fresen.) Sacc. and *Alternaria alternata* (Fr.) Keissl were the most common fungi (**Singh and Rai 1980**).

Phylloplane microfungi were isolated from young, mature, senescent, and dead sugarcane leaves by using two different methods. A total of 67 species of fungus were discovered. The number of fungi rose as the leaves became older. The mycoflora was dominated by dematiaceous hyphomycetes (**Sharma 2004**).

Ocimum sanctum L., *Withania somnifera* (L.) Dunal in DC., *Catharanthus roseus* (L.) G. Don., and *Azadirachta indica* A. Juss. were studied for the presence of phylloplane fungi on the leaf surface of four significant medicinal plants. Leaf print (LPT) and leaf washing (LWT) procedures were used to isolate a total of 20 fungal species from thirteen taxa. Fungal species from the genera *Aspergillus* and *Penicillium* were found to be the most prevalent (**Jalander and Gachande 2012**).

Two techniques, agar plate and moist chamber, to isolate and enumerate phylloplane and endophytic fungal diversity from one ornamental plant, *Mangifera indica*, with the host relationship. *Cladosporium herbarum*, *Penicillium digitatum*, *Moniliella* sp., *Candida* sp., *Trichoderma* sp., and *Aspergillus niger* were identified as phylloplane and *Aspergillus niger*, *Aspergillus flavus*, *Penicillium digitatum*, and *Trichoderma* sp. as endophytes using the agar plate method. *Colletotrichum* sp.,

Curvularia sp., *Aspergillus niger*, *Stachybotrys* sp., and *Trichoderma* sp. were recognized as phylloplane fungi in the moist chamber method, 14 while *Aspergillus niger*, *Botrytis* sp., *Cladosporium resinae*, Grey sterile mycelia, *Trichoderma* sp (Nayak 2015).

Some phylloplane fungi isolated from the leaves surface of five medicinal plants, including *Ocimum sanctum*, *Ficus bengalensis*, *Datura metel*, *Butea monosperma* and *Stevia rebaudiana*. A dilution plating approach was used to isolate 44 fungal species from 28 genera from surface-sterilized leaf segments. *Ocimum sanctum*'s phylloplane yielded 04 species and 03 genera, *Butea monosperma*'s yielded 17 species and 07 genera, *Datura metel*'s yielded 03 species and 03 genera, *Ficus bengalensis* yielded 05 species and 05 genera, and leaf surfaces yielded 15 species and 10 genera (Garg et al., 2019).

The formation and evolution of phylloplane microbial communities are influenced by phylloplane topography, microclimatic conditions around the phylloplane, and leaf exudates. The phylloplane microfungi of *Solanum nigrum* L. dried-decaying leaves were studied in this study. The fungus Deuteromycotina was the most prevalent (Chauhan and Jain 2020).

The phyllosphere mycoflora exhibits seasonal, qualitative, and quantitative change. By using the leaf wash and leaf print methods, a total of 21 fungal species were identified in this study. *Cladosporium fulvum* was discovered to have the highest percent frequency of occurrence (19.2 % LW and 27.3 % LP), followed by *Gibberella avenacea* (14.4 % and 13.2 %). *Fusarium oxysporum* was found to have the lowest percent frequency (0.1 % and 0.28 %, respectively) in both approaches (Satpute and Vanmare 2017).

The isolation and enumeration of *Sapindus mukorossi* phylloplane, as well as endophytic fungal diversity. *Sapindus mukorossi* isolated 14 phylloplane and endophytic fungus species from 9 genera. *Alternaria alternata*, *Collectotrichum orbiculare*, *Torulla herbarium*, *Epicoccum nigrum*, and *Candida* sp. are phylloplane fungi, as are *Cladosporium herbarum*, *Penicillium expansum*, *Fusarium oxysporum*, *Fusarium* sp., *Alternaria alternata*, *Collectotrichum orbiculare*, *Torulla herbarium*, *Epicoccum nigrum*, and *Candida* spp., *Epicoccum nigrum*, *Aspergillus niger*, *Aspergillus flavus*. Endophytic fungi were identified as *Penicillium digitatum* and *Penicillium* sp. (Varpe (2020).

CONCLUSION

The study on Phylloplane mycoflora of medicinal plants is of great importance, because nowadays plants are easily infested by fungi. Leaf samples of different medicinal plants showed varied level of incidence of mycoflora. It indicates the ability of fungi developing an association with broad spectrum of leaves irrespective of their types. The actual diversity may depend on the methods used for gathering and handling of leaf samples, size of the leaf fragment and culture. From the present study,

it may be concluded that phylloplane flora is highly sensitive to environmental factors. Study of phylloplane mycoflora is highly interdisciplinary in nature and has tremendous scope to find the significant applications in the field of medicine, epidemiology, disease forecasting and plant pathology.

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