

Plants of the Phyllosphere Microflora in the Mysuru Districts of Karnataka, India: A Selection of Medicinal, Garden, Terrestrial, and Aquatic Species

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Abstract:

Dust and other particles settle on plant surfaces, particularly the phyllosphere of leaves, where a normal microflora takes root. Epiphytic microbes, which may affect the development of diseases, find a home on the surfaces of aerial plant components. Understanding the persistence of plant disease-causing bacteria and fungi is critical for creating novel methods to restrict their spread, which is why microbial life in the phyllosphere is of considerable economic relevance to the agricultural business. The microflora studied here came from a variety of plant sources, including those used in medicine, the garden, on land, and in water. In this study, nutrient-rich media are used to identify actinomycetes, bacteria, and fungus by washing the leaves. The current investigation was mostly conducted using biochemical test techniques and microorganism observation and identification under a microscope. Using keys and manuals, the current study identifies fifteen mycoflora (fungi), fifteen bacterial strains, and nine actinomycetes.

Key words: Gardens, phyllosphere, microflora, mycoflora, medicinal, land, water

Introduction

The phylloplane is the surface of the leaf, while the phyllosphere is the area on the leaf where the microbes live. The normal microflora, which includes bacteria, fungus, and microalgae, is formed when dust and other particles come into contact with plant surfaces, particularly leaf surfaces. Exudates from leaf cells include a variety of amino acids, glucose, fructose, and sucrose, which help in the formation of microflora. Ruinen, a Dutch microbiologist, saw a dense microbial epiphytic association on the leaves of forest vegetation in Indonesia and came up with the word "phyllosphere" [1] to describe it. All the things in the immediate vicinity, whether physical, chemical, or biological, make up the phyllosphere's habitat. The most current assessment of phyllosphere microbial ecology by Vorholt [2] emphasized basic research that explains how microbes live on sections of plants

that are above ground. The The leaf microbiome is quite varied, including several bacterial species, yeasts, filamentous fungus, algae, and, on rare occasions, protozoa and nematodes. Epiphytic microbes, which may affect the development of diseases, find a home on the surfaces of aerial plant components. At the beginning of the growth season, most freshly enlarged leaves are inhabited by bacteria, but later on, filamentous fungi and yeasts take over [3]. The surface area of a leaf that is three-dimensional is called the phyllosphere. The microflora that inhabits the phylloplane, a unique environment on the surface of leaves, has recently received a lot of research interest. In addition to a wide variety of bacteria, yeasts, filamentous fungi, algae, and, on occasion, protozoa and nematodes, the phyllosphere is home to resident populations of protozoa and nematodes, as well as non-pathogenic fungi that feed on nutrients released by leaves or dusted with atmospheric nutrients [4,5]. The aero-mycoflora of any given region might

fluctuate according to a wide variety of physical, chemical, and biological variables; moreover, several fungal species are region-specific [6,7]. The agricultural sector places a high value on research into the phyllosphere's microbial life features because a better knowledge of the survival mechanisms of plant disease-causing bacteria and fungi is critical for the development of novel approaches to restrict their spread. And incidents of food illness due to bugs like *Salmonella* and *E. coli* in tainted produce have been on the increase recently. Recent research has shown that culture-dependent approaches of phyllosphere community characterization are likely to be erroneous and understate diversity [8]. Using culture-independent methods has shown that the phyllosphere is home to a significantly more diverse range of communities than was previously thought, even though previous assumptions about the dominating occupants were mostly true. Numerous microbes, such as yeast, filamentous fungus, and bacteria, call the phylloplane, the surface of plant leaves, their home on Earth [9–13]. By revealing patterns of microbial distribution, interrelationships, and the existence of non-recoverable or uncultivable species, microscope-based observation of surface bacteria may bolster indirect approaches like culture or DNA analysis of surface washings. Mycetes known as phylloplane fungus inhabit the spaces between leaves. Fungi may be classified into two categories: residents and casuals. The host plant is unaffected by the residents' multiplication on healthy leaves. In contrast, casuals settle on the surface of the leaf but are unable to grow. In comparison to endophytes, saprobes, and pathogenic fungi, phylloplane fungi have received less attention from researchers. Many scholars from different areas of the globe have studied the phylloplane flora of garden plants and cultivated plants' leaf surfaces extensively [14–18]. Fungi found on various plant leaf surfaces (phyllosphere and phylloplane) were also detailed by El-Said [19].

Finding and comparing mycoflora from the medicinal, terrestrial, garden, and aquatic phyllospheres is the focus of this investigation. Unlike garden plants, which are intentionally

cultivated and stored in a controlled environment, medicinal plants are grown in natural settings. The current research will demonstrate any variation in the microflora of aquatic and terrestrial plants, as well as in the leaf flora of naturally occurring medicinal plants and garden plants that have been intentionally preserved. This study set out to identify the phyllosphere microflora in a small number of plants, including some that are medicinal, some that are terrestrial, some that are aquatic, and a couple that are garden plants. The plants included *Cymbopogon*, *Citrus*, *Nerium oleander*, *Centella asiatica*, *Morinda citrifolia*, *Colasta esculantum*, *Eichhornia crassipes*, *Cyperus* sp., *Alocasia macrorrhizas*, and *Polygonum glabrum*, as well as *Datura metel*, *Antigonon leptopus*, *Polyalthia logifolia*, *Lablab purpureus*, and *Caryota mitis*, and a small number of garden plants, *Ficus religiosa*, *Ricinus com*, *Cyperus munis*, *Tecoma*, *Hamelia patens* and *Millettia pinnata*).

Materials and Methods

Collection of sample

For the present investigation 10 plants representing two different groups *viz.*, medicinal and garden plants and 11 plants representing 2 different groups *viz.*, terrestrial and aquatic plants were selected. The studies were undertaken from the month of January 2019 to April 2019. The medicinal and garden leaf samples were collected from plants growing in and around Mysuru District, Karnataka, India.

Method for isolation of phyllosphere mycoflora

The phyllosphere microflora was isolated by using the leaf washing method. In this method for the isolation of phyllosphere microflora of terrestrial, aquatic, medicinal and garden plant of leaves samples were prepared and wash the leaves for the preparation of the suspension. All the glass wears used in the present work will be sterilized by using autoclave at 121°C at 15 mins.

A leaf washing method for isolation of phyllosphere

microflora

Take 10 gm healthy and fresh leaves, don't rub their surfaces, cut them into small bits and suspend them into 100 ml of sterile distilled water in a conical flask. Shake thoroughly for 5 minutes. Take ten clean and sterilized Petri plates and mark the sample name and date of inoculation for further reference. Pour 15 ml of sterilized PDA media for each Petri plate. Cover them and allow them to cool and become semisolid. Take 1 ml of suspension from the conical flask and pour it in the Petri dishes. Gently mix and keep them in an incubator at 37 °C. Observe after 2-3 days.

Preparation of Potato Dextrose Agar (PDA)

Add 39 gm of commercial prepared potato dextrose agar powder in 1 liter of distilled water then add a pinch of Chloramphenicol powder. Boil while mixing to dissolve. Sterilize the dissolved mixture using autoclave at 121 °C for 15 minutes.

Identification of fungi

After a week observe the mold culture with a hand lens or stereomicroscope recording their colony morphology. Prepare a wet mount by suspending some of the fungal colonies in a few drops of the cotton blue

stain without damaging the fungal structure. Examine the preparation under low power or high power magnification of microscope and record the observation. Identify the fungi using keys and manuals.

Identification of Bacteria

The staining of Bacteria for identification is done by using gram staining and negative staining. Examine the preparation under low power or high power magnification with the aid of a microscope and record the observation. Identify the fungi using manuals.

Fermentation test: Carbohydrate Fermentation

This fermentation test aims to find the ability of microorganisms to degrade and ferment carbohydrates with the production of acid and gas. Most microorganisms use carbohydrates differently depending on their enzyme's components. The pH indicator Phenol Red is used to detect the production of acid, which is red at a neutral pH 7 and changes to yellow at a slightly acidic pH of 6.8. This indicates a positive reaction. Table 1 shows the expected results of the Glucose and Sucrose fermentation test [20].

Glucose	Sucrose
Fermented with acid production only Eg. <i>S. aureus</i>	Fermented with acid production only Eg: <i>S. aureus</i>
Fermented with acid and gas production Eg. <i>E. coli</i> , <i>Klebsiella</i>	Fermented with acid and gas production Eg: <i>E. coli</i> , <i>Klebsiella</i>
Non- Fermenting Eg. <i>Acinetobacter</i>	Non- Fermenting Eg: <i>S. typhi</i> <i>S. paratyphi</i> <i>Pseudomonas</i> sp.

Table 1: Glucose and Sucrose fermentation

Triple Sugar Iron Agar test

Triple Sugar Iron Agar test is to find the microorganisms based on the ability to ferment the carbohydrates (Glucose, Sucrose, and Lactose)(Table 2). The triple sugar- iron agar test is designed to differentiate among the different groups or genera of the *Enterobacteriaceae*, which are all Gram-negative bacilli capable of fermenting glucose with the production of acid and to distinguish them from other gram- negative intestinal bacilli. This differentiation is based on the differences in carbohydrate fermentation patterns and hydrogen sulphide production

by the various groups of intestinal organisms. Carbohydrate fermentation is indicated by the presence of gas and a visible colour change of the pH indicator, phenol red. The production of hydrogen sulphide in the medium is indicated by the formation of a black precipitate that will blacken the medium at the bottom of the tube.

Observation	Interference	Examples
A/A without gas and H ₂ S production	Acid Slant / Acid butt without gas & H ₂ S production	<i>Staphylococcus aureus</i>
A/A with gas and without H ₂ S production	Acid Slant / Acid butt with gas & without H ₂ S production	<i>E. coli</i> , <i>Klebsiella</i>
K/A with gas and without H ₂ S production	Alkaline slant / Acid butt with gas & without H ₂ S production	<i>Salmonella paratyphi</i>

Table 2: Triple sugar Iron Agar test

Casein hydrolysis test

In casein hydrolysis test to find if an organism can produce the exoenzyme casease. Casease is an exoenzyme produced by some bacteria to degrade casein. This test is conducted on milk agar which is a complex media containing casein, peptone and beef extract. If an organism can produce casein, then there will be a zone of clearing around the bacterial growth. A positive reaction is indicating by clearing in the media surrounding the colonies. *Pseudomonas aeruginosa* will hydrolyze casein and may produce a yellow to green diffusible pigment.

Gelatin hydrolysis test

Gelatine hydrolysis test is used to detect the ability of an organism to produce gelatinase the liquefy gelatine. Hydrolysis of gelatine indicates the presence of gelatinases. This test is used to decide the ability of an organism that produces gelatinases. This test is useful in identifying and differentiating species of *Serratia*, *Proteus*, *Bacillus*, *Pseudomonas*, and *flavobacterium*.

Gram staining technique and KOH test

By using the Gram staining technique, The Bacteria which keep the primary stain appear dark blue or violet and not decolorized when stained with Gram's method are called Gram-positive, whereas those that lose the crystal violet used counterstain, safranin appears red are called as Gram-negative. In this way by using Gram staining to differentiate Gram-positive and Gram-negative strains of Bacteria. The Gram stain uses different reagents in the order, crystal violet, iodine solution, alcohol, and safranin.

Preparation of Starch casein Agar for identification of Actinomycetes

Ingredients	Gms/ml
Casein Powder	1.00
Starch	10.00
Sea Water	37.00
Agar	15.00

Table 3: Starch casein Agar

The above ingredients (Table 3) are mixed in 1 liter of distilled water. Boil while mixing to dissolve. Autoclave the dissolved mixture at 121°C for 15 minutes.

Catalase Test

Catalase mediates the breakdown of hydrogen peroxide H_2O_2 into oxygen and water. To find out if a particular bacterial isolate is able to produce catalase enzyme. Add a drop of H_2O_2 to the smeared cell culture on a slide in a case of catalase-positive bacteria (CAT+) bubbles will appear (Most of G- bacteria are CAT+ and *Staphylococcus* and *Bacillus* are CAT+ too).

Coagulase Test

The bound coagulase is also known as the clumping factor. It cross-links α and β chain of fibrinogen in plasma to form a fibrin clot that deposits on the cell wall. As a result, individual coccus sticks to each other and clumping is observed. This test is useful in differentiating *S. aureus* from other coagulase-negative *Staphylococci*.

Result and discussion

Fungi

Examine the colonies of microorganisms in Petri dishes and list all microflora of the phyllosphere. It was observed that different leaves show different microflora some of the common mycoflora (fungal) of the phyllosphere are listed in Fig. 1 and Table 4.

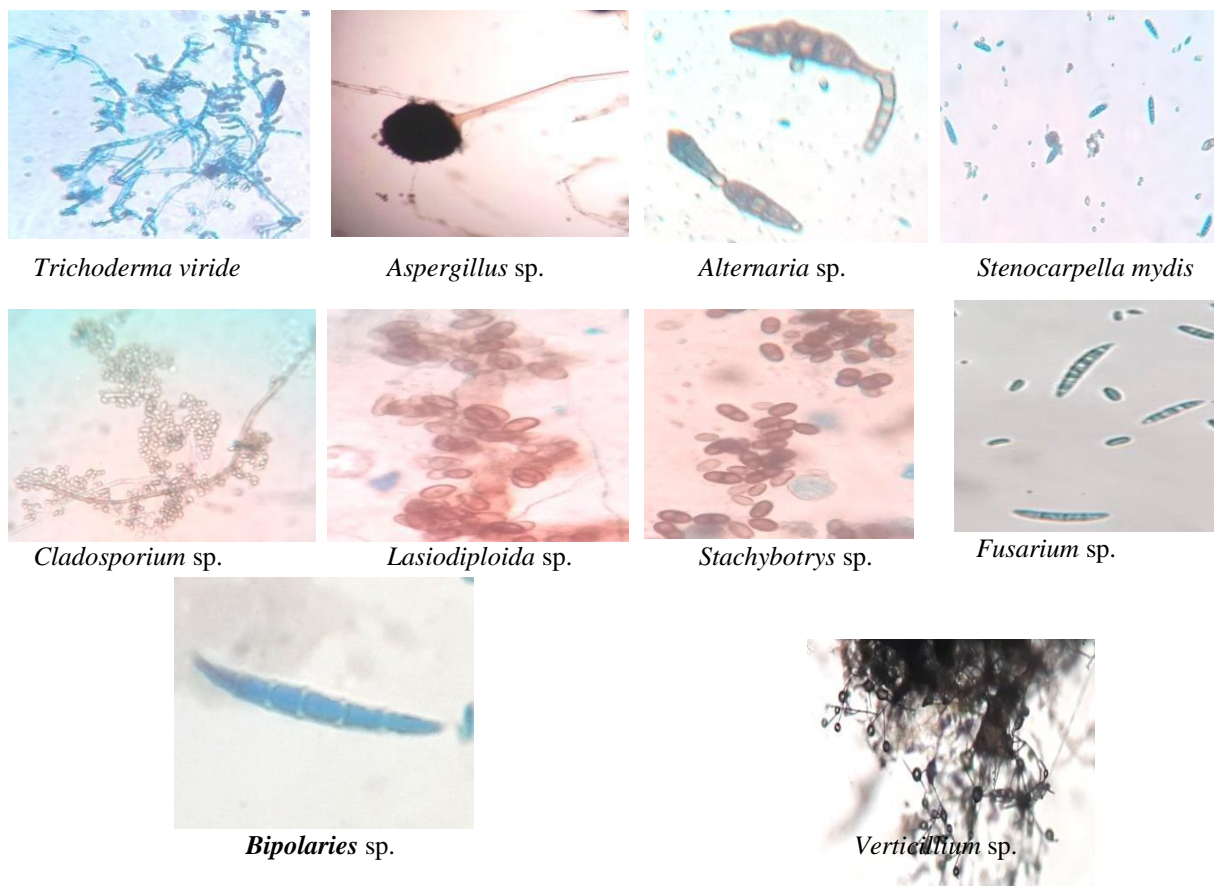


Fig 1: Showing identified fungus on phyllosphere of some medicinal, terrestrial, aquatic and garden plants

Phyllosphere mycoflora of garden plant			
Sl. No.	Name of the plant		Genera representing on the leaf surface
	Common name	Botanical name	Fungi

1	Sacred fig	<i>Ficus religiosa</i>	<i>Trichoderma viride</i> and <i>Lasioidiploida</i>
2	Ricinus	<i>Ricinus communis</i>	<i>Aspergillus</i> and <i>Trichoderma viride</i>
3	Trumpet bushes	<i>Tecoma</i> sp.	<i>Cladosporium</i> and <i>Stachybotrys</i>
4	Firebush	<i>Hamelia patens</i>	<i>Aspergillus</i> and <i>Trichoderma viride</i>
5	Pongamoil tree	<i>Millettia pinnata</i>	<i>Alternaria</i> , <i>Cladosporium</i> and <i>Stenocarpella mydis</i>
Phyllosphere mycoflora of medicinal plants			
1	Lemmon grass	<i>Cymbopogon</i>	<i>Aspergillus</i> and <i>Trichoderma viride</i>
2	Citrus	<i>Citrus</i> sp.	<i>Aspergillus</i> , <i>Alternaria</i> and <i>Cladosporium</i>
3	Nerium	<i>Nerium leander</i>	<i>Aspergillus</i> and <i>Trichoderma viride</i>
4	Centella	<i>Centella asiatica</i>	<i>Cladosporium</i> , <i>Aspergillus</i> and <i>Trichoderma viride</i>
5	Noni	<i>Morinda citrifolia</i>	<i>Trichoderma viride</i> , <i>Aspergillus</i> and <i>Lasioidiploida</i>
Phyllosphere mycoflora of aquatic plants			
1	Alocasia	<i>Alocasia macrorrhizas</i>	<i>Verticillium</i> sp., <i>Aspergillus</i> sp. and <i>Stachybotrys</i> sp.
2	Colacasia	<i>Colacasia esculantum</i>	<i>Alternaria</i> sp. and <i>Aspergillus</i> sp.
3	Water hyacinth	<i>Eichhornia crassipes</i>	<i>Trichoderma</i> sp., <i>Stachybotrys</i> sp., and <i>Aspergillus</i> sp.
4	Cyperus	<i>Cyperus</i> sp.	<i>Cladosporium</i> sp. and <i>Aspergillus</i> sp.
5	Knotweed, Knotgrass, Smartweed, etc	<i>Polygonum glabrum</i>	<i>Trichoderma</i> sp., <i>Cladosporium</i> sp., and <i>Aspergillus</i> sp.
Phyllosphere mycoflora of terrestrial plants			
1	Thorn apple	<i>Datura metel</i>	<i>Verticillium</i> sp., <i>Fusarium</i> sp., and <i>Aspergillus</i> sp.
2	Coral vine	<i>Antigonon leptopus</i>	<i>Trichoderma</i> sp., <i>Stachybotrys</i> sp. and <i>Cladosporium</i> sp.
3	Ashoka tree	<i>Polyalthia logifolia</i>	<i>Verticillium</i> sp., <i>Bipolaris</i> sp. and <i>Aspergillus</i> sp.
4	Lablab	<i>Lablab purpureus</i>	<i>Cladosporium</i> sp. and <i>Aspergillus</i> sp.
5	Fish tail palm	<i>Caryota mitis</i>	<i>Cladosporium</i> sp. and <i>Aspergillus</i> sp.

Table 4: Phyllosphere mycoflora of garden, medicinal, aquatic and terrestrial plants.

In the present work phyllosphere, mycoflora of few garden plants i.e., *Ficus religiosa*, *Ricinus communis*, *Tecoma* sp., *Hamelia patens* and *Millettia pinnata* were identified. Phyllosphere mycoflora of few medicinal plants were identified (*Cymbopogon*, *Citrus* sp., *Nerium leander*, *Centella asiatica* and *Morinda citrifolia*). Meanwhile, few aquatic and terrestrial plants phyllosphere mycoflora were also tried to identify. For this work, aquatic plants

are *Alocasia macrorrhizas*, *Colacasia esculantum*, *Eichhornia crassipes*, *Cyperus* sp. and *Polygonum glabrum* were selected. Similarly for the study of terrestrial plants, *Datura metel*, *Antigonon leptopus*, *Polyalthia logifolia*, *Lablab purpureus* and *Caryota mitis* were preferred.

Ninety-two species in addition to two varieties that belong to 32 genera were collected from the phyllosphere and phylloplane of *Triticum vulgare* [21] and 59 species, 22 genera of fungi were collected from the phyllosphere of few fern plants [14, 16]. The study of fungal phyllosphere also helps with the biological regulation of fungal diseases. In this mode effects of microflora composition in the phyllosphere on biological regulation of grapevine fungal diseases were carried out earlier by Sackenheim *et. al.*, [22].

Bacteria

The bacterial isolates were identified as *Klebsiella* sp., *Pseudomonas* sp., *Micrococcus* sp. *Bacillus anthracis*, *Fusobacterium – moniliformis*, *Corynebacterium* sp. *Staphylococcus aureus*, *Clostridium* sp., *Salmonella* sp. and the Gram-ve bacteria are dominant in the phyllosphere of the various aquatic, terrestrial, medicinal and garden plants (Fig 4 and Table 5). Different types of the test were conducted to find out the type of strains (Fig 2 A, B and C, Fig 3 and Fig 4). Similar observations also recorded by earlier workers [23]. In glucose fermentation test if acid is produced identified the strain as *S. aureus*, if it is produced acid with gas the strain is considered as *Klebsiella* sp. and if it non-fermented the strain should be *Actinoetobacter*. In the present glucose fermentation investigation *S. aureus* and *Klebsiella* sp. were collected and identified. A similar type of observation was done in sucrose fermentation test and the results obtained in glucose fermentation test are confirmed due to similar types of strains were collected in both. In Triple Sugar Iron test reveals that the presence of *Salmonella* sp. and *Klebsiella* sp. Similarly different type of test reveals different types of bacterial strains and listed in Table 5 and Fig 3, 4 and 5

Fermentation test: Carbohydrate Fermentation



Fig 2 A. Glucose fermentation

(First test tube uninoculant, Middle-*Klebsiella* and Last –*Staphylococcus aureus*)



Fig 2 B. Sucrose fermentation
(First test tube – *Staphylococcus aureus*, Middle-*Klebsiella*, and Last –uninoculant)



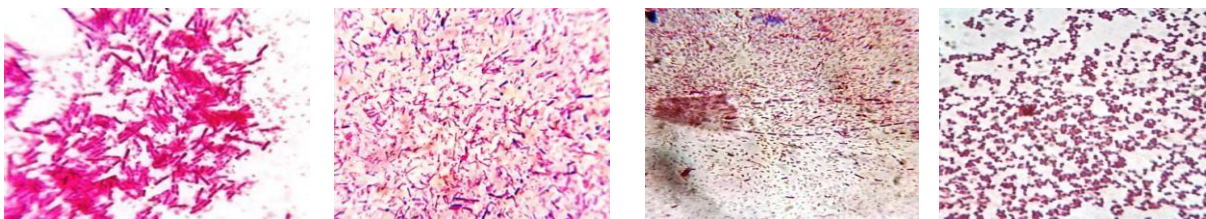
Fig 2 C. Triple sugar Iron fermentation test

(First test tube –*Klebsiella*, Middle- *Salmonella* sp. and Last –uninoculant)

Casein hydrolysis and Gelatin hydrolysis test: *Pseudomonas aeruginosa* will hydrolyze casein and may produce a yellow to green diffusible pigment (Fig 3 A). Gelatin hydrolysis test useful in identifying and differentiating species of *Serratia*, *Proteus*, *Bacillus*, *Pseudomonas*, and *flavobacterium* (Fig 3 B).



Fig 3 A. Casein Hydrolysis (green colonies shows *Pseudomonas aeruginosa*). B. Gelatin Hydrolysis test



Klebsiella sp.

Corynebacterium

Bacillus anthracis

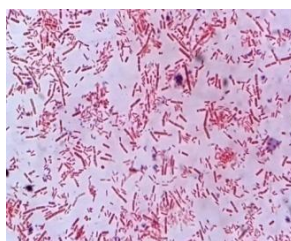
Sarcina sp.

Staphylococcus aureus

Pseudomonas

*Fusobacterium –
nucleatum*

Clostridium sp.



Salmonella sp.

Fig 4. Showing identified bacterial strains on phyllosphere of some medicinal, terrestrial, aquatic and garden plants

Phyllosphere bacterial strains in the garden plant			
Sl. No.	Name of the plant		Genera representing on the leaf surface
	Common name	Botanical name	Bacteria
1	Sacred fig	<i>Ficu sreligiosa</i>	<i>Pseudomans</i> sp., <i>Sarcina</i> sp. and <i>Corynebacterium</i> sp.
2	Ricinus	<i>Ricinus communis</i>	<i>Bacillus anthrcis</i>
3	Trumpet bushes	<i>Tecoma</i>	<i>Klebsiella</i> sp. and <i>Pseudomonas aeruginosa</i>
4	Firebush	<i>Hamelia patens</i>	<i>Bacillus anthrcis</i>
5	Pongamoil tree	<i>Millettia pinnata</i>	<i>Fusobacterium nucleatum</i>
Phyllosphere bacterial strains of medicinal plants			
1	Lemmon grass	<i>Cymbo pogon</i>	<i>Fusobacterium nucleatum</i> and <i>Corynebacterium</i> sp.
2	Citrus	<i>Citrus</i>	<i>Pseudomonas</i> sp. and <i>Staphylococcus aureus</i>
3	Nerium	<i>Nerium oleander</i>	<i>Pseudomonas aeruginosa</i> and <i>Salmonella</i> sp.
4	Centella	<i>Centella asiatica</i>	<i>Bacillus anthrcis</i> and <i>Corynebacterium</i> sp.
5	Noni	<i>Morinda citrifolia</i>	<i>Staphylococcus aureus</i> and <i>Klebsiella</i> sp.
Phyllosphere bacterial strains of an aquatic plant (emergent plant)			
1	Alocasia	<i>Alocasia macrorrhizas</i>	<i>Sarcina</i> sp. and <i>Corynebacterium</i> sp.
2	Colacasia	<i>Colacasia esculantum</i>	<i>Klebsiella</i> sp., <i>Fusobacterium nucleatum</i>
3	Water hyacinth	<i>Eichhornia crassipes</i>	<i>Pseudomonas</i> sp. and <i>Listeria</i> sp.
4	Cyperus	<i>Cyperus</i> sp.	<i>Pseudomonas</i> sp. and <i>Bacillus anthrcis</i>
5	Knotweed, Knotgrass, Smartweed, etc	<i>Polygonum glabrum</i>	<i>Pseudomonas</i> sp. and <i>Colastridium</i> sp.
Phyllosphere bacterial strains of terrestrial plants			
1	Thorn apple	<i>Datura metel</i>	<i>Corynebacterium</i> sp. and <i>Clostridium</i> sp.
2	Coral vine	<i>Antigonon leptopus</i>	<i>Klebsiella</i> sp., <i>Fusobacterium nucleatum</i> and <i>Salmonella</i> sp.

3	Ashoka tree	<i>Polyalthia logifolia</i>	<i>Sarcina</i> sp., <i>Pseudomonas</i> sp. and <i>Staphylococcus aureus</i>
4	Lablab	<i>Lablab purpureus</i>	<i>Staphylococcus aureus</i> and <i>Pseudomonas</i> sp.
5	Fish tail palm	<i>Caryota mitis</i>	<i>Pseudomonas</i> sp. and <i>Fusobacterium nucleatum</i>

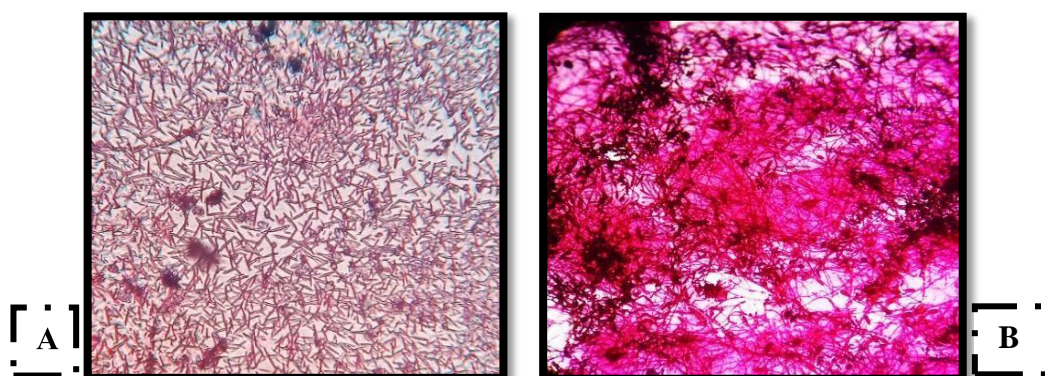
Table 5: Phyllosphere bacterial strains in the garden, medicinal, aquatic and terrestrial plants.

The bacterial population was predominant of leaf surfaces of all plants and amongst bacteria gram -ve were more in number [24]. They investigate the phyllosphere microflora of some common plants representing crop plants, forest trees, plantation crops and weeds. Numerous biotic and abiotic factors, including the plant itself, drive microbial community structure in the phyllosphere and most phyllosphere microorganisms are bacteria. The phyllosphere is a discrete habitat and is a model system for understanding the relationships between microorganisms and hosts. An improved understanding of phyllosphere microbiology is also of practical importance for biocontrol of the phyllosphere [2].

Actinomycetes

In the present work, the identified actinomycetes are *Bifidobacter* sp., *Norcodia* sp., *Micromonospora* sp., *Enterobacter* sp., *Actinomyces pyogenes* and *Micromonospora chalcea* (Fig 5 and Table 6). The isolation and screen non-pathogenic phyllosphere actinomycetes of rice which are capable of controlling BLB disease in rice were carried out by Ilsan *et.al.*, 2016. Leaf washing method was used to isolate bacteria and actinomycetes from groundnut leaves [25]. Phylloplane microflora plays important role affecting the plant-microbe interactions and thereby contribute significantly for disease suppression and qualitative and quantitative composition of phylloplane microflora depends on change in various parameters such as host characteristics, leaf architecture, chemical environment of the corresponding leaf surface and altering micro and macro climatic conditions [26].

The aerial habitat colonized by these microbes is termed the phyllosphere and most work on phyllosphere microbiology has focused on leaves, a more dominant aerial plant structure. Bacteria are by far the most numerous colonists of leaves [27].



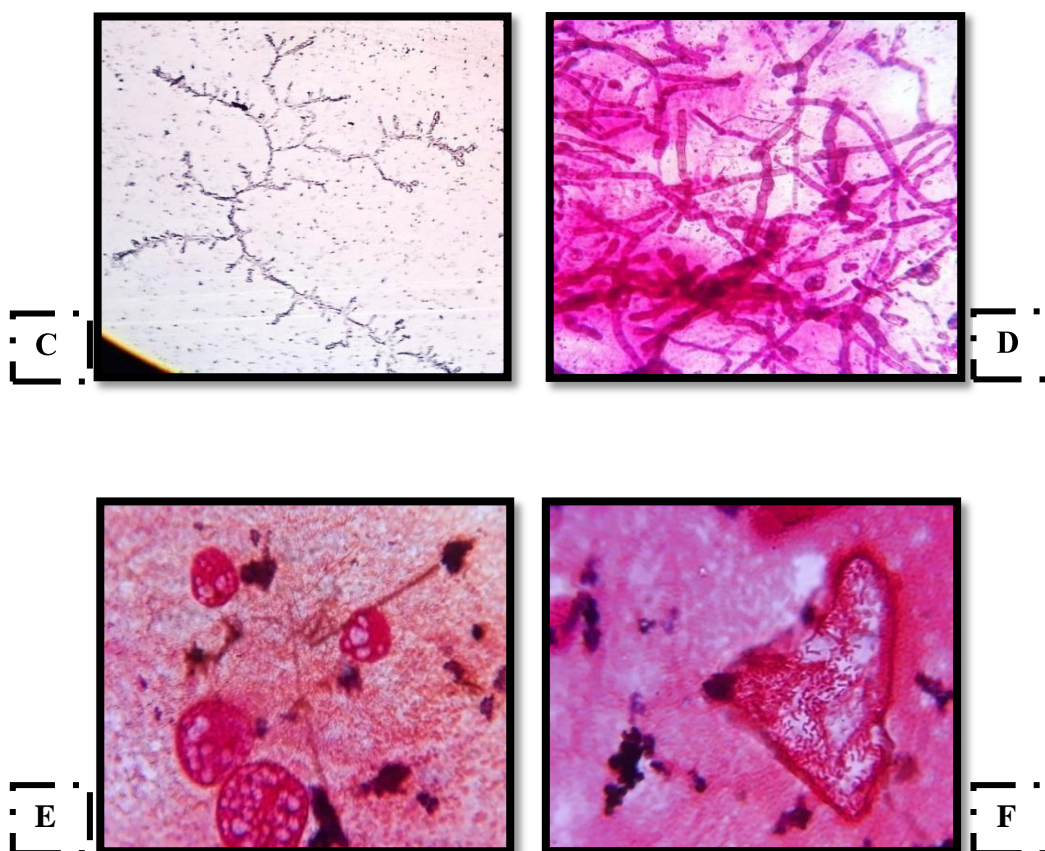


Fig 5: Show isolated actinomycetes. A – *Bifidobacter* sp. B – *Norcodia* sp. C – *Micromonospora* sp. D – *Micromonospora chalcea*. E - *Enterobacter* sp., F - *Actinomyces pyogenes*

Phyllosphere actinomycetes of garden plant			
Sl. No.	Name of the plant		Genera representing on the leaf surface
	Common name	Botanical name	Actinomycetes
1	Sacred fig	<i>Ficus religiosa</i>	<i>Bifidobacter</i> sp.
2	Ricinus	<i>Ricinus communis</i>	<i>Micromonospora chalcea</i> .
3	Trumpet bushes	<i>Tecoma</i> sp.	<i>Bifidobacter</i> sp.
4	Firebush	<i>Hamelia patens</i>	<i>Norcodia</i> sp.
5	Pongamoil tree	<i>Millettia pinnata</i>	<i>Micromonospora</i> sp.
Phyllosphere actinomycetes of medicinal plants			
1	Lemmon grass	<i>Cymbopogon</i>	<i>Micromonospora</i> sp. and <i>Norcodia</i> sp.
2	Citrus	<i>Citrus</i> sp.	<i>Bifidobacte</i> rsp.
3	Nerium	<i>Nerium oleander</i>	<i>Bifidobacter</i> sp.
4	Centella	<i>Centella asiatica</i>	<i>Bifidobacter</i> sp. and <i>Micromonospora chalcea</i> .
5	Noni	<i>Morinda citrifolia</i>	<i>Micromonospora</i> sp. and <i>Norcodia</i> sp.
Phyllosphere actinomycetes of an aquatic plant (emergent plant)			
1	Alocasia	<i>Alocasia macrorrhizas</i>	<i>Actinomyces pyogenes</i>

2	Colacasia	<i>Colacasia esculantum</i>	<i>Enterobacter</i> sp.
3	Water hyacinth	<i>Eichhornia crassipes</i>	<i>Norcodia</i> sp.
4	Cyperus	<i>Cyperus</i> sp.	<i>Nocardia</i> sp. and <i>Bifidobacter</i>
5	Knotweed, Knotgrass, Smartweed, etc	<i>Polygonum glabrum</i>	<i>Enterobacter colacae</i>
Phyllosphere actinomycetes of terrestrial plants			
1	Thorn apple	<i>Datura metel</i>	<i>Micromonospora</i> sp. and <i>Bifidobacter</i> sp.
2	Coral vine	<i>Antigonon leptopus</i>	<i>Bifidobacter</i> sp.
3	Ashoka tree	<i>Polyalthia logifolia</i>	Unknown
4	Lablab	<i>Lablab purpureus</i>	<i>Actinomyces pyogenes</i>
5	Fish tail palm	<i>Caryota mitis</i>	<i>Micromonospora chalcone</i>

Table 6: Phyllosphere actinomycetes of the garden, medicinal, aquatic and terrestrial plants.

Reference

In Plant and Soil, Volume 15, Issue 1, Pages 81–109, Ruinen J. discusses the environmentally overlooked milieu known as the phyllosphere. The phyllosphere as a habitat for microbes [2] Vorholt J.A., Nature Reviews Microbiology, 10, 828–840, 2012. Oecologia, 71, 1987, 405–408; Kinkel, Andrews, Berbee, and Nordheim, 1987. Leaves as islands for bacteria. Within the volume Phyllosphere Microbiology, edited by S.E. Lindow, E.I. Hecht-Poinar, and V.J. Elliot (St. Paul, 2002), Belanger and Avis (2002) discuss the ecological processes and interactions that take place in leaf surface fungus. In a 2002 study published in Microbiology, Inacio et al. estimated the variety and abundance of phylloplane mycobiota on certain plants in a Portuguese environment typical of the Mediterranean. The study was conducted by Pereira, de Carvalho, Fonseca, Amaral-Collaco, and Spencer-Martins. (6) Verma K.S., Indian Journal of Aerobiology, 3(1), 1990, pp. 79–82. A study conducted by Bajwa, Rukhsana, Farooq, Muhammad, and Javaid A. on the aeromycoflora of Lahore found that air mycoflora in less populated, non-commercialized regions varied with the seasons. Chapters 113–122 of Biota, volume 1, written in 1997. The diversity of bacterial endophytes colonizing sweet pepper (*Capsicum annum* L.) is affected by chilling and cultivar type, according to a study published in the Canadian Journal of Microbiology (52, 2006b, 1036–1045) [8]. *Acerplj antanoides* leaf fungal community seasonal study [9] by Breeze E.M. and

Dix N.J. The referenced article is from the Trans. Br. Mycol. Soc., volume 77, issue 1981, pages 321–328. Phylloplane and litter fungi of *Ilex aquifolium*, in: Trans. Br. Mycol. Soc., vol. 77, no. 3, 1981, pp. 329–337, by Mishra R.R. and Dickinson C.H. Referenced in [11] Andrews J.H. and Harris R.F.'s article "The ecology and biogeography of microorganisms of plant surfaces" published in the Annual Review of Plant Pathology in 2000, pages 145–180. Can. J. Bot. 80: 460–469, 2002. Osono T. Phyllosphere fungi on *Fagus sylvatica* leaf litter: occurrence, colonization and succession. Phyllosphere fungus on live and decomposing leaves of giant dogwood, Mycoscience 45, 2004, 35–41, by Osono T.M., Bhatta B.K., and Takeda H. The phylloplane and phyllosphere fungi of Saudi Arabian wheat [14] Abdel-Hafez S.I.I. No. 75, 1981, pages 33–38, Mycopathologia. The phylloplane and chlorosphere fungus of four species of ferns native to Saudi Arabia [15] Mycopathologia 85 (1984): 45–82. In their 1985 publication "Leaf Surface Fungi of *Argemone mexicana* Growing in Saudi Arabia," Abdel-Hafez S.I.I. compiled a list of 69 species of fungus. The cellulolytic capacity and mycoflora of the mature sugarcane (*Saccharum officinarum* L.) plant and its leaves, stems, bagasse, and juice in Egypt was studied by Abdel-Hafez, El-Said, and Gherabawy in 1995. The study was published in the Bulletin of the Assiut University of Science and was numbered 24. In Trans. Brit. Mycol. Soc., 67, 1976, 275–28, Ecker A. discussed the non-parasitic mycoflora of *Panicum polyanthes*'s phylloplane and litter. [19] In

Mycology, 29(4), 2001, 210–217, EI–Said A.H.M. reports on the cellulolytic ability of phyllosphere and phylloplane fungi found in bananas grown in Upper Egypt. The Microbiology Laboratory Manual, edited by Varghese and Joy, P.P., [20]. Published by the Pineapple Research Station of Kerala Agricultural University, pages 1–76, in 2014. [21] Mycopathologia, 75, 1981, 33–38; Abdel-Hafez S.I.I., Phyllosphere and phylloplane fungus of Saudi Arabian wheat. The effects of the phyllosphere microflora composition on the biological control of grapevine fungal infections were discussed in a 1994 article by Sackenheim, Weltzien, and Kast in the Vitis Journal of Grapevine Research, volume 33, issue 4, pages 235–240. In the Biology of Nitrogen Fixation, edited by A. Quispel and published in 1974 by North Holland Publishing Company in Amsterdam, Ruinen J. discusses nitrogen fixation in the phytosphere and its effects on plant growth and development (pp. 121–167). The phyllosphere

microflora of some common plants was published in the Mysore Journal of Agricultural Sciences in 1978 and can be found in volume 12, pages 398–403, by Bopaih, Wani, and Rai. Changes in bacterial and actinomycetes diversity of groundnut Phyllosphere with reference to plant age, varieties of leaves, and seasons applying culture dependant approach [25] Karthick R.N., Selvaraj, Sahayaraj, and Kitherian. Internet Journal of Microbiology, 61998, 1-6. In a study published in the European Journal of Biological Research in 2016, the authors Tanti, Amarjyoti, Bhattacharyya, Pranaba, Dutta, Prasanta, Sarmah, Satya, Madhab, Mausomi, Saikia, Dipiman, Kachari, Anita, Berceroyjyrwa, and Rahnam examined the diversity of phylloplane microflora in several tea cultivars from Assam, which is located in northeastern India. Microbiology of the Phyllosphere, by Steven E.L. and Maria T.B., published in Applied and Environmental Microbiology in 2003, volume 69, issue 4, pages 1875–1883.