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Phyllosphere Microflora of Few Medicinal, Garden, Terrestrial and Aquatic Plants in and Around Mysuru Districts, Karnataka

Veerabhadra Swamy AL*[1], Ananth R and Yamuna SP[2]

*[1]Corresponding author *Assistant Professor, Post Graduate Department of Botany, J.S.S. College of Arts, Commerce and Science, B.N. Road, Mysuru – 570 025, Karnataka, India,*

[2] Post Graduate Students, Post Graduate Department of Botany, J.S.S. College of Arts, Commerce and Science, B.N. Road, Mysuru – 570 025, Karnataka, India

Correspondence author

Veerabhadra Swamy AL
Assistant Professor, Post Graduate Department of Botany, J.S.S. College of Arts, Commerce and Science, B.N. Road, Mysuru – 570 025, Karnataka, India
vsbotany@gmail.com

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Abstract:

The plant surface especially the leaf surface i.e., phyllosphere is exposed to dust and other particulates resulting in the establishment of a typical microflora. The surfaces of aerial plant parts provide habitat for epiphytic micro-organisms, many of which also influence the growth of pathogens. Microbial life in the phyllosphere is of great commercial importance to the agricultural industry for understanding the survival of plant disease-causing bacteria and fungi is vital for developing new ways to control their spread. In the present investigation, microflora isolated from the surface of a leaf of different medicinal, garden, terrestrial and aquatic plants. In this work fungus, bacteria and actinomycetes are isolated by using leaf washing methods on suitable nutritive media. Generally, the present work carried out by observation and identification of microflora under the microscope and biochemical test methods. The present work reveals that fifteen mycoflora (fungi), fifteen bacterial strains and nine actinomycetes were identified using manuals and keys.

I. Introduction

The leaf surface has been termed phylloplane and the zone on leaves inhabited by the microorganisms as phyllosphere. The plant surface especially the leaf surface is exposed to dust and other particulates resulting in the establishment of a typical microflora consisting of bacteria, fungi, and microalgae. The growth of microflora is aided by exudates of leaf cell which consists of various amino acids, glucose, fructose, and sucrose. The term "Phyllosphere" was coined by the Dutch microbiologist, Ruinen [1] from her observations on Indonesian forest vegetation where thick a microbial epiphytic association exists on leaves. The environment of the phyllosphere includes physical, chemical and the biological components occupying the surrounding space. In the most recent review of microbial ecology in the phyllosphere, Vorholt [2] highlighted fundamental studies elucidating conservation mechanisms through which microorganisms survive on above-ground plant parts. The

microbial communities of leaves are diverse and include many generals of bacteria, filamentous fungi, yeasts, algae, and less often, protozoa and nematodes.

The surfaces of aerial plant parts provide habitat for epiphytic micro-organisms, many of which also influence the growth of pathogens. Bacteria are generally the main first inhabitants of newly expanded leaves, while yeasts and filamentous fungi dominate later in the growing season [3]. The phyllosphere is the three-dimensional space on the leaf surface. In recent years, much 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 many genera of bacteria, filamentous fungi, yeasts, algae, and less often protozoa and nematodes which may form resident populations on leaves and the non-pathogenic fungi that inhabit the phyllosphere depend on nutrients exuded from the leaf or those deposited from the atmosphere [4,5]. 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 a particular area with specific environmental conditions [6,7].

Research into the characteristics of microbial life in the phyllosphere is of great commercial importance to the agricultural industry for understanding the survival of plant disease-causing bacteria and fungi is vital for developing new ways to control their spread. And there has been a recent rise in the number of food poisoning cases associated with fruit and vegetables contaminated with bacteria, such as Salmonella and *E. coli*. Recent studies have demonstrated that profiling of phyllosphere communities based on culture-dependent methods is likely to be inaccurate and to underestimate diversity [8]. In the case of the phyllosphere, the use of culture-independent approaches has shown that although assumptions about the dominant inhabitants are largely correct, the diversity of phyllosphere communities is far greater than before recognized. 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 [9-13].

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. A 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 [14-18]. El-Said [19] also reported the fungi identified from different plant leaf surfaces (phyllosphere and phylloplane).

The aim of the present work is to identify and comparative study of the medicinal, terrestrial, garden and aquatic phyllosphere mycoflora. The medicinal plant is grown in natural conditions since the garden plant is grown in artificially and conserved in the garden area. The present works will show at any difference in terrestrial and aquatic, natural conditionally growing medicinal plants leaf flora and artificially conserved garden plants leaf microflora. In the present investigation, experiments were carried out to isolate the phyllosphere microflora in few medicinal (*Cymbopogon, Citrus, Nerium oleander, Centella asiatica,* and *Morinda citrifolia*), few terrestrial (*Colacasia esculantum, Eichhornia crassipes, Cyperus* sp., *Alocasia macrorrhizas,* and *Polygonum glabrum*), few aquatic (*Datura metel, Antigonon leptopus, Polyalthia logifolia, Lablab purpureus* and *Caryota mitis*) and few garden plants (*Ficus religiosa, Ricinus communis, Tecoma, Hamelia patens* and *Millettia pinnata*).

II. Materials and Methods

2.1 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.

2.2 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 121^0C at 15 mins.

2.3 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 ^{0}C. Observe after 2-3 days.

2.4 Preparation of Potato Dextrose Agar (PDA)

Add 39 gm of commercial prepared potato dextrose agar powder in l liter of distilled water then add a pitch of Chloramphenicol powder. Boil while mixing to dissolve. Sterilize the dissolved mixture using autoclave at 121 ^{0}C for 15 minutes.

2.5 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.

2.6 Identification of Bacteria

The staining of Bacteria for identification is done by using gram stating 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.

2.7 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. *S. aureus*	Fermented with acid production only Eg: *S. aureus*
Fermented with acid and gas production Eg. *E. coli, Klebsiella*	Fermented with acid and gas production Eg: *E. coli, Klebsiella*
Non- Fermenting Eg. *Acinoetobacter*	Non- Fermenting Eg: *S. typhi* *S. paratyphi* *Pseudomonas* sp.

Table 1: Glucose and Sucrose fermentation

2.8 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 H2S production	Acid Slant / Acid butt without gas & H2S production	*Staphylococcus aureus*
A/A with gas and without H2S production	Acid Slant / Acid butt with gas & without H2S production	*E. coli, Klebsiella*
K/A with gas and without H2S production	Alkaline slant / Acid butt with gas & without H2S production	*Salmonella paratyphi*

Table 2: Triple sugar Iron Agar test

2.9 Casein hydrolysis test

In casein hydrolysis test to find if an organism can produce the exoenzyme casesase. 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.

2.10 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.*

2.11 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.

2.12 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 l liter of distilled water. Boil while mixing to dissolve. Autoclave the dissolved mixture at 121^0C 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).

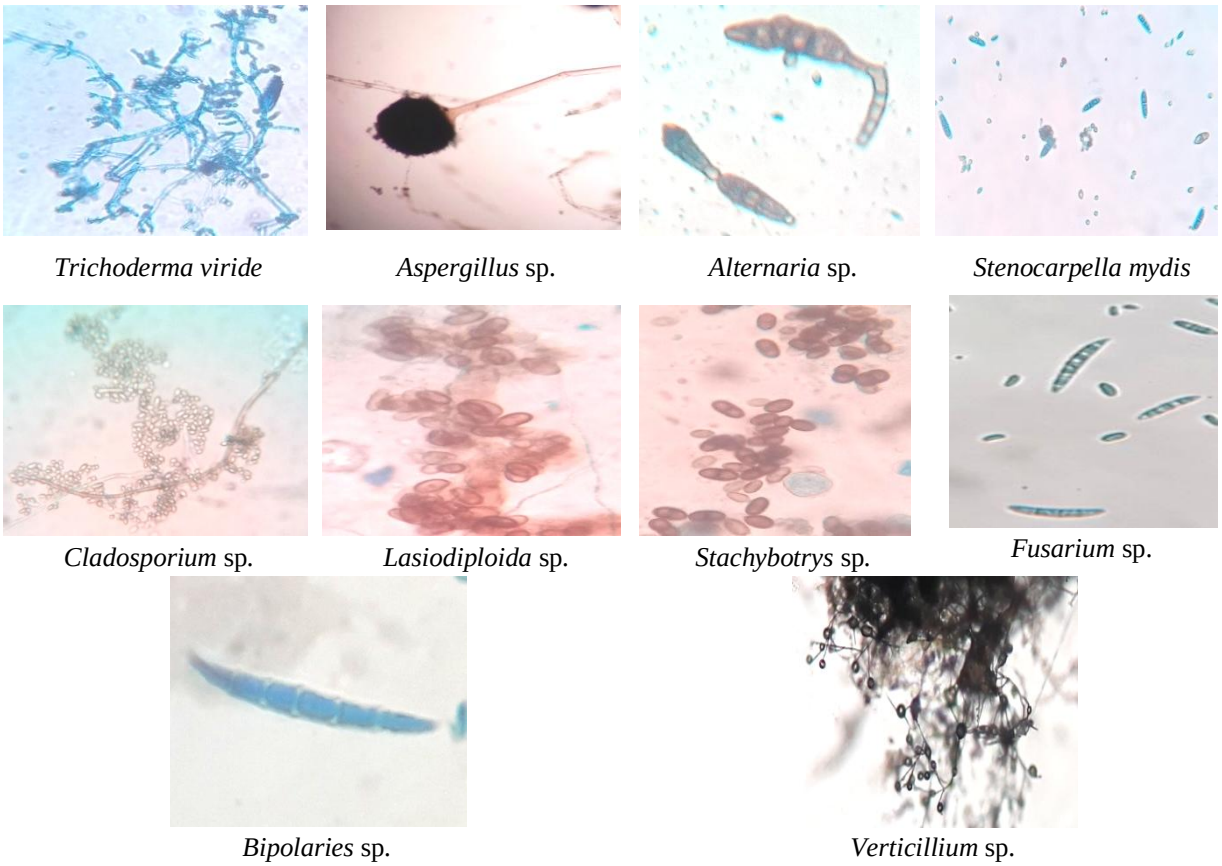
Coagulase Test

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*.

III. 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.



Trichoderma viride | *Aspergillus* sp. | *Alternaria* sp. | *Stenocarpella mydis*

Cladosporium sp. | *Lasiodiploida* sp. | *Stachybotrys* sp. | *Fusarium* sp.

Bipolaries sp. | *Verticillium* sp.

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

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Fermentation test: Carbohydrate Fermentation

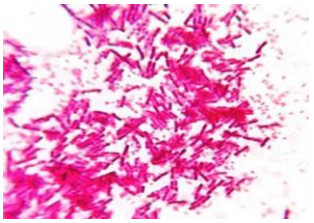
Fig 2 A. Glucose fermentation
(First test tube uninoculant, Middle-Klebsiella and Last –Staphylococcus aureus)

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

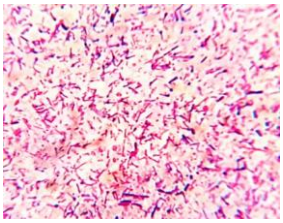
Fig 2 C. Triple sugar Iron fermentation test
(First test tube –*Klebsiella,* Middle- *Salmonella* sp. and Last –uninoculant)

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).

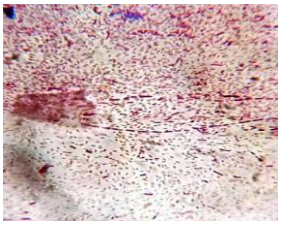
Fig 3 A. Casein Hydrolysis (green colonies shows *Pseudomonas aeruginosa)*. B. Gelatin Hydrolysis test



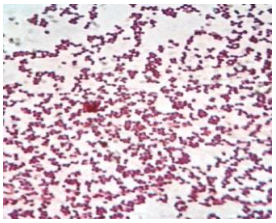
Klebsiella sp.



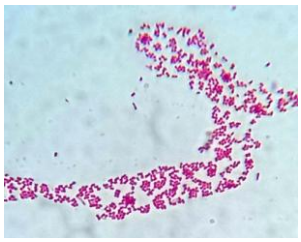
Corynebacterium



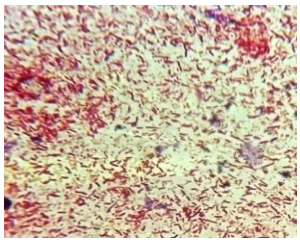
Bacillus anthrcis



Sarcina sp.

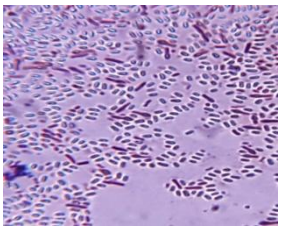


Staphylococcus aureus

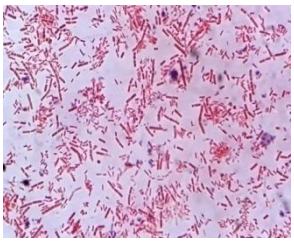


Pseudomonas

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	*Ficu sreligiosa*	*Pseudomans* sp., *Sarcina* sp. and *Corynebacterium* sp.
2	Ricinus	*Ricinus communis*	*Bacillus anthrcis*
3	Trumpet bushes	*Tecoma*	*Klebsiella* sp. and *Pseudomonas aeruginosa*
4	Firebush	*Hamelia patens*	*Bacillus anthrcis*
5	Pongamoil tree	*Millettia pinnata*	*Fusobacterium nucleatum*
Phyllosphere bacterial strains of medicinal plants			
1	Lemmon grass	*Cymbo pogon*	*Fusobacterium nucleatum* and *Corynebacterium* sp.
2	Citrus	*Citrus*	*Pseudomonas* sp. and *Staphylococcus aureus*
3	Nerium	*Nerium oleander*	*Pseudomonas aeruginosa* and *Salmonella* sp.
4	Centella	*Centella asiatica*	*Bacillus anthrcis* and *Corynebacterium* sp.
5	Noni	*Morinda citrifolia*	*Staphylococcus aureus* and *Klebsiella* sp.
Phyllosphere bacterial strains of an aquatic plant (emergent plant)			
1	Alocasia	*Alocasia macrorrhizas*	*Sarcina* sp. and *Corynebacterium* sp.
2	Colacasia	*Colacasia esculantum*	*Klebsiella* sp., *Fusobacterium nucleatum*

3	Water hyacinth	*Eichhornia crassipes*	*Pseudomonas* sp. and *Listeria* sp.
4	Cyperus	*Cyperus* sp.	*Pseudomonas* sp. and *Bacillus anthrcis*
5	Knotweed, Knotgrass, Smartweed, etc	*Polygonum glabrum*	*Pseudomonas* sp. and *Colastridium* sp.
Phyllosphere bacterial strains of terrestrial plants			
1	Thorn apple	*Datura metel*	*Corynebacterium* sp. and *Clostridium* sp.
2	Coral vine	*Antigonon leptopus*	*Klebsiella* sp., *Fusobacterium nucleatum* and *Salmonella* sp.
3	Ashoka tree	*Polyalthia logifolia*	*Sarcina* sp., *Pseudomonas* sp. and *Staphylococcus aureus*
4	Lablab	*Lablab purpureus*	*Staphylococcus aureus* and *Pseudomonas* sp.
5	Fish tail palm	*Caryota mitis*	*Pseudomonas* sp. and *Fusobacterium nucleatum*

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].

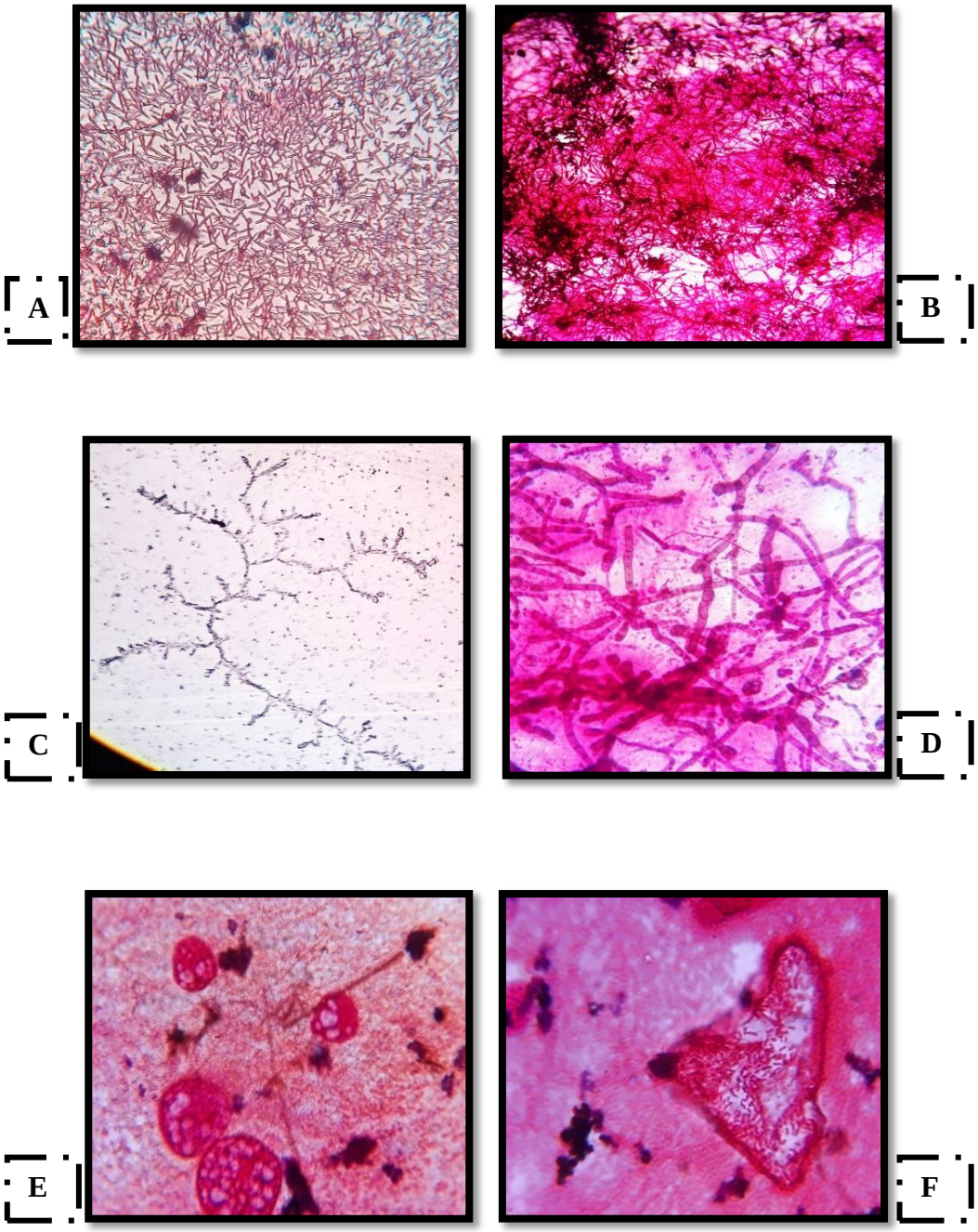


Fig 5: Show isolated actinomycetes. A – *Bifidobacter* sp. B – *Norcodia* sp. C – *Micromonospora* sp. D – *Micromonospora chalcea.* E - *Enterobacter* sp., F - *Actinomyces pyogenes*

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	*Ficus religiosa*	*Bifidobacter* sp.
2	Ricinus	*Ricinus communis*	*Micromonospora chalcea.*
3	Trumpet bushes	*Tecoma* sp.	*Bifidobacter* sp.
4	Firebush	*Hamelia patens*	*Norcodia* sp.
5	Pongamoil tree	*Millettia pinnata*	*Micromonospora* sp.
Phyllosphere actinomycetes of medicinal plants			
1	Lemmon grass	*Cymbopogon*	*Micromonospora* sp. and *Norcodia* sp.
2	Citrus	*Citrus* sp.	*Bifidobacte r*sp.
3	Nerium	*Nerium oleander*	*Bifidobacter* sp.
4	Centella	*Centella asiatica*	*Bifidobacter* sp. and *Micromonospora chalcea.*
5	Noni	*Morinda citrifolia*	*Micromonospora* sp. and *Norcodia* sp.
Phyllosphere actinomycetes of an aquatic plant (emergent plant)			
1	Alocasia	*Alocasia macrorrhizas*	*Actinomyces pyogenes*
2	Colacasia	*Colacasia esculantum*	*Enterobacter* sp.
3	Water hyacinth	*Eichhornia crassipes*	*Norcodia* sp.
4	Cyperus	*Cyperus* sp.	*Nocardia* sp. and *Bifidobacter*
5	Knotweed, Knotgrass, Smartweed, etc	*Polygonum glabrum*	*Enterobacter colacae*
Phyllosphere actinomycetes of terrestrial plants			
1	Thorn apple	*Datura metel*	*Micromonospora* sp. and *Bifidobacter* sp.
2	Coral vine	*Antigonon leptopus*	*Bifidobacter* sp.
3	Ashoka tree	*Polyalthia logifolia*	Unknown
4	Lablab	*Lablab purpureus*	*Actinomyces pyogenes*
5	Fish tail palm	*Caryota mitis*	*Micromonospora chalcea*

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

Reference

[1] Ruinen J., The phyllosphere, I. An ecologically neglected milieu, *Plant and Soil*, 15, 1961, 81- 109.

[2] Vorholt J.A., Microbial life in the phyllosphere, *Nat. Rev. Microbial.*,10, 2012, 828-840.

[3] Kinkel L.L., Andrews, J.H., Berbee, F.M. and Nordheim, E.V., Leaves as islands for microbes, *Oecologia*, 71, 1987, 405-408.

[4] Belanger R.R. and Avis T.J, Ecological processes and interactions occurring in leaf surface fungi, in: *Phyllosphere Microbiology* (S.E. Lindow, E.I. Hecht-Poinar and V.J. Elliot (eds),. APS Press: St. Paul,2002), 193-207.

[5] Inacio J., Pereira P., de Carvalho M., Fonseca A., Amaral-Collaco M.T. and Spencer-Martins I., Estimation and diversity of phylloplane mycobiota on selected plants in a Mediterranean-type ecosystem in Portugal, *Microb Ecol*, 44, 2002, 344–353.

[6] Verma K.S., *Ind. J. Aerobial.*, 3(1), 1990, 79-82.

[7] Bajwa, Rukhsana and Farooq, Muhammad and Javaid A., Aeromycoflora of Lahore. I: Seasonal variation in air mycoflora of non-commercialized, less populated areas. *Biota.*, 1, 1997, 113-122.

[8] Rasche F., Trondl R., Naglreiter C., Reichenauer T.G. and Sessitsch A., Chilling and cultivar type affect the diversity of bacterial endophytes colonizing sweet pepper (*Capsicum anuum* L.), *Can. J. Microbiol.,* 52, 2006b, 1036– 1045.

[9] Breeze E.M. and Dix N.J., Seasonal analysis of the fungal community on *Acerplj antanoides* leaves. *Trans. Br. Mycol.Soc.*,77, 1981, 321-328.

[10] Mishra R.R. and Dickinson C.H., Phyllosplane and litter fungi of *Ilex aquifolium, Trans. Br. Mycol. Soc.,* 77, 1981, 329-337.

[11] Andrews J.H. and Harris R.F. The ecology and biogeography of microorganisms of plant surfaces, *Annu. Rev. phytopathol.*,38, 2000, 145-180.

[12] Osono T., Phyllosphere fungi on leaf litter of *Faguscrenata*: occurrence, colonization and succession, *Can. J. Bot.,* 80, 2002, 460-469.

[13] Osono T.M., Bhatta B.K. and Takeda H, Phyllosphere fungi on living and decomposing leaves of giant dogwood, *Mycoscience,* 45, 2004, 35-41.

[14] Abdel-Hafez S.I.I., Phyllosphere and phylloplane fungi of wheat cultivated in Saudi Arabia. *Mycopathologia.*,75, 1981, 33-38.

[15] Abdel-Hafez S.I.I., Phyllosphere and phylloplane fungi of four fern plants growing in Saudi Arabia, *Mycopathologia.*,85, 1984, :45-82.

[16] Abdel-Hafez S.I.I., Leaf surface fungi of *Argemonemexi-cana* growing in Saudi Arabia, Cryptogamie, *Mycol.,* 6, 1985, 69-78.

[17] Abdel-Hafez S.I.I., El-Said A.H.M. and Gherabawy Y.A.M.H., Mycoflora of leaf surface stem, bagassw and juice of adult sugarcane (*Saccharum officinarum* L.) plant and cellu-lolytic ability in Egypt (Bull. Fac. Sci. Assiut Univ. 24, 1995), 113-130.

[18] Eicker A., Non-parasitic mycoflora of the phylloplane and litter of *Panicum coloarum, Trans. Brit. Mycol. Soc.,* 67, 1976, 275- 28.

[19] EI-Said A.H.M., Phyllosphere and Phylloplane Fungi of Banana Cultivated in Upper Egypt and their Cellulolytic Ability, *Mycology,* 29(4), 2001, 210- 217.

[20] Varghese, Naveena and Joy P.P., Microbiology Laboratory Manual. Kerala Agricultural University, Pineapple Research Station. [2014] 1- 76.

[21] Abdel-Hafez S.I.I, Phyllosphere and phylloplane fungi of wheat cultivated in Saudi Arabia, *Mycopathologia,* 75, 1981, 33-38.

[22] Sackenheim R., Weltzien H.C., Kast W.K., Effects of microflora composition in the phyllosphere on biological regulation of grapevine fungal diseases, *Vitis. Journal of Grapevine Research,* 33(4), 1994, 235-240.

[23] Ruinen J., Nitrogen fixation in the Phyllosphere, In: *the Biology of Nitrogen fixation*. (Ed: A Quispel, North Holland publishing company, Amsterdam. Oxford American Elsevier publishing company Inc. N. Y. Chay., 1974), 121-167.

[24] Bopaih B.M., Wani S.P. and Rai P.V., Phyllosphere microflora of some common plants, *Mysore Journal of Agricultural Sciences,* 12, 1978, 398-403.

[25] Karthick R.N., Selvaraj, Sahayaraj, and Kitherian, Changes in bacterial and actinomycetes diversity of groundnut Phyllosphere with reference to plant age, kinds of leaves and seasons adapting culture dependent method, *Internet Journal of Microbiology*, 61998, 1-6.

[26] Tanti, Amarjyoti, Bhattacharyya, Pranaba, Dutta, Prasanta, Sarmah, Satya, Madhab, Mausomi, Saikia, Dipiman, Kachari, Anita, Berceroyjyrwa and Rahnam, Diversity of phylloplane microflora in certain tea cultivars of Assam, North-East India, *European Journal of Biological Research,* 2016, 287-292.

[27] Steven E.L. and Maria T.B., Microbiology of the Phyllosphere, *Applied and Environmental Microbiology,* 69(4), 2003, 1875-1883.