

INCIDENCE OF Alternaria alternata (Fr.) Keissl. ON PHYLLOPLANE OF SOME SHRUBS AND TREES OF MALL ROAD, LAHORE

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Abstract

In the present investigation, an attempt was made to investigate myco-flora of forty trees, selected for the collection of infected leaves from Mall Road Lahore. Morpho-anatomical descriptions and illustrations were recorded after thorough analysis. Identification was done by light microscopy, stereomicroscopy and scanning electron microscopy (SEM). Among all the isolated fungi, *Alternaria alternata* (Fr.) Keissl.and *Cladosoprium cladosporioides* (Fresen.) G.A. de Vries were found to be the most dominant upon the leaves. It is expected that there could be a large number of leaf-associated fungi present on leaves of these trees.

Key words: Alternaria alternata, Mall Road, Epiphytes, Phylloplane, Scanning Electron Microscopy

Novelty statement: In current study, 19 plants species have been first time reported to be affected by *Alternaria alternata*.

Introduction

The external surfaces of plant have been recognized as important habitats for epiphytic microorganisms. The above-portions of plants as the habitat of microbes can be expressed by the term phyllosphere e.g. phylloplane (leaves), caulosphere (stems), anthosphere (flowers), and carposphere (fruits).

In the phylloplane, the growth of microorganisms is dependent on nutrients from plant metabolites that are secreted to the phylloplane or on compounds in materials from external sources that drop on the plant surface. The plant metabolites are organic substances, mostly simple sugars e.g., glucose, fructose and sucrose, while the materials from external sources are inorganic nutrients (Limtong & Koowadjanakul, 2012). The phylloplane supports an abundant and varied microflora, with yeasts, filamentous fungi, and bacteria dominating at different times during the various stages of leaf development (McCormack, 1994).

According to Saha *et al.* (2013), phylloplane fungi have been poorly studied as compared to endophytic, saprobic and pathogenic fungi. Phyloplane fungi mostly belong to filamentous fungal group. Filamentous fungi are made up of long, slender, branched thread like hyphae which can be septate or aseptate.

In this study, we characterized and identified phyllopalne fungal flora of trees growing along the Mall Road Lahore. By selecting the 40 trees of Lahore's primary and most famous road The Mall (Shahrah-e-Quaid-e-Azam), infected plants were found belonging to 21 families. These plant families were, Acanthaceae, Amaranthaceae, Anacardiaceae, Annonaceae, Apocynaceae, Asteraceae, Bignoniaceae, Burseraceae. Combretaceace. Convolvulaceae. Euphorbiaceae, Cucurbitaceae. Ebenaceae. Fabaceae, Lythraceae, Malvaceae, Moraceae, Myrtaceae, Rhamnaceae, Rosaceae and Sapotaceae.

Materials and Methods

1. Description of sampling site: The infected leaf samples were collected in the month of October 2014 when temperature was approximately 31° C. Trees growing along the Mall road, Lahore were covered with dust but most of them were in good conditions.

2. Collection of infected leaf samples: After careful inspection of trees, infected and contaminated leaves were selected. Only yellowish-brown, pale-yellowish, and spotted leaves were collected from the trees of Mall Road. Collected infected leaves were used to isolate and study the fungi present on them. Collected leaves were placed inside the paper envelops and later on pressed with the help of plant presser.

3. Photography of infected leaf samples: Collected

leaves of plants were photographed by digital camera. For morphological study, infected parts on leaves were first observed under stereomicroscope and then photographed under it.

4. Microscopy and slide preparation: Microscopy of infected portion was done by slide preparation. For this purpose, material was collected from the infected part of the leaf with the help of a needle by observing under stereo-microscope. A drop of 10% Lactic acid was dropped on glass slide and isolated material was put into it. Material was covered by a cover-slip and observed under Laborned compound microscope (at $100 \times$ and $400 \times$ magnification). Fungal structures e.g. spore, conidia, conidiophores and other elements were measured with the help of stage and ocular micrometer. Descriptions were prepared and light micro-graphs were taken. Illustrations were first made on plain paper with the help of Camera Lucida then sketched on tracing paper. Scanning Electron Micrographs were also obtained for conidia of some fungal taxa.

Results

A total of 9 fungal species were characterized morpho-anatomically and identified. Among them, dominant and completely identified taxa were *Alternaria alternata* (Fr.) Keissl., *A. brassicae* (Berk.) Sacc., *Aspergillus niger* Tiegh., *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, *C. heterophragmatis* S.A. Khan & M. Kamal, *Curvularia lunata* (Wakker) Boedijn. However some of the taxa which were identified up to genus level were, *Alternaria* sp., *Aspergillus* sp. and *Curvularia* sp. (Table 1, Plates 1-40).

Discussion

From Pakistan, it is the first report about the occurance of phylloplane myco-flora on the trees leaves of Alstonia scholaris (L.) R. Br., Bauhinia variegata (L.) Benth., Broussonetia papyrifera (L.) L'Herit ex Vent., Bursera serrata Wall. ex Colebr., Cassia sp., Cassia fistula L., Chrysanthemum indicum L., Diospyros kaki Thunb., Erythrina suberosa Roxb., Ficus sp., F. elastica Roxb. ex Hornem., F.

benghalensis L., F. racemosa L., F. religiosa L., F. virens Aiton, Hibiscus rosa-sinensis L., Ipomoea batatas (L.) Lam., Jatropha integerrima Jacq., Justicia adhatoda L., Lagerstroemia speciosa (L.) Pers., Mangifera indica L., Millettia pinnata (L.) Pierre, Minusops elengi L., Plumeria obtusa L., Pongamia sp., Prunus armeniaca L., Senna surattensis (Burm. f.) H. S. Irwin & Barneby, Syzygium cumini (L.) Skeels., Tacoma stans (L.) Juss. ex Kunth, Terminalia arjuna (Roxb.) Wight & Arn., T. chebula Retz., Ziziphus jujuba Mill.. Previously, no data was reported about the existence of any kind of phylloplane myco-flora on *Alternanthera ficoidea* (L.) Sm. and Causonia sp., Combretum indicum (L.) DeFilipps, Lagerstroemia indica L., and Polyalthia longifolia Sonn. B.Xue & R.M.K.Saunders.

In the present work, efforts were made to investigate myco-flora of some trees of Mall Road, Lahore. Phylloplane myco-flora of forty trees were described and illustrated morpho-anatomically with of different techniques. the help Fungal characterization and identifications were made by using microscope, stereomicroscope and scanning electron microscope (SEM). This investigation was conducted to find out the diversity and species richness of filamentous fungi on leaves. In this study, Alternaria alternata and Cladosporium cladosporioides were reported on leaf surface of Albizia lebbeck and were characterized morphoanatomically. According to Mushtaq et al. (2005), 15 yeast species belonging to 9 genera were isolated from 40 slime flux samples collected from the leaves of Acacia nilotica (L.) Willd. ex Delile, Albizzia lebbeck and Aralia cachemirica Dcne., trees. Species isolated during his investigation from Albizzia lebbeck were Candida valdiviana Grinb. & Yarrow, Debaryomyces castellii Capr., D. yamadae (Van der Walt & Johannsen) Van der Walt, M.T. Sm. & Y. Yamada, Fibulobasidium inconspicuum Bandoni, Phaffia rhodozyma M.W. Mill., Yoney. & Soneda, P. anomala, P. rabaulensis and Sporidiobolus ruineniae Holzschu, Tredick & Phaff. In the present investigation Alternaria sp. and Curvularia lunata were reported on the leaf surface of Alstonia scholaris and characterized morpho-anatomically.

Table 1: A morpho-anatomical comparison of fungal taxa isolated from the phylloplane of tree species of Mall Road, Lahore

Sr. No.	Fungal species	s Conidiophore		Vesicle and phialides	sicle and phialides Spores/ conidia			Host plants
		Size	Colour		Size and septa	Colour and shape	Texture	
1.	Alternaria alternata	47-106 x 0.78-1.22 μm	Septate, branched, olive brown	Absent	26-78 x 2.4-16.5 μm/ 3-5 cross septa	Obclavate cylindrical beak to ellipsoidal and light olive brown to pale brown	Smooth to warty or verrucose	Albizia lebbeck, Alstonia scholaris, Bauhinia variegata, Broussonetia papyrifera, Bursera serrate, Cassia fistula, Causonia sp., Chrysanthemun indicum, Diospyros kaki, Erythrina suberosa Ficus sp., Ficus benghalensis, F. elastica, F. racemosa, Ficus virens, Ipomoea batatas, Jatropha integerrima, Justicia adhatoda, Lagerstroemia indica, L. speciosa, Mangifera indica, Millettia pinnata, Polyalthia longifolia, Pongamia sp., Sema surattensis, Syzygium cumini, Tecoma stans, Terminalia arjuna, Terminalia chebula, Ziziphus jujuba.
2.	Alternaria brassicae	190 x 4-11 μm	Branched, erect	Absent	52.52-1.30 μm/ Up to 10 cross septa	Obclavate to ellipsoidal with short beak Olive-brown to brown	Verrucose	Ficus elastica
3.	Alternaria sp.	26-54 x 0.78-1.3 μm		Absent	2.35 x 0.6 µm/ 3-5 cross septa	Light pale yellow	Smooth and divided into several cells by transverse and vertical cells	Albizia lebbeck, Alstonia scholaris, Bursera serrate, Cassia sp., Combretum indicum, Diospyros kaki, Erythrina suberosa, Ficus benghalensis, Ficus elastica, Ficus religiosa, Ficus virens, Justicia adhatoda, Millettia pinnata, Mimusops elengi, Morus alba, Pongamia sp., Prunus armeniaca, Terminalia arjuna, Terminalia chebula.
4.	Aspergillus niger	444-445 x 0.78 μm	Hyaline, dark near the vesicle	Swollen vesicle, flask shaped phialides, 26.2 x 26.5 µm.				Mimusops elengi
5.	Aspergillus sp.	184-210 x 0.5-0.78 μm	Hyaline Septate, branched	Flask shaped phialides, 26.78-27.04 x 27.3- 27.6 μm Golden in colour Swollen vesicle				Cassia sp., Ficus sp., Ficus religiosa, Mangifera indica, Plumeria obtusa, Polyalthia longifolia, Senna surattensis, Terminalia arjuna, Aspergillus sp.
6.	Cladosporium cladosporiodies	300-522 μm x 0.4- 0.52 μm	Hyaline	Absent	1.56-2.08 x 0.52- 0.78 μm	Round, oval, cylindrical and blackish-brown	Smooth wall, Single celled	Albizia lebbeck, Alternanthera ficoidea, Bauhinia variegate, Broussonetia papyrifera, Cassia sp., Cassia fistula, Ficus elastica, F. religiosa, F. virens, Hibiscus rosa-sinensis, Jatropha integerrima, Lagerstroemia indica, Mangifera indica, Prunus armeniaca,
7.	Cladosporium heterophragmatis	28.08 x 1.04 μm	Dark brown, cylindrical, smooth	Absent	15-15.6 x 0.9	Ellipsoidal to limoni	Smooth walled	Ficus elastica
8.	Curvularia lunata				26-26.5 x 0.78-1.0 μm/ 3-5 cross septa, Pointy beak	Pale to dark brown	Smooth, Pointy beak divided into several cells.	Bursera serrata, Causonia sp., Erythrina suberosa, Ficus elastica, Ficus racemosa, Ficus virens, Ipomoea batatas, Pongamia sp.
9.	Curvularia sp.	106 x 0.78 μm		Absent	26.5-28.4 x 1.04- 1.3 μm/ 3-5 cross septa	Olive-brown, smooth, pointy beak	Smooth, pointy beak, 1 celled.	Cassia fistula, Causonia sp., Diospyros kaki, Ficus sp., Ficus elastica, F. racemosa, F. religiosa, Ipomoea batatas, Pongamia sp., Prunus armeniaca, Senna surattensis, Tecoma stans, Ziziphus jujuba



Plate 1: Albizia lebbeck A. Infected Leaf; B. Infection Site; C. Microscopic photograph of conidia of Alternaria alternata; D,E. Conidia of Cladosporium cladosporioides; F. Conidia of Alternata; sp. G. SEM of conidia of A. alternata; H. Illustration of A. alternata; I, J. Illustrations of Cladosporium cladosporioides; Scale Bar (1cm) A= 2.3cm; B= 0.6cm; C= 7.8µm; D= 1.67µm; E= 1.45µm; F= 2.48µm; H= 3.6µm; I=2.62µm; J= 2.77µm



Plate 2: Alstonia scholaris A. Infected Leaf; B. Infection Site; C,D. Microscopic Photograph of Conidia of Alternaria sp. E. Conidia of Alternaria alternata; F, H. Illustrations of Conidia of A. sp. G. Illustration of Conidia of A. alternata; Scale Bar (1cm) A= 2.2cm; B= 0.1cm; C= 4 μ m; D= 7.7 μ m; E= 10.2 μ m; F= 3.90 μ m; G= 3.1 μ m; H= 6.8 μ m



Plate 3: *Alternanthera ficoidea* A. Infected Leaf; B. Infection Site; C. Microscopic Photograph of Conidia of *Cladosporium cladosporioides*; D. Illustration of Conidia of *Cladosporium cladosporioides*; Scale Bar (1cm) A= 1.4cm; B= 0.2cm; C= 0.7µm; D= 0.47µm



Plate 4: *Bauhinia variegata* A. Infected Leaf; B. Infection Site; C-E. Microscopic photo of conidia of *Alternaria alternata*; F. SEM of conidia of *Cladosporium cladosporioides*; G-I. Illustrations of Conidia of *A. alternata*; Scale Bar (1cm) A= 1.4cm; B= 0.1cm; C=7.2µm; D= 6.72µm; E= 9.41µm; G= 3.6µm; H= 3.6µm; I=3.9µm



Plate 5: *Broussonetia papyrifera* A. Infected Leaf; B. Infection Site; C. Microscopic photo of conidia of *Cladosporium cladosporioides*; D. Microscopic photo of conidia of *Alternaria alternata*; E. Illustration of *C. cladosporioides*; F. Illustration of Conidia of *A. alternata*; Scale Bar (1cm) A= 1.5cm; B= 0.1cm; C= 1.33µm; D= 3.62µm; E= 1.33µm; F= 3.3µm



Plate 6: *Bursera serrata* A. Infected Leaves; B. Infection Site; C. Microscope photo of conidia of *Alternaria* sp. D. Illustration of conidia of *A.* sp. E. Unidentified sp. F. Conidia of *Curvularia lunata*; G. Conidia of *Alternaria alternata*; H. Illustration of unidentified sp. I. Illustration of conidium of *Curvularia lunata*; J. Illustration of conidia of *A. alternata*; Scale Bar (1cm) A= 2.8cm; B= 0.3cm; C= 2.52µm; D= 3µm; E= 6.11µm; F= 4µm; G= 6.3µm; H= 5µm; I= 4µm; J= 5.2µm



Plate 7: Cassia sp. A. Infected Leaves; B. Infection Site; C. Microscopic Photo of Conidiophore of Cladosporium cladosporioides; D. Aspergillus sp. E. Illustration of A. sp. F. Illustration of C. cladosporioides Scale Bar (1cm) A= 2.3cm; B= 0.5cm; C= 14.34μm; D= 61.44μm; E= 17μm; F= 50μm



Plate 8: Cassia fistula A. Infected Leaf; B. Infection Site; C, I. Conidium of Alternaria sp.; D. Conidium of Cladosporium cladosporioides; E, F. Conidium of A. alternata; G. Conidium of Curvularia sp. J, K. Illustration of conidium of Alternaria sp. L. Illustration of conidium of Cladosporium cladosporioides; M, N. Illustration of conidium of A. alternata; O. Illustration of conidium of Curvularia sp. Scale Bar (1cm) A= 1.9cm; B= 0.4cm; C= 7.65µm; D= 5.1µm; E= 16µm; F= 7.8µm; G= 3.3µm; I= 11µm; J= 9µm; K= 4.5µm; L= 3.9µm; M= 7.1µm; N= 7µm; O= 4µm



Plate 9: Cousonia sp. A. Infected Leaves; B. Infection Site; C. Microscopic-photo of conidium of Curvularia lunata; D, E. Conidia of Curvularia sp. F. Conidium of Alternaria alternata; G. Illustration of conidium of Curvularia lunata H, I. Illustration of conidia of Curvularia sp. J. Illustration of conidium of Alternaria alternata; Scale Bar (1cm) A= 4.0cm; B= 0.4cm; C= 5.4µm; D= 7.2µm; E= 3.4µm; F= 4µm; G= 4.3µm; H= 5µm; I= 4.2µm; J= 4.13µm



Plate 10L Chrysanthemum indicum A. Infected Leaf; B. Infection Site; C, F, G. Microscopic photo of conidia of Alternaria alternata; D, E. Conidium of Alternaria sp. H. SEM of A. alternata; I, J. Illustration of A. sp. K, L, M. Illustration of A. alternata; Scale Bar (1cm) A= 1.5cm; B= 0.13cm; C= 8.2µm; D= 15µm; E= 4.4µm; F= 7.8µm; G= 7.5µm; H= 8.8µm; I= 6.75µm; J= 4.9µm; K= 8.37µm; L= 8.8µm



Plate 11: Combretum indicum A. Infected Leaf; B. Infection Site; C. Microscopic Photograph of conidia of Alternaria sp. D. Illustration of conidia of A. sp. Scale Bar (1cm) A= 1.4cm; B= 0.2cm; C= 10.7μ m; D= 9.2μ m



Plate 12: Diospyros kaki A. Infected Leaves; B. Infection Site; C. Microscopic Photograph of conidia of Alternaria sp. D, E. Unidentified sp. F. Conidia of A. alternata; G. conidia of Curvularia sp. H, J. Illustration of unidentified sp. I. Illustration of conidia of A. sp. K. Illustration of A. alternata; L. Illustration of Curvularia sp. Scale Bar (1cm) A= 1.5cm; B= 0.3cm; C= 6.9µm; D= 1.52µm; E= 6.6µm; F= 8µm; G= 4µm; H= 2.67µm I= 8.4µm; J= 5.8µm; K= 4.2µm; L= 5.8µm



Plate 13: Erythrina suberosa A. Infected Leaf; B. Infection Site; C, D. Microscopic photograph of Alternaria sp. E. Conidia of Curvularia lunata; F. SEM of Alternaria alternata; G. Illustration of C. lunata; H-I. Illustration of Alternaria sp. Scale Bar (1cm) A= 1.6cm; B= 0.1cm; C= 7.4 μ m; D= 10 μ m; E= 4.10 μ m; G= 5.5 μ m; H= 7.6 μ m; I= 6.00 μ m



Plate 14: Ficus sp. A. Infected Leaf; B. Infection Site; C. Microscopic photo of conidia of Alternaria alternata; D. Conidiophore of A. alternata; E. Unidentified sp.; F. Conidia of Curvularia sp. G. SEM of Aspergillus sp. H. Aspergillus sp. I. Illustration of conidia of A. alternata; J. Illustration of unidentified fungal sp. K. Illustration of Aspergillus sp. L. Illustration of conidiophore of A. alternata; Scale Bar (1cm) A= 1.4cm; B= 0.4cm; C= 10.20µm; D= 10.6µm; E= 8.1µm; F= 5.7µm; H= 15µm; I= 7.36µm; J= 7.4µm; K= 4.3µm; L= 15.7µm; M= 10.74µm



Plate 15: *Ficus benghalensis* A. Infected Leaves; B. Infection Site; C, D. Microscopic photograph of *Alternaria alternata*; E, F. Conidia A. sp. G. SEM of conidia of A. *alternata*; H, I. Illustration of A. *alternata*; J, K. Illustration of A. sp. Scale Bar (1cm) A= 1.5cm; B= 0.4cm; C= 4.5µm; D= 4.1µm; E= 8µm; F= 10µm; H= 4.9µm; I= 3.9µm; J= 7.21µm; K= 9.10µm



Plate 16: Ficus elastica A. Infected Leaf; B. Infection Site; C. Microscopic photograph of conidia of Alternaria brassicae; D. Conidia of A. sp.; E. Conidia of A. sp. F. Conidia of Curvularia sp. G. Cladosporium heterophragmatis; H. Conidia of Curvularia lunata; I. Conidia of Cladosporium cladosporioides; J. SEM of conidia of A. alternata; K. Illustration of A. alternata; L. Illustration of A. sp.; M. Illustration of A. sp. N. Illustration of Curvularia sp. O. Illustration of Cladosporium eladosporioides; Scale Bar (1cm) A= 2.1cm; B= 0.6cm; C= 9.8µm; D= 5.1µm; E= 8.8µm; F= 3.7µm; G= 11µm; H= 8µm; I=1.8µm; K= 7.4µm; L= 4.8µm; M= 7µm; N= 3µm; O= 9µm; P= 6µm; Q= 0.79µm



Plate 17: Ficus racemosa A. Infected Leaf; B. Infection Site; C. SEM of Conidiophore; D. Microscopic photograph of conidia of Alternaria alternata and conidiophore; E. Conidia of Curvularia sp. F. Conidia of Curvularia lunata G. Illustration of C. lunata; H. Illustration of C. sp. I. Illustration of A. alternata and conidiophores Scale Bar (1cm) A= 1.9cm; B= 0.3cm; D= 11 μ m; E= 6.7 μ m; F= 5.2 μ m; G= 7.1 μ m; H= 9.2 μ m; I= 5.6 μ m



Plate 18: Ficus religiosa A. Infected Leaf; B. Infection Site; C. Microscopic photograph of conidia of *Cladosporium cladosporioides*; D, H. Microscopic photograph of conidia of *Alternaria* sp. E. *Curvularia* sp. G, J. *Aspergillus* sp. I. Conidiophore of *Alternaria* sp M. Illustration of *A.* sp. N. Illustration of *Curvularia* sp. K. Illustration of *Conidiophore* of *A.* sp. L. Illustration of *Conidiophore* of *A.* sp. L. Illustration of *Conidiophore* of *A.* sp. L. Illustration of *Conidiophore* of *A.* sp. 1. Illustration of *Conidiophore* of *A.* sp. 1. Illustration of *Conidiophore* of *A.* sp. 1. Illustration f *Conidiophore* of *A.* sp. 5.60 m; *C* = 5.20 m; *C* = 3.20 m; *C* = 3.20 m; *C* = 5.20 m; *C* = 5.20



Plate 19: Ficus virens A. Infected Leaf; B. Infection Site; C. Microscopic photograph of conidia of Alternaria sp. D. SEM of Cladosporium cladosporioides; E. Microscopic photograph of conidia of Alternaria alternata; F. Microscopic photograph of conidia of Curvularia lunata; G. Illustration of A. alternata; H. Illustration of C. lunata; I. Illustration of A. sp. Scale Bar (1cm) A= 2.01cm; B= 0.08cm; C= 7.64 µm; E= 5.63 µm; F= 5.02 µm; G= 5.50 µm; H= 4.0 µm; I= 6.8 µm



Plate 20:*Heterophragma adenophyllum* A. Infected Leaf; B. Infection Site; C. Microscopic photograph of conidia of *Curvularia* sp. D. Illustration of conidia of *Curvularia* sp. Scale Bar (1cm) A= 4.6cm; B= 0.04cm; C= 2.26μm; D= 2.29μm



Plate 21: *Hibiscus rosa-sinensis* A. Infected Leaves; B. Infected Site; C. Microscopic photograph of conidiophore of *Cladosporium cladosporoiodes*; D. Illustration of conidiophore of *C. cladosporoiodes* Scale Bar (1cm) A= 1.0cm; B= 0.3cm; C= 58.6μm; D= 50.08μm



Plate 22: *Ipomoea batatas*. A. Infected Leaf; B. Infection Site; C. Microscopic photograph of conidia of *Curvularia* sp. D-G. Microscopic photograph of conidia of *Alternaria alternata*; F, H. Microscopic photograph of conidia of *C. lunata*; I. SEM of conidia of *A. alternata*; J. Illustration of conidia of *C. sp. K*, M. Illustration of conidia of *C. lunata*; Scale Bar (1cm) A= 1.8cm; B= 0.08cm; C= 4.7µm; D= 4.6µm; F= 4.9µm; G= 3.42µm; H= 4.9µm; J= 4.0µm; K= 4.9µm; L= 4.9µm; M= 4.03µm; N= 5.55µm



Plate 23: Jatropha integerrima A. Infected Leaf; B. Infection Site; C. Microscopic photograph of conidia of Alternaria alternata; D. SEM of conidia of A. alternata; E. Conidia of Cladosporium cladosporioides; F. Illustration of A. alternata; G. Illustration of C. cladosporioides; Scale Bar (1cm) A= 1.2cm; B= 0.1cm; C=3µm; E=7.0µm; F=4.7µm; G=5.3µm



Plate 24: Justicia adhatoda A. Infected Leaf; B. Infection Site; C-E. Microcsopic photograph of Alternaria alternata; F. Microcsopic photograph of conidia of A. sp. G. SEM of conidia of A. alternata; H-J. Illustration of Conidia of A. alternata; K. Illustration of A. sp. Scale Bar (1cm) A= 1.4cm; B= 0.14cm; C= 3.12 μ m; D= 3.2 μ m; E=4.5 μ m; F= 5.23 μ m; H= 3.4 μ m; I= 3.12 μ m; J= 3.2 μ m; K= 7.7 μ m



Plate 25: Lagerstroemia indica A. Infected Leaves; B. Infection Site; C. Microscopic photograph of conidia of Alternaria alternata; D. Conidia of Cladosporium cladosporioides E. SEM of C. cladosporioides; F. Illustration of A. alternata; G. Illustration of C. cladosporioides; Scale Bar (1cm) A= 0.9cm; B= 0.12cm; C= 7.3µm; D= 0.5µm; F= 2.56µm; G= 1.01µm



Plate 26: Lagerstroemia speciosa. A. Infected Leaves; B. Infection Site; C. Microscopic photograph of conidia of Alternaria alternata; D. Illustration of conidia of A. alternata; E. SEM of conidia of A. alternata; Scale Bar (1cm) A= 2.7cm; B= 0.3cm; C= 6.64μm; D= 6.9μm



Plate 27: Mangifera indica A. Infected Leaf; B. Infection Site; C,D. Aspergillus sp. E. Conidia of Alternaria alternata; F. Illustration of A. alternata; G. Cladosporium cladosporioides; H. Illustration of C. cladosporioides; I,J. Illustration of Aspergillus sp. Scale Bar (1cm) A= 1.60cm; B= 0.2cm; C= 41.6 μ m; D= 43.91 μ m; E= 2.34 μ m; F= 4.0 μ m; G= 52.83 μ m; H= 44.21 μ m; I= 47.93 μ m; J= 43.91 μ m



Plate 28: *Millettia pinnata* A. Infected Leaf; B. Infection Site; C. Conidia of *Alternaira* sp. D. Conidia of *Alternaira alternata*; E. Unidentified sp. F. SEM of Conidia of *A. alternata*; G. Illustration of conidia of *A. sp.* H. Illustration of conidia of *A. alternata*; I. Illustration of unidentified sp. Scale Bar (1cm) A= 1.2cm; B= 0.09cm; C= 3.70 μ m; D= 3.23 μ m; E= 6.3 μ m; G= 3.20 μ m; H= 4.7 μ m; I=6.10 μ m



Plate 29: *Mimusops elengi* A. Infected Leaves; B. Infection Site; C. *Aspergillus niger*; D. Conidia of *Alternaria* sp. E. Illustration of *Aspergillus* sp. F. Illustration of Conidia of *A.* sp. Scale Bar (1cm) A= 1.4cm; B= 0.3cm; C= 16.07 μ m; D= 4.36 μ m; E= 16.89 μ m; F= 4.31 μ m



Plate 30: *Morus alba* A. Infected Leaf; B. Infection Site; C. Microscopic photograph of conidia of *Alternaria* sp. D. Illustration of *A.* sp. Scale Bar (1cm) A= 1.2cm; B= 0.3cm; C= 14.5µm; D= 8.2µm



Plate 31: *Plumeria obtusa*. A. Infected Leaf; B. Infected Site; C. Microscopic photograph of *Aspergillus* sp. D. Illustration of *Aspergillus* sp. Scale Bar (1cm) A= 1.5cm; B= 0.1cm; C= 12.71μm; D= 13.14μm



Plate 32: Polyalthia longifolia. A. Infected Leaf; B. Infection Site; C. Aspergillus sp. D. Illustration of Aspergillus sp. E, F. Conidia of Alternaria alternata; G, H. Illustration of A. alternata Scale Bar (1cm) A= 2.3cm; B= 0.2cm; C= 8.58µm; D= 9.71µm; E= 7.4µm; F= 8.68µm; G= 7.4µm; H= 8.4µm



Plate 33: *Pongamia* sp. A. Infected Leaf; B. Infection Site; C. Microscopic photograph of Conidia of *Curvularia lunatu*; D. Conidia of *Alternaria* sp. E. Conidia of *Curvularia* sp. F. Conidia of *Alternaria alternata*; G. Illustration of conidia of *C. lunata*; H. Illustration of *A.* sp. I. Illustration of conidia *C.* sp. J. Illustration of *A. alternata* Scale Bar (1cm) A= 1.4cm; B= 0.1cm; C= 4.1µm; D= 8.86µm; E= 3.31µm; F= 8.72µm; G= 5.65µm; H= 7.4µm; I=5.5µm; J= 7.47µm



Plate 34: *Prunus armeniaca* A. Infected Leaves; B. Infection Site; C, E. Conidia of *Alternaria* sp. D. Conidia of *Curvularia* sp. F. SEM of Conidia of *Cladosporium cladosporioides*; G, I. Illustration of conidia of *A*. sp. H. Illustration of conidia of *C*. sp. Scale Bar (1cm) A= 1.3cm; B= 0.6cm; C=4.29µm; D= 3.28µm; E= 4.29µm; G= 2.83µm; H= 4.54µm; I= 4.64µm



Plate 35: Senna surattensis A. Infected Leaf; B. Infection Site; C. Aspergillus sp. D. Illustration of A. sp. E. SEM of A. sp. F. Conidia of Alternaria alternata; G. Conidia of Curvularia sp. H. Conidiophore of A. alternata; I. Illustration of conidiophore of A. alternata; J. Illustration of Conidia A. alternata K. Illustration of conidiophore of Curvularia sp. Scale Bar (1cm) A= 1.4cm; B= 0.4cm; C= 14.22µm; D=15µm; F= 9.8µm; G=9.8µm; I= 4.5µm; J= 5.8µm; K= 45.40µm



Plate 36: Syzygium cumini A. Infected Leaf; B. Infected Spot; C. Microscopic photograph of Alternaria alternata; D. Illustration of Alternaria alternata; Scale Bar (1cm) A= 1.6cm; B= 0.2cm; C= 5.64µm; D=6.64µm



Plate 37: Tacoma stans A. Infected Leaf; B. Infection Site; C, E. Microscopic photograph of conidia of Cladosporium cladosporioides; D. Alternaria alternata; F. Unidentified sp. G. SEM of Alternaria alternata; H. Illustration of Alternaria alternata; I, J. Illustration of Cladosporium cladosporioides; Scale Bar (1cm): A= 2.8cm; B=0.3cm; C= 0.33µm; D= 3.32µm; E= 0.33µm; F=6.28µm; H= 3.56µm; I= 0.50µm; J= 0.56µm; K=9.96µm



Plate 38: *Terminalia arjuna* A. Infected Leaf; B. Infection Site; C. *Alternaria* sp.; D. *Alternaria alternata*; E. *Aspergillus* sp. F. Illustration of *Alternaria* sp.; G. Illustration of *Alternaria alternata*; Scale Bar (1cm): A= 2.1cm; B= 0.05cm; C= 6.52µm; D= 4.87µm; E= 10.02µm; F= 5.85µm; G= 4.87µm



Plate 39: *Terminalia chebula* A. Infected Leaf; B. Infected Site; C. *Alternaria* sp. D. Conidium of *A. alternata*; E. SEM of *A. alternata*; F. Illustration of *A.* sp. G. Illustration of *A. alternata*; Scale Bar (1cm): A= 1.7cm; B= 0.2cm; C= 5.19µm; D=3.12µm; F= 5.70µm; G= 3.7µm



Plate 40: *Zizipnus Jujuba* A. Infected Lear; B. Infection Site; C. Microscopic photo of *Aspergillus* sp. D. Illustration of *Aspergillus* sp. E. SEM of *Alternaria alternata*; F. Conidia of *Curvularia*; G. Illustration of conidia of *Curvularia*; Scale Bar (1cm): A=2.7cm; B= 0.14cm; C= 12µm; D= 12.40µm F.=4.87µm; G= 4.41µm



Cladosporium cladosporioides was observed on the leaf surface of *Alternanthera ficoidea* and is characterized morpho-anatomically. *A. ficoidea* is native to Mexico, South America. It is cultivated in Lahore as an ornamental plant (Online Source 2). No previous report found about the existence of any kind of phylloplane mycoflora on this plant.

Alternaria alternata and Cladosporium cladosporioides were observed on the leaf surface of Bauhinia variegata and characterized morphoanatomically in the present research. Valarini et al., (2007) studied the leaf yeast populations by using the spore-fall method from the phyllopsphere of the B. forticata. Same taxa (Alternaria alternata and Cladosporium cladosporioides) were observed on the leaf surface of Broussonetia papyrifera. B. papyrifera is native of Japan, China and South east Asia; introduced and cultivated in Russia, Indo-Pakistan subcontinent, West Asia, tropical Africa, Europe, Polynesia, Philippines, U.S.A. and many other countries as an avenue tree (Online Source 3).

On the leaf surface of *Bursera serrata, Curvularia lunata* and some tentative species in-addition to *Alternaria alternata, Cladosporium cladosporioides,* were observed. *B. serrata* is native of E. Pakistan and Assam and cultivated in Lahore (Online Source 4). No previous reports were found regarding any kind of phylloplane mycoflora from Pakistan.

On the leaf surface of *Cassia* sp., *Aspergillus* sp. and *Cladosporium cladosporioides* were observed and characterized. *Alternaria alternata*, *C. cladosporioides* and *Curvularia* sp., were also observed on leaf surface of *Cassia fistula*, characterized morpho-anatomically. On the work of Limtong and Koowadjanakul (2012) in Thailand, 2 fungal species isolated from *Cassia fistula* were *Candida etchellsii* (Lodder & Kreger-van Rij) S.A. Mey. & Yarrow, and *Aureobasidium pullulans* (de Bary & Löwenthal) G. Arnaud.

In current study, *Alternaria alternata*, *Curvularia lunata* were also observed on the leaf surface of *Causonia* sp. and characterized morpho-anatomically. No previous reports were found about the existence of any kind of phylloplane mycoflora on this plant.

In the present survey, *Alternaria alternata* and *Alternaria* sp. were observed on the leaf surface of *Chrysanthemum indicum* and characterized. *C. indicum* is native to Asia and northeastern Europe and distributed to southern China (Liu *et al.*, 2012). No previous report was found about the existence of any

kind of phylloplane mycoflora.

Combretum indicum is native to tropical Asia and widely cultivated throughout the tropics and subtropics, mainly as an ornamental plant, and has become naturalized in many localities (Online Source 5). In the present search, only *Alternaria* sp. was observed on leaf surface of *Combretum indicum* and characterized. No previous report was found about the existence of any kind of phylloplane myco-flora from Pakistan.

In the present investigation, *Alternaria alternata*, *Curvularia lunata* and tentatively identified sp. were observed on leaf surface of *Diospyros kaki* and characterized morpho-anatomically. *D. kaki* is introduced and cultivated throughout Eastern Asia, USSR, Japan and China (Online Source 6). No previous report was found about the existence of any kind of phylloplane mycoflora.

In the present study, *Alternaria alternata* and *Curvularia lunata* were observed on the leaf surface of *Erythrina suberosa* and characterized morphoanatomically. *E. suberosa* is commonly cultivated in Pakistan and India (Online Source 7). No previous report was found about the existence of any kind of phylloplane mycoflora from Pakistan on this tree.

In the present investigation, *Alternaria alternata*, *Aspergillus* sp., *Cladosporium cladosporioides* and *Curvularia* sp. and tentative sp. were observed on the leaf surface of *Ficus* sp., while *Alternaria brassicae*, *A. helianthi*, *Cladosporium cladosporioides*, *C. heterophragmatis*, *Curvularia lunata*, and *Curvularia*. sp., were observed on the leaf of *F. elastica* and characterized.

In the present investigation, *Alternaria alternata* was observed on the leaf surface of *Ficus benghalensis* and characterized morpho-anatomically. However, *Alternaria alternata, A. brassicae, Cladosporium cladosporioides* and *Curvularia lunata* were observed on leaves surfaces of *F. racemosa* and characterized.

On the leaf surface of *Ficus religiosa*, Alternaria alternata, A. helianthi, Aspergillus sp., Cladosporium cladosporioides and Curvularia lunata were observed and characterized. Limtong and Koowadjanakul (2012) isolated Curvularia tropicalis from *F. religiosa* (Limtong & Koowadjanakul, 2012).

On the phylloplane of *Ficus virens*, *Alternaria alternata*, *Cladosporium cladosporioides*, *C. herbarum*, *Curvularia lunata* and *Curvularia* sp. were observed. Wang *et al.* (2008) collected the fallen leaves of *F*. *altissima*, *F. virens*, *F. benjamina*, *F. fistulosa* and *F. semicordata*, in Chiang Mai Province in northern Thailand and examined for existance of fungi (Wang *et al.*, 2008).

Curvularia sp. was also found on leaf surface of *Heterophragma adenophyllum*. Khan and Kamal (1962) isolated a new species *Cladosporium heterophragmatis* S.A. Khan & M. Kama from the *Hetrophragma adenophyllum* wallich ex. G. Don., and described the structure (Khan & Kamal, 1962).

Phylloplane of *Hibiscus rosa-sinensis* was observed and found the occurrence of only *Cladosporium cladosporioides* on leaf surface. Limtong and Koowadjanakul (2012) isolated fungal species from *H. rosa-sinensis*, i.e., *Candida glabrata* (Limtong & Koowadjanakul, 2012).

Leaf surfaces of *Ipomoea batatas* were investigated and found the existence of In the present investigation, *Alternaria alternata, Curvularia lunata* and *Curvularia* sp. *I. batatas* is an American root crop and grown throughout the tropical and subtropical regions of the world. It is commonly cultivated for the edible tubers (Online Source 8). No previous report was found on this plant about the existence of any kind of phylloplane mycoflora from Pakistan.

In the present investigation, *Alternaria alternata* and *Cladosporium cladosporioides* were observed on the leaf surface of *Jatropha integerrima* and characterized morpho-anatomically. Venkatesan (2013) studied the biodiversity of mycoflora associated with decomposing leaf litter of *Jatropha curcas* (Biodiesel plant) with the pattern of fungal colonization. A total of 79 fungal species belonging to 59 genera were isolated and 4 main groups of taxa were identified (Venkatesan, 2013).

Alternaria alternata was also observed on the leaf surface of Justicia adhatoda and characterized on the basis of microscopy. J. adhatoda is distributed in Panama (probably introduced), Indonesia, Malaya, S.E. Asia, India and Pakistan. In Pakistan, it does well on waste lands up to 1300 m; it is also cultivated as an ornamental in Lahore (Online Source 9).

In this study, on the leaf surface of *Lagerstroemia indica*, *Alternaria alternata* and *Cladosporium cladosporioides* were observed and identified. *L. indica* is extensively planted in the sub-continent (Online Source 10). No previous data was found about the existence of any kind of phylloplane mycoflora from Pakistan. Alternaria alternata was observed on the leaf surface of Lagerstroemia speciosa and characterized morpho-anatomically. L. speciosa is native in Assam and South India and cultivated elsewhere (Online Source 11). This is the first report about the existence of any kind of phylloplane mycoflora from Pakistan on this host.

In the current investigation, *Alternaria alternata*, *Aspergillus* sp. and *Cladosporium cladosporioides* were observed on the leaf surface of *Mangifera indica*. This tree was observed by Limtong and Koowadjanakul (2012) and collected and evaluated 91 leaf samples from Thailand. Isolated species was *Hanseniaspora opuntiae* Čadež, Poot, Raspor & M.T. Sm. (Limtong & Koowadjanakul, 2012).

In the current investigation, *Alternaria alternata* and Tentatively identified fungal sp. were observed on leaf surface of *Millettia pinnata*, while *Alternaria alternata*, *Curvularia lunata*, and *Curvularia* sp. were observed on the leaf surface of *Pongamia* sp. and characterized morpho-anatomically. Ten new species of ascomycetes collected in Taiwan were described, illustrated and compared with closely related taxa by Hsieh *et al.* (1995), from which *Schizothyrium millettiae* Sivan. & W.H. Hsieh, was present on *Millettia nitida*.

On the phylloplane of *Morus alba* in this study, *Alternaria* sp. was identified. Previously from Pakistan, Abbas *et al.* (2010) reported 5 fungal species on *Morus alba* viz; *Tetracoccosporium aerium* P.C. Misra & P. Srivast., *Gliomastix novae-zelandiae* S. Hughes & C.H. Dickinson, *Septoria cytisi* Desm., *Pseudocercospora mori* (Hara) Deighton, and *Lasiodiplodia undulata* (Berk. & M.A. Curtis) Abbas, B. Sutton, Ghaffar & Abbas.

In this research, only *Aspergillus* sp. was observed on the leaf surface of *Plumeria obtusa* and characterized morpho-anatomically. Previously, two fungal species were isolated from *Plumeria* sp. during the work of Limtong & Koowadjanakul (2012) viz. *Candida tropicalis* (Castell.) Berkhout and *Starmerella meliponinorum* A.C.P. Teixeira, M.M. Marini, Lachance & C.A. Rosa.

In this research, *Alternaria* alternata and *Aspergillus* sp. were observed on leaves surfaces of *Polyalthia longifolia*. *P. longifolia* is distributed in Indigenous to Sri Lanka, cultivated in India, Malaya, Pakistan and Tropical East Africa. The Mast Tree is indigenous to Sri Lanka, elsewhere it is cultivated. In Pakistan, it is commonly cultivated in Sindh as an avenue tree or in gardens, also occasionally planted in

Punjab. It rarely flowers in Karachi (Online Source 12). No previous data about the existence of any kind of phylloplane mycoflora on this host plant was reported.

In the current study, *Alternaria* sp., *Cladosporium cladosporioides* and *Curvularia* sp. were observed on the leaf surface of *Prunus armeniaca* and characterized morpho-anatomically.

In the present investigation, *Alternaria alternata*, *Aspergillus* sp. and *Curvularia* sp. were observed on the leaf surface of *Senna surattensis*. *S. surattensis* origin remains obscure. It was previously thought by Bentham to be native to coastal north Australia and often cultivated as an ornamental, in many tropical and subtropical regions including Southeast Asia, Africa, and the West Indies, and is known to have escaped into the wild, naturalizing in many of these places (Online Source 13).

Two species of *Alternaria* such as *A. alternata* and *A. brassicae* were observed on the leaf surface of *Syzygium cumini* and characterized morphoanatomically. Sudheep and Sridhar (2010) collected the canopy samples such as trapped leaf litter, trapped sediment, stemflow and through fall from Kaiga forest India to evaluate the occurrence of water-borne hyphomycetes.

In the present investigation, Alternaria alternata, Cladosporium cladosporioides and Tentatively identified fungal sp. were observed on the leaf surface of Tecoma stans. T. stans, a native of tropical South America, is widely cultivated in the tropics and subtropics (Online Source 14). Alternaria alternata, A. brassicae and Aspergillus sp. were isolated from Terminalia arjuna while Alternaria alternata and A. brassicae were isolated from T. chebula and characterized. Valarini et al. (2007) studied the leaf yeast populations from the phyllopsphere of the T. catappa by using the spore-fall method. A small subset of isolated fungi was Aureobasidium pullulans (de Bary & Löwenthal) G. Arnaud, Pseudozyma antarctica (Goto, Sugiy. & Iizuka) Boekhout, Cryptococcus albidus (Saito) C.E. Skinner, Candida guilliermondii (Castell.) Langeron & Guerra, and Candida sp.

On the leaf surface of Ziziphus jujuba, Aspergillus sp., Cladosporium cladosporioides, and Curvularia sp. were observed and characterized. Limtong & Koowadjanakul (2012) worked on Z. mauritiana and isolated C. tropicalis and Pichia kudriavzevii Boidin, Pignal & Besson, fungal species.

Percentage abundance of mycoflora on each family was following: Fabaceae (57.5%), Apocynaceae

(7.5%), Moraceae (65%), Lythraceae (7.5%), Bignoniaceae (10%),Combretaceace (15%), Amaranthaceae (2.5%),Burseraceae (10%), Cucurbitaceae (7.5%), Asteraceae (5%), Ebenaceae (10%), Malvaceae (2.5%), Convolvulaceae (7.5%), Euphorbiaceae (5%), Acanthaceae (5%), Anacardiaceae (7.5%), Sapotaceae (5%), Annonaceae (5%), Rosaceae (7.5%), Myrtaceae (2.5%),Rhamnaceae (7.5%).

The relative abundance of mycota (Simpson's Index) on 40 plants is 0.15 whereas the species diversity (Shannon Weiner's Index) on 40 plants is 1.9. *Alternaria alternata* had the highest abundance ratio and *Cladosporium cladosporiodes* had the second highest abundance rate. So, we concluded that *Alternaria alternata* and *Cladosporium cladosporiodes* were found to be dominant on the leaves of selected trees.

Calculation of Species Richness and Diversity for Phylloplane Fungi (Tables 2 & 3; Figures 1 & 2): Species Richness and Diversity for phylloplane fungi was calculated by Simpson's Index and (D) Shannon-Wiener Index (H).

Simpson Index	Shannon- Wiener		
-	Index		
Simpson's Diversity	This diversity measure		
Index is a measure of	came from information		
diversity which takes	theory and measures the		
into account the number	order (or disorder)		
of species present, as	observed within a		
well as the relative	particular system. This		
abundance of each	order is characterized by		
species. As species	the number of		
richness and evenness	individuals observed for		
increase, so diversity	each species in the		
increases. The value of	sample plot. The value		
Simpson Index ranges	of Shannon- Wiener		
between 0 and 1. With	Index ranges between 1		
this index, 1 represents	and 5. With this index, 1		
infinite diversity and 0,	represents low species		
no diversity so more the	richness and 5 represents		
value of D, the lower the	high species richness. It		
diversity.	increases as diversity		
$\sum n(n-1)$	increases.		
$D = \frac{N(N-1)}{N(N-1)}$	s S		
()	$H = \sum_{i=1}^{n} - (P_i \times \ln P_i)$		

Species Name	Observed Abundance	Percentage Abundance
Alternaria alternate	25	29%
Alternaria brassicae	1	1%
Alternaria sp.	10	11%
Aspergillus niger	1	1%
Aspergillus sp.	9	10%
Cladosporium cladosporioides	14	16%
Cladosporium heterophragmatis	1	1%
Curvularia lunata	9	10%
Curvularia sp.	12	14%
Unidentified Fungal Species 1	3	3%
Unidentified Fungal Species 2	1	1%
Unidentified Fungal Species 3	1	1%

Table 2: Species Richness and Diversity of Phylloplane Fungi

Table 3: Percentage Abundance of Different

Total No. of Tree s	Total No. of Fungal Species	Alpha Biodiversity					
		Simpson Index	Simpson's Index of Diversity (1- D)	Simpson's Reciprocal Index (1/D)	Shannon Wiener Index (H)		
40	12	0.15	0.85	7.77	1.9		









Fig. 1 Species Abundance Bar Graph



Fig. 2 Community Structure Pi Graph

Conclusion

Here we present the morpho-anatomical, species richness and abundance data of filamentous and yeast fungal myco-biota that occurred on the phylloplane of selected tree species growing in Mall Road Lahore. A total of 40 tree species were analyzed and investigated for the diversity of myco-flora, using conventional morphological and microscopic tools. Among all the isolated fungal strains, *Alternaria alternata* and *Cladosporium cladosporiodes* were the dominant fungal species of phylloplane in current study.

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- http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id =200006341 (online source 3)
- http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id =200014643 (online source 10)
- http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id =200014648 (online source 11)
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