

## PHYLLOPLANE FUNGI OF *VITEX NEGUNDO* LINN.

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Phylloplane fungi associated with the leaves of *Vitex negundo* Linn., (Perennial Medicinal Plant) collected from Bhadrachalam forest localities of Telangana region has been worked out for a period of 12 months (2015-2016) at monthly intervals using dilution plate technique and leaf impression methods respectively. Fungi have been identified up to species level. Altogether 127 fungal species belonging to 61 genera are recorded. There is not much difference in the fungal species composition of abaxial and adaxial leaf surfaces. 16 fungi have been reported as new additions to the fungi of Telangana state. This is the first report of phylloplane fungi of *Vitex negundo* Linn.

**Keywords:** Bhadrachalam, Dilution plate, Leaf impression, phylloplane, *Vitex negundo*.

Plant leaf is a part of the plant which is exposed to air and atmospheric fungi get associated in numerable counts on to the leaf surfaces and they have been termed as phyllosphere fungi by Last (1955) and Ruinen (1956) Last and Deighton (1965) more precisely defined this as phylloplane. Kerling (1964) and Gregory (1961) considered phylloplane as spore trap. Several workers have reported fungi associated with leaf surface of phylloplane from time to time (Ajay Krishna *et al.* 2013, Bhat and Kaveriappa 2009, Bhat and Anusree 2015, Borgohain *et al.* 2014, Cox and Hall 1978, Dalal 2014, El kady 1997, Girivasan and Suryanarayanan 2004, Grbic *et al.* 2015, Hawksworth 2004, Jayasinghe and Fernando 2000, Last and Deighton 1965, Lindsey 1976, Manjit Kumar *et al.* 2014, Nayak 2015a,b, Osono 2007, Prabakaran and Pannerselvam 2011, Ruinen 1961, Seth 1970, Subramaniam 1971, Thom and Raper 1945, Thomas *et al.* 2013, Ushasri 2016, Uzma Bashir and Sobia Mushtaq 2012, Vardavikas 1988, Waill *et al.* 2016). Therefore an account of phylloplane fungi associated with *Vitex negundo* Linn. has been provided as it has not been worked out.

### MATERIALS AND METHODS

*Vitex nigundo* Linn. is a large shrub with three

foliolate leaves, leaflets lanceolate, acuminate and flowers blue in terminal thyrasoid panicle, fruit is drupe, it belongs to Verbenaceae. The chemical constituents are mostly flavones and iridoides. The leaves are used in aromatic tonic preparation as vermifuge, antiparasitic and anodyne. Fruit is used as a nervine tonic and vermifuge and flowers are used as cool astringent. The oil from leaf is used in rheumatism and also to improve hair growth. *Vitex nigundo* has been selected from Bhadrachalam forest localities of Telangana region. leaf samples of uniform size were collected at monthly intervals for one year (2015-2016) under aseptic conditions.

### Collection of samples

Leaf samples were collected (irrespective of age) and processed for the isolation of fungi. The screening for fungi was done by dilution plate techniques and leaf impression methods

### Isolation of fungi

#### Dilution Plate Method:

Five grams of leaf collected in fresh polythene bags were transferred to 50 ml distilled water in flasks. Later the contents were shaken mechanically for 20 minutes to get a homogenous suspension. Serial dilutions were made. 1 ml was incorporated into each sterile



**Figure 1-** *Vitex negundo* Plant with flowering parts in sampling area.

petridish media. Petriplates were incubated in room temperature or  $28 \pm 2^\circ\text{C}$  up to 5-7 days, having different media and triplicates were maintained as recommended by Dickinson (1965). Fungi were isolated and identified. Observation was made up to 20 days after plating. Potato sucrose agar medium, vegetable agar medium, glucose yeast extract medium and leaf extract agar medium were the media employed. 0.01% streptopenicillin was added in the media to avoid contaminant.

#### **Leaf impression method:**

The leaf impression technique as recommended by Potter (1910) was followed for the enumeration of phylloplane fungi qualitatively. Individual leaves were placed on the solidified potato sucrose agar and vegetable agar media and pressed to make the contact with the medium and removed. Both abaxial and adaxial surfaces were pressed. The petridishes were incubated at room temperature  $28 \pm 2^\circ\text{C}$  and enumerated for fungal species.

#### **Identification of Fungi:**

Czepek-Dox Agar medium (CZA) was used for the identification of *Aspergilli* and *Penicillia* species and identified using with the help of manuals written by Thom and Roper 1945, Raper and Fennel (1965) and Raper and Thom

(1949). Species of *Fusarium* are grown on potato sucrose agar medium and identified with the help of the manual written by Booth (1971, 1977). Remaining fungi were grown on various media were identified with the help of the keys provided by Barron (1968), Barnett and Hunter 1972, Ellis (1971, 1976), Gilman (1957), Nagamani *et al.* (2006), Onions and Barron (1967), Rifai (1969), Seth (1970) and Subramanian (1971) in their respective manuals and other available literature.

## **RESULTS AND DISCUSSION**

Altogether 127 fungal species belong to 61 genera were recorded from phylloplanes of *Vitex nigundo* for 12 months. It is clear that the dominant species belong to fungi imperfecti followed by *Aspergilli*, *Penicillia*, Ascomycetes and *Fusaria*. The presence of all such fungi indicates that leaf surface acts as an ecological niche for saprophytic and biotrophic fungi. The genus *Alternaria* was represented by 7 species, *Aspergillus* by 26, *Chaetomium* by 5, *Cladosporium* by 5, *Curvularia* by 12, *Drechslera* by 5, *Fusarium* by 8, *Mucor* by 3, *Myrothecium* by 2, *Nigrospora* by 2, *Paecilomyces* by 2, *Penicilium* by 2, *Spicaria* by 6, *Torula* by 3, *Trichoderma* by 2, and remaining genera by single species each

(Fig.2). There is not much difference in the composition of fungal species associated with abaxial and adaxial surfaces

The following is the list of fungal species associated with leaf surface of *Vitex negundo*.

*Alternaria alternata* (Fr.) Keissl, *Aternaria atra* (Preuss) Woundenb and Crous, *Aternaria echinulata* E.G. Simmons & C.F. Hill, *Aternaria humicola* Oudem, *Aternaria longipes* (Ellis & Everh.) E.W. Mason, *Aternaria tenuissima* (Kunze) Wiltshire, *Aspergillus caespitosus* Raper and Thom, *Aspergillus candidus* Link, *Aspergillus chevalieri* Thom & Church, *Aspergillus clavatus* Desm., *Aspergillus conicus* Blochwitz., *Aspergillus flavipes* (Bainier & RS Artory) Thom & Church, *Aspergillus flavus* Link, *Aspergillus fumigates* Fresenius, *Aspergillus funiculosus* G. Sm., *Aspergillus humicola* Chaudhuri, *Aspergillus luchuensis* Inui, *Aspergillus nidulans* Eidam, *Aspergillus niger* Tiegh, *Aspergillus ochraceus* G.Wilhi, *Aspergillus repens* (Corda) Sacc., *Aspergillus restrictus* Raper & Thom., *Aspergillus sparsus* raper & Thom., *Aspergillus sulphurous* Desm., *Aspergillus sydowii* Bainer & Sartory, *Aspergillus tamarii* Kita, *Aspergillus terreus*

Thom, *Aspergillus unguis* (Emile Weil & Gauden) Thom & Raper, *Aspergillus ustus*, *Aspergillus versicolor* (Vuill) Tiraboschi, *Aspergillus violaceo-fuscus* Gasperini, *Aureobasidium pullulns* (de Bary & Lowental) G. Arnand, *Cephalophora irregularis* Thaxt., *Chaetomella raphigera* Swift., *Chaetomium erraticum* L.M. Ames., *Chaetomium globosum* Kunze, *Chaetomium reflexum* Skolko & J.W. Groves, *Cladosporium cladosporioides* (Fresen) G.A. de Vries, *Cladosporium herbarum* (Pers.) Link, *Cladosporium oxysporum* Berk. M.A. Curtis, *Cladosporium variabile* (Cooke) G.A. de Vries, *Cunninghamella blakesleeana* Lendn., *Curvularia clavata* Jain, *Curvularia eragraotidis* Itenn & Mayer, *Curvularia geniculata* (Tracy & Earle) Boedijn., *Curvularia harveyi* Shipton, *Curvularia lunata* (Walker)Boedijn, *Curvularia lunata* var. *Aeria* (Bat.J.A Lima & C.T. Vascont.) M.B.Ellis, *Curvularia pallescens* Boedijn, *Curvularia prasadii* R.L. Mathur & B.L Mathur, *Curvularia trifolii* (Kauffman) Boedijn, *Curvularia tuberculata* B.L. Jain, *Curvularia verruculosa* Tandon & Bilgrami ex M.B. Ellis, *Dendryphiella vinosa* (Bark. & Curt)

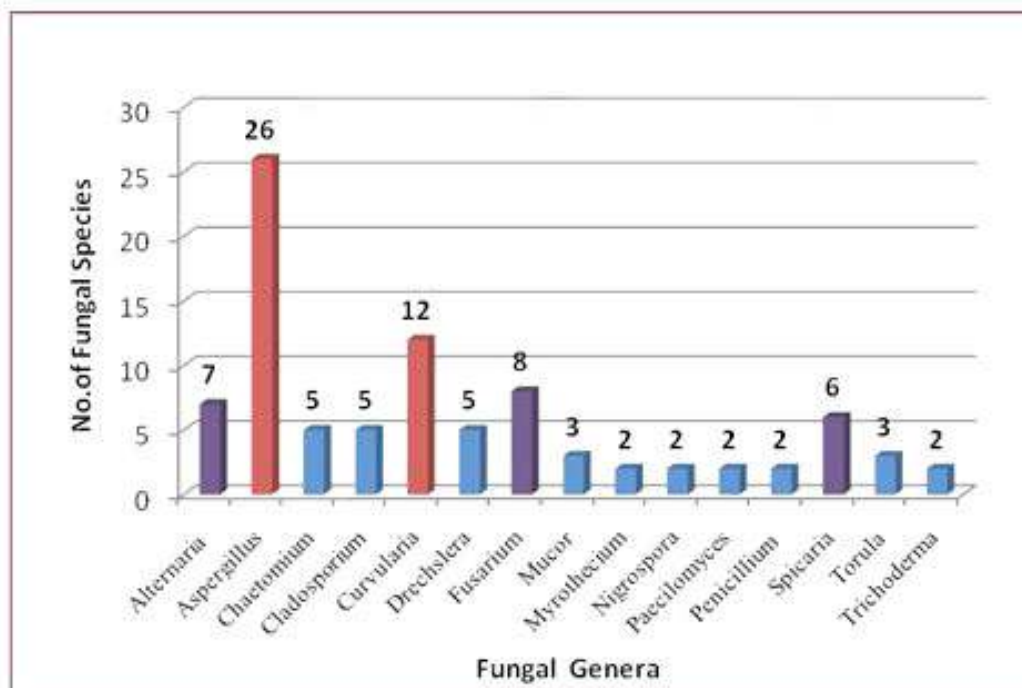


Figure 2- Number of fungal species in each Genus of Phylloplane



Reisinger, *Doratomyces microspores* (Sacc.) F.J. Marten & G.Sm., *Drechslera australiensis* (Bugnicourt) Subr. & Jain, *Drechslera bicolor* (Mitza.) Subr. & B.L. Jain, *Drechslera halodes* (Drechsler) Subr. & Jain, *Drechslera hawaiiensis* Bugnic. Ex M.B. Ellis, *Epicoccum nigrum* Link, *Fusarium avenaceum* (Fr.) Sacc., *Fusarium dimrum* Penzig, *Fusarium equiseti* (Corda) Sacc., *Fusarium nivale* (Fr.) Sarauer, *Fusarium oxysporum* Schltdl, *Fusarium poae* (Peck) Wollenw., *Fusarium semitectum* Berk & Ravenel, *Fusarium solani* Sacc., *Humicola grisea* Traaen, *Monodictys glauca* (Cooke & Harkn.) S.Hughes, *Mucor racemosum* Fresen, *Mucor varians* (H. Mart.) Fr., *Myrothecium carmichaelii* Grev., *Myrothecium gramineum* Lib., *Nigrospora oryzae* (Berk. & Broome) Petch, *Nigrospora sacchari* (Speg.) E.W. Mason, *Paecilomyces fusisporus* S.B. Saksena, *Paecilomyces humicola* Onions G L Barron, *Penicillium chermesinum* Biourge, *Penicillium decumbens* Thom, *Penicillium diversum* Raper & Fennell, *Penicillium fellutanum* Biourge, *Penicillium funiculosum* Thom., *Penicillium herquei* Bainier & Sartory, *Penicillium implicatum* Biourge, *Penicillium lilacinum* Thom., *Penicillium miczynskii* K.M. Zaleski, *Penicillium restrictum* J.C. Gilman & E.V. Abbott, *Penicillium rubrum* Stoll, *Penicillium ramulosum* Visagie & K.Jacobs, *Penicillium tardum* Thom., *Penicillium variabile* Wehmer, *Penicillium varians* G. Sm., *Periconia atropurpurea* (Berk. & M.A. Curtis) M.A. Litv., *Phoma ficuzzae* Brackel, *Phoma glomerata* (Corda) Wollenw. & Hochapfel, *Phoma humicola* J.C. Gilman & E.V. Abbott., *Pithomyces atro-olivaceus* Cooke & Harkn.) M.B.Ellis, *Pithomyces chartarum* (Berk. & M.A. Curtis) M.B. Ellis, *Pithomyces flavus* Berk. & Broome, *Pithomyces maydicus* (Sacc.) M.B.Ellis, *Rhinochadiella basitona* (de Hoog) Arzanlou & Crous, *Rhizoctonia bataticola* (Taubenth.) E.J. Butler, *Rhizopus nigricans* Ehrenb., *Rhizopus nodosus* Namyst., *Sclerotium oryzae* Catt., *Scolecobasidium constrictum* E.V. Abbott, *Scolecobasidium humicola* G.L. Barron & L.V. Busch, *Scopulariopsis brevicaulis* (Sacc.)

Bainier, *Spicaria divaricata* (Thom) J.C. Gilman & E.V. Abbott., *Spicaria elegans* (Corda) Harz, *Spicaria fumosorosea* (Wize) Vassiljevsky, *Spicaria griseola* Sacc. *Spicaria silvatica* Oudem, *Sporotrichum roseolum* Oudem. & Beij., *Stachylidium bicolor* Link, *Thielaviopsis paradoxa* (De Seynes) Hohn., *Torula allii* (Harz.) Sacc., *Torula herbarum* (Pers.) Link, *Trichocladium canadense* S. Hughes, *Trichoderma koningi* Oudem, *Trichoderma viride* Pers., *Trichurus spiralis* Hasselbr., *Verticillium puniceum* Cooke & Ellis, *Wardomyces inflatus* (Marchal) Hennebert, Yeast, *Zygorhynchus moelleri* Vuill.

Altogether 16 fungi namely *Aternaria echinulata* E.G. Simmons & C.F. Hill, *Aspergillus caespitosus* Raper and Thom., *Aspergillus chevalieri* Thom & Church, *Aspergillus funiculosus* G. Sm., *Aspergillus sulphureus* Desm., *Curvularia harveyi* Shipton, *Myrothecium carmichaelii* Grev., *Penicillium diversum* Raper & Fennell, *Penicillium implicatum* Biourge, *Periconia atropurpurea* (Berk. & M.A. Curtis) M.A. Litv., *Rhinochadiella basitona* (de Hoog) Arzanlou & Crous, *Spicaria elegans* (Corda) Harz., *Sporotrichum roseolum* Oudem. & Beij., *Trichocladium canadense* S.Hughes, *Trichurus spiralis* Hasselbr., and *Wardomyces inflatus* (Marchal) Hennebert. form new additions to Telangana state.

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