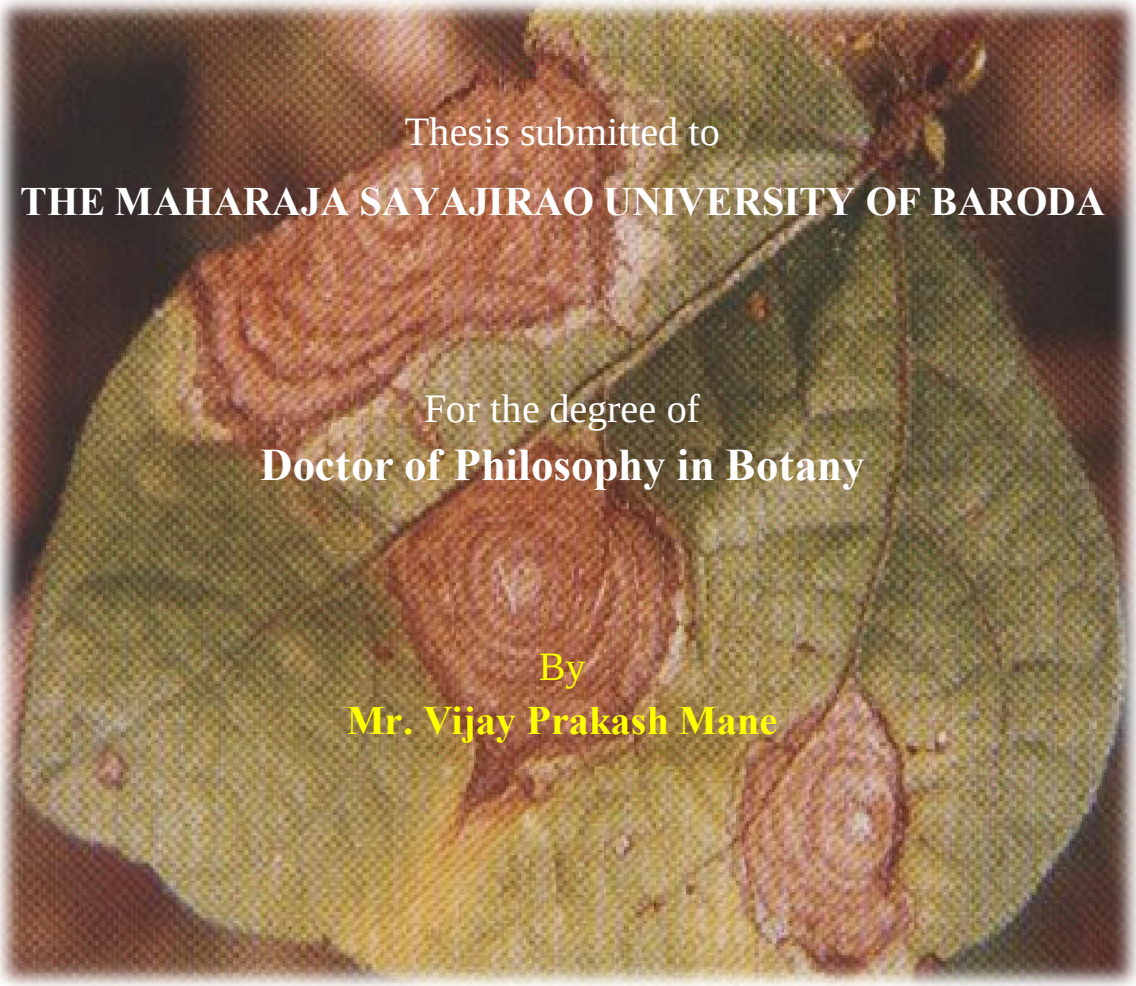
# Foliicolous Fungi of Certain Forest Trees and Their Ecofriendly Management



Thesis submitted to

**THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA**

For the degree of

**Doctor of Philosophy in Botany**

By

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# *Certificate*

**The thesis entitled "Foliicolous Fungi of certain Forest Trees and their Ecofriendly Management" submitted by Mr. Vijay Prakash Mane, contains the original research work carried out by him in the Phytopathology laboratory of the Department of Botany, The Maharaja Sayajirao University of Baroda. It has been prepared in accordance with the University norms under my direct supervision. It is further certified that this work has not been submitted earlier to any other University/ Institute for any degree.**

**Prof. Arun Arya**
Guide & Head,
Dept. of Botany

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*Making a thesis is a hard work, what makes it possible is the support we get from those around us, as always, our learned teachers and beloved friends. While we cheerfully share the credit for the accurate aspects of the project; the mistakes and omissions we have to claim as our own.*

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*(Vijay Prakash Mane)*

# ABBREVIATIONS

εg/l Microgram/ litre

$^{0}$C Degree in Celsius

h hour

g Grams

mg Milligram

mg/g Milligram/gram

ml Millilitre

mm Millimetre

PDA Potato Dextrose Agar

pH Potentials of Hydrogen Ion

wt Weight

WLS Wild Life Sanctuary

ppm Parts per million

# Foliicolous Fungi of Certain Forest Trees and Their Ecofriendly Management

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Ecosystems are responsible for much of our Climate Physiology. The Ecosystem is a core function of a working Forest However, simply planting trees will not create a working Forest Ecosystem. To accomplish that you must have virtually all the plant species that Nature provides from the smallest Flowers through woody shrubs and under Forest Ecosystems are responsible for much of our Climate Physiology. The Ecosystem is a core function of a working Forest However, simply planting trees will not create a working Forest Ecosystem. To accomplish that you must have virtually all the plant species that Nature provides from the smallest Flowers through woody shrubs and understory trees. Then you add the Birds, Animals and Insects. Only then will the synergy of these elements begin a working Forest Ecosystem.

The working forest Ecosystem is a virtual clean climate machine. It cleans the air removes particulate matter, cools the air and adds moisture. The forests absorb existing air polluted separating the elements freeing and releasing the oxygen disposing of the minor elements and using the $CO_2$ for food synthesis and further growth. Forests release water during transpiration vapour which rises and form clouds.

As ecological threats to forest health and sustainability intensify and new threats are emerging, forest pathology plays an increasingly important role in recent times.

**Forests**

The forest in the state may be classified into eleven categories (after Champion and Seth, 1968). Important categories are: Tropical moist deciduous forests, mixed dry deciduous forests (dry-teak and non-teak forest), Scrub forests and mangrove forests.

**Type of Forests in Gujarat:**

The main forest types of Gujarat are as follow

Sub-group 3-B -**South Indian moist deciduous forests**: Dangs, Valsad and Surat.

1. Sub- group 4-B-**Swamp forests or tidal forests**: Kachchh, Jamnagar, Bhavnagar, Bharuch and Surat.
2. Sub-group 5-A-**Southern Tropical dry deciduous forests**; Dangs, Gir-Girnar, Sabarkantha and Panchmahals..
3. Sub-group 6-B **Northern tropical thorn forests**: Saurashtra, Kachchh and North Gujarat, Little Rann of Kutch (LRK) and Great Rann of Kachchh (GRK) and Banni.

**Forest Cover of Gujarat:**

1. Open Forest (canopy density between 10 to 48%) 5504Sq.km (29.23%).
2. Dense Forest (Canopy density 48%) 6430 Sq.km (34.15%)
3. Scrub/Degraded/Cultivation/Water body/Saline area 4463 Sq.km. (23.7%)
4. Grasslands 1428 Sq.km. (7.45%)
5. Mangroves 1031 Sq.km. (5.48%)

**(i) Tropical moist deciduous forests**

These forests are found in sub humid southern most hilly areas of the state viz. Dangs, Valsad, Vyara in Surat, Bharuch and Narmada districts where average rainfall is not more than 1025 mm. these forests are not evergreen, top layer of trees are more than 8m tall and they shed their leaves during March and April, though middle layer and ground cover generally remain fairly green. The dominant trees in these forests are:

*Tectona grandis* L.f. and *Shorea robusta* Gaertn.f. are more dominant. The other evergreen associates are *Acacia chundra* (Rottler) Willd., *Anogeissus latifolia (*DC) Wall ex. Bedd, *Butea monosperma* (Lam.) Taub., *Dendroclalamus strictus* (Roxb.) CG.Nees, *Diospyros melanoxylon* Roxb., *Mitragyna parvifolia* L., *Terminalia crenulata* Roth., *T. alata* Hewne ex. Roth., *T. chebula, Lagerstroemia parvifolia* L., *Pterocarpus marsupium* Roxb., *Schleichera oleosa* (Lour) Oken., *Dillenia pentagyna* Roxb., *Miliusa tomentosa* (Roxb.) Sinclair, *Trema orientalis* (L.) Bl., *Kydia calycina* Roxb., *Clausena heptaphylla* Roxb., *Olea diocia* Roxb., *Oroxylum indicum* Vent., *Bambusa arundinacea* (Retz.) Willd., etc. During monsoon, these forests resemble just as evergreen forests, however, some species shed their leaves during March- April except *Tectona grandis* which shed its leaves during winter season. The common woody climbers reported in these areas are *Diploclisia glaucescens, Dioscorea belophylla, D. hipsida, D. oppositifolia, Cayratia carnosa, Ventilago maderaspatana, Derris heyneana, Hyptage benghalensis,* etc.

A number of epiphytes and terrestrial orchids, characteristics of moist forest, further enrich the photodiversity of these beautiful forests. The important orchid species in these forests are: *Aerides crispum* Lind., *A. maculosum* Lind., *Dendrobium barbatulum* Lind., *D. microbulbon, D. Ovatum* (L.) Krazl., *D. peguanum, Habenaria commelinifolia* (Roxb.) Wall. Ex Lind., *H. Furcifera* Lind., *H. gibsonii var. foliosa* Hook. F., *H. grandifloriformis* Blat.and Mclan, *H. longicorniculata, H. marginata var. fusifera* (Colebr.)Lind. Ex Wall., *Malaxis mackinnonii, Nervilia aragoana, N. discolor, Oberonia falconeri, Peristylus lawii* (Wight.) Hook.f., *Platanthera susannae* (L.) Lind., *Vanda tessellate* (Roxb.) Hk. Ex G Don., *Zeuxine strateumatica* (L.) Schlechter, etc. the ground flora is dominated mainly by shade loving species of *Ageratum, Abutilon, Cassia, Cleome, Eupatorium, Cynoglossum* and other grass species, etc.

**(ii) Mixed dry deciduous forests**

These forests are mixed growth of trees and remain deciduous during dry season. The lower canopy in these forests is also deciduous with occasional evergreen being present in the moist areas. The under growth is comprising shrubs and ground flora flourish well during rains. These forests can be further divided into ódry teak forestsô and ódry non-teak forestsô. The former type occurs in Rajpipla, Chhota-udepur, Panchmahal, Sabarkantha in north-east and Junagadh and Amreli district in Saurashtra. The ódry non-teak forestsô are found in some parts of Bansakantha district in north Gujarat and Rajkot district in Saurashtra. The main difference between these forests types is that in dry teak forest, *T. grandis*, dominates the vegetation, while in latter type it is very poorly represented and dominating species is *A. latifolia* or *A. chundra*. The other associates of these are: *B. monosperma, D. melanoxylon, Mitragyna parvifolia, Schleichera oleosa, Lannea coromandelic, Terminalia crenulata*, etc. *Boswellia serrata* becomes most dominant tree on the top of hills in some forest ranges. The other scattered tree species reported in these forests are: *Haldina cordifolia, Albizzia lebbeck* (L.) Benth *, A. odoratissima, Bauhinia racemosa* Linn.*, Bridelia retusa* Willd.*, Buchanania lanzan* Spreng.*, Carea arborea* Roxb.*, Cochlospermum religiosum* (L.) Alston.*, Dalbergia latifolia* Roxb.*, D. paniculata* Roxb.*, Embilica officinalis* L.*, Garuga pinnata* Roxb.*, Hymenodictyon excelsum* (Roxb.) Wall.*, Sterculia urens* Roxb.*, Pterocarpus marsupium, Desmodium oojcinensis* (Roxb.) H. ohashi, etc. Most of these trees are 8 m or more high and are heliophytes. The trees or shrubs which are rather less tall and constitute middle layer are: *Holarrhena pubescens, Wrightia tinctoria, Aegle marmelos* (L.) Corr. Serr.*, Alangium salvifolium* Lam.*, Casearia elliptica* Willd.*, Flacourtia indica* (BrumF.) Mer.*, Gardenia turgida* J. Ellis.*, Mallotus philippensis* (Lam.) Muell. Arg.*, Zizipus xylopyrus* (Retz.) Willd. etc.

On the top of some of these forest ranges *Dendrocalamus strictus* clumps are associated with *Boswellia serrata*. On low and isolated hillock the common shrubs present are: *Carissa*

*congesta, Capparis sepiaria* L.*, Maytenus emarginatus* Willd.*, Mimosa hamata* Willd.*, Cassia auriculata* L.*, Trema orientalis* (L.) Bl.*, Xeromphis spinosa* (Thum.) Keay.*, Woodfordia fruticosa* (L.) Kurz., etc. The common climbers and twinners associated with these plants are: *Abrus precatorius* L.*, Acacia pinnata, Asparagus racemosus* Willd.*, Aspidopterys parvifolia, Cansjera rheedii* J. Gmelin.*, Celastrus paniculata* Wild*, Cissus repanda* Wahl.*, Clematis hedysarifolia* DC.*, Combretum ovalifolium* Loefl.*, Cryptolepis buchanani* R & S*, Cylista scariosa, Hiptage benghalensis, Millettia racemosa* (Roxb.) Benth.*, Wattakaka volublis* (L.f.) Stapf. *etc.*

The ground flora which makes its appearance green during monsoon comprises of following common species: *Acanthospermum hispidum* DC.*, Achyranthes aspera* L.*, Anisochilus carnosa, Barleria prattensis* Sant.*, Bidens biterbata* (Lour.) Merr. and Sherff.*, Blainvillea acmella* L.*, Cassia absus* L.*, C. Tora* L.*, Clitoria biflora* Dalz.*, Curcuma inodora* Blat.*, Dicliptera roxbhurghii* Nees.*, Trachyspermum stictocarpum* (C.B. Clarke) H. Wolff*, Sida* spp., etc. The common grasses intermingled with these herbaceous plants are: *Apluda mutica* L.*, Dicanthium annulatum* (Forssk.)Stapf and species of *Arthraxon, Eragrostis, Digitaria, Brachiaria and Panicum*, etc.

Fossil records of parasitic fungi date back to Devonian period, which possibly suggest that plant disease originated along with the plants; much before man came on earth. When man started growing food they also unwittingly started culturing the parasites. The outbreak of plant diseases, then must have occurred much before recorded history. Mention of plant diseases are found in ancient Greek and Indian citations though they knew little about them.

First authentic records of plant disease are found in the writings of Theophrastus ï ñThe Father of Botanyò. While describing trees, cereals, pulses he also described their diseases. He recorded the harmful effects of wind, weather location. He and other Greek philosophers of the time believed that the diseases originated from the plants or from the environment.

An Italian botanist Micheli in 1729 made an extensive study of fungi and their reproductive structures. He discovered the role of spores and experimentally proved that the fungi originated from their spores. This was the first experimental proof that fungi are autonomous organisms which produce seed ï like bodies and not capricious creatures of the spontaneous generation.

**I**ndia is one of the 12 mega-biodiversity countries of the world. About 70% of total geographical India area has been surveyed so far, in which 46,000 plants have been described. A healthy forest needs protection from all kinds of disturbances caused by a variety of biotic & abiotic stresses (Sarbhoy, 2005).

Forest is a dense growth of trees, together with other plants, covering a large area of land. It is an ecosystemð a community of plants and animals interacting with one another and with the physical environment. Gujarat state has 19.6 m. ha. forest cover, which is 9.61% of the geographical area of the state.

The south and south-eastern parts of the state support the growth of a tropical deciduous forest typified by *Tectona grandis* L.f., *Shorea robusta* Gaertn. f. for which the district of Valsad is well known. The forest of the state can be divided into the following broad categories, depending upon their environmental adjustments and the general morphological character of the representative species. It is estimated that the forest products contribute about 1% of world gross domestic product (GDP).

Gujarat has about 19.66 lakh hectares of land under forest. A large part of the forest cover which is economically exploitable is distributed in the districts of Dangs, Panchmahals, Broach, Surat, Bulsar, Junagadh, Sabarkantha and Banaskantha. Dangs, Surat and Broach,

which are the three southern districts of the state have a sizable area under forest. The districts of Panchmahals and Sabarkantha in north-east Gujarat and Junagadh in Saurashtra are other important areas of forest cover. The south and south-eastern parts of the state support the growth of a tropical deciduous forest typified by teak, *Shorea robusta* for which the district of Valsad is well known.

All shades trees are attacked by one or more fungi that cause scattered, rather definite round to oval, angular or irregular shaped spots on the leaves. These spots become usually become conspicuous from late June through August. Leaf spots are most common diseases of shade trees. Most of the diseases are favored by cool weather, light and frequent rains, fog or heavy dews, high humidity, and crowded or shady plantings. Leaf spot infections that start early in the growing season can lead to premature defoliation. If it occurs over two or more successive years, it can seriously weaken a tree, reduce its growth, and its susceptibility to bark borers, winter injury and other diseases. Leaf spot commonly increase in number and size in late summer and early autumn as the leaf begin to senesce. The occurrence of leaf spot disease late in the growing season generally does not seriously affect the health of a tree. Certain leaf spots have special name such as anthracnose, black pot, downy spot or white mold, ink spot, anthracnose, leaf blister, scab, shot hole, sooty blotch and tar spot. (Patacky, 1998)

**Moist Deciduous Forests**

Moist Deciduous Forests occur in Dangs and parts of Vyara in Surat division. These forests are not evergreen and shed their leaves during March and April, though the under-wood and shrub cover are fairly green. Teak is an important species which drops its leaves only in the cold weather in localities which are relatively dry or cold, but is almost evergreen in the moist parts of its distribution. Teak needs a moderately good rainfall and a well-drained

terrain. The associates of teak in the moist deciduous forests are *Terminalia tomestosa* and *Anogeissus latifolia*.

**Dry Deciduous Forests**

There are mixed growth of trees which are deciduous during the dry season. The lower canopy in these forests is also deciduous with occasional evergreen or sub greens being present in the moister area. There is an undergrowth of shrubs, but the light reaches the surface allowing the growth of grass which occasionally develops into a savanna-type grass field. Bamboos are not luxuriant. Other trees of the dry deciduous forests are teak, *Boswellia serrata, Anogeissus latifolia* and *Diospyros melanoxylon*. Dry deciduous forests with teak occur in north-east Gujarat, particularly in Sabarkantha district. The forests of Junagadh are valuable for their yield of timber and of grass growing on their outer margin.

## Plants selected for the study

***Tectona grandis* L.f.**

The plant *Tectona grandis* is native to India and Myanmar and is found in the monsoon vegetation forest. It is commonly known as teak. It is a large deciduous tree with a height up to 35 meters, leaves simple, opposite, broadly elliptical or acute or acuminate, with minute glandular dots; the Flowers are white in color and small with a pleasant smell.

Teak is an important species which drops its leaves only in the cold weather in localities which are relatively dry or cold, but is almost evergreen in the moist parts. Teak needs a moderately good rainfall and a well-drained terrain. The associates of teak in the moist deciduous forests are *Terminalia tomestosa* (Roxb.)W & A. and *Anogeissus latifolia* (DC.)Wall.ex Bedd. Trees of the dry deciduous forests are teak, *Boswellia serrata* Triana and

Planch, *Anogeissus latifolia* (DC.)Wall.ex Bedd and *Diospyros melanoxylon* Roxb found with grasses and bamboos.

**Chemical Constituents**

The various phytoconstituents reported are tectoquinone, 5-hydroxylapacol, tectol, betulinic, aldehyde, betulinic acid, squalin, lapachol**.** The plant is used in the treatment of urinary discharge, bronchitis, common cold and headache, as a laxative, sedative, diuretic and in scabies. We have earlier reported that the methanolic extract of the leaves showed a significant wound healing, analgesic and anti inflammatory activity. The extract was investigated to determine the nature of the phytoconstituents responsible for these activities. Literature survey has revealed that plant metabolites like phenolic compounds (simple phenols, phenolic acids, Flavonoids, tannins etc), and sterols play an important role in many of the activities like wound healing, analgesic, anti inflammatory and anti microbial activity. This paper reports the isolation and identification of phenolic compounds gallic acid, ellagic acid, rutin and quercitin.

**Uses**

Teak is used extensively in India to make doors and window frames, furniture, and columns and beams in old type houses. It is very resistant to termite attacks. Mature teak fetches a very good price. It is grown extensively by forest departments of different states in forest areas.

Leaves of the teak wood tree are used in making Pellakai gatti (jackfruit dumpling), where batter is poured into a teak leaf and is steamed. This type of usage is found in the coastal district of Udupi in the Tulunadu region in South India. The leaves are also used in gudeg, a

dish of young jackfruit made in Central Java, Indonesia, and give the dish its dark brown color. Teak is used as a food plant by the larvae of moths of the genus *Endoclita* including *E. aroura*, *E. chalybeatus*, *E. damor*, *E. gmelina*, *E. malabaricus*, *E. sericeus* and *E. signifer* and other Lepidoptera including Turnip Moth.

Teak is used extensively in boat decks, as it is extremely durable and requires very little maintenance. The teak tends to wear in to the softer 'summer' growth bands first, forming a natural 'non-slip' surface. Any sanding is therefore only damaging. Use of modern cleaning compounds, oils or preservatives will shorten the life of the teak, as it contains natural teak-oil a very small distance below the white surface. Wooden boat experts will only wash the teak with salt water, and re-caulk when need this cleans the deck, and prevents it from drying out and the wood shrinking. The salt helps it absorb and retain moisture, and prevents any mildew and algal growth. People with poor knowledge often over-maintain the teak, and drastically shorten its life.

***Terminalia arjuna*** (Roxb.) Wight & Arn.

A large evergreen tree with smooth grey bark exfoliating in large, thin, irregular sheets, often tinged with green and red. The leaves are oblong, opposite and sub-opposite. The leaves on the lower surface usually carry a pair of prominent glands close to the top of the leaf-stalk. The flowers are pale-yellowish-white, cup-shaped, resembling myrobalan, small and crowded on a long axis. It flowers from March to June when its honey attracts swarms of bees. The fruit is a winged nut, the leathery wings are usually five in number and are closely veined, the veins not spreading horizontally but tending to curve upwards. The fruit is tan-coloured when dry.

**Chemical - Constituents**

Plant contains triterpene arjunolitin. Roots and root bark contain triterpenoid glycoside arjunoside I-IV, triterpene terminic acid. Stem bark contains flavone arjunolone, arjunglucoside, tannin, arjunic acid, arjunetin, arjungenin. Fruits contain Beta-sitosterol, friedelin, methyloleanolate, gallic acid, ellagic acid, arjunic acid, ceresidin,arjunone.

**Uses**

Its bark is astringent and is used in fever and in fractures and convulsions; it is also taken as a cardiac tonic. Bark styptic, tonic, febrifuge and anti-dysenteric; pulverised bark gives relief in symptomatic hypertension and acts as a diuretic in cirrhosis of liver. Fruits are tonic and deobstruent. Juice of leaves is used in earache.

***Bambusa arundinacea*** (Retz.) Willd

Native to tropical and subtropical areas of Asia, especially in the monsoon and wet Tropics. A tall, thickset bamboo; stem up to 33 m high, tufted on a stout rootstock. Leaves up to 20 cm long, linear-lanceolate. Inflorescence an enormous panicle often occupying the whole stem.

*Bambusa* is a large genus of clumping bamboos. The plant grows wild all over India, mainly in forests of western and southern parts of the country. *Bambusa arundinacea* can grow up to 1500 ï 2000 meters of elevation. It is an erect, 15-35 meter tall, thorny grass, with many stems. The plant is hollow between the joints, with 2-3 alternate thorns on the stem. Their leaves are sheathing, linear, 20 cm long and 2 cm broad, lanceolate, tapering in the pointed tips. The flowers are produced in bunches, yellow or yellowish green in long panicles. The fruits are oblong grains, resembling like yava fruits, hence called as vamsayava.

**Chemical - Constituents**

Young shoots contain cholin, betain, enzymes, benzoic acid and a cyanogenetic glycoside, (taxiphyllin), oxalic acid, reducing sugars, resins, waxes, silica and other minerals. Juice of sprout contains benzoic and hydrocyanic acids. The plant also contains silicic acid, oxides of iron, potash and lime (Ghani, 2003).

**Uses**

The stem and leaves are cooling and laxative; useful in diseases of blood, leucoderma and inflammatory conditions. The sprouts are laxative and beneficial in strangury. The siliceous concretion found in the joints of the female bamboo is largely used in homeopathic medicine as tonic and aphrodisiac; useful in cough and cold, congestion and asthma. The leaves are considered emmenagogue; given to check diarrhoea in cattle and horses. Thin green layer of the bark is used to arrest bleeding. The roots are used to treat joint pains.

***Madhuca indica* J. F. Gmel**

Mahua: The Honey Tree

*Madhuca indica* is the botanical name of Madhuka tree which belongs to family Sapotaceae.

*M. indica* is a medium to large deciduous tree fast growing upto 20m height (Figð ). Tree possesses evergreen or semi-evergreen leaves which cluster near ends of branches, elliptic or elliptic-oblong, pubescent and turn to glabrous at maturity. Young leaves are pinkish red. Flowers white to cream colour with tubular, fleshy and juicy corolla, clustered at the end of branches. Fruits berry ovoid, green at maturity and turn pinkish yellow when ripe. Fruits are pulpy with large ovoid seed, numbers of seeds vary from 1 to 4, seed color brown to black.

Fruits occur in single or bunch up to 30-40 (Figð ). Leaf fall takes place between February to April and at the same time flowering commences. Fruits mature generally in the months of May-June. Tree blooms at night and in early morning hours flowers fall on the ground and collected by local tribal population for commercial use.

In Sanskrit, the plant is known as Madhuka. Mahua is its Hindi name. It is used for timber, flowers and fruits. It has several medicinal properties.

**Medicinal uses**

Roasted leaves of the tree are mixed with sesame oil and applied on swelling and inflammation. Patients suffering from piles are supplemented with 12-15 drops of seed oil. It works as laxative. Bark decoction is good in diabetes. Topical application of seed oil is recommended for stiffness and arthritis. Seed oil provides soothing effect to the skin. A decoction of the bark can be given internally in rheumatic diseases. The leaves of *Madhuca* are effective in the treatment of eczema. Flowers used as expectorant are good cure for curing bronchial asthma. Flowers are used as sweet, some ethnic food like chapati are prepared by tribal women. Mahua cake is used as manure; it has pesticidal properties.

**Uses:**

*Madhuca* fat is satisfactory for production of washing soaps. The oil extracted from seeds is used in cooking, soap making and manufacture of margarine**.** It is used for edible purposes culinary, hair oil, illumination, keeps body glossy and warm.

*Madhuca* cake can be used as cheap organic manure and possesses insecticidal property. Also used with shikakai for hair-wash.

The flowers used as vegetable, for making vinegar and liquor.

Seed paste is applied to cure muscle fatigue and relieve pain in the muscle and joints to improve the texture and vigor of skin. Bark decoction is used in curing bleeding gums and ulcers. *Madhuca* oil extracted from the seeds has laxative properties. It helps to cure piles by relieving chronic constipation. The leaves of *Madhuca* are effective in the treatment of eczema.

***Diospyros melanoxylon* Roxb.**

Coromandel Ebony or East Indian Ebony (*Diospyros melanoxylon*) is a species of flowering tree in the family Ebenaceae that is native to India and Sri Lanka.

*D. melanoxylon* is a medium-sized tree or shrub up to 25 m, in height and 1.9 m girth. The bark is pelican in colour, exfoliating in rectangular scales. The primary root is long, thick and fleshy at first, afterwards woody, greyish, often swollen in upper part near ground level. The roots form vertical loops in sucker-generated plants. Leaves opposite or alternate and coriaceus, up to 35 cm long, tomentose on both sides when young, becoming glabrous above when fully grown. Male flowers are mauve in colour, tetramerous to sextamerous, 1-1.5 cm long, sessile or nearly sessile in short peduncles, mostly 3-flowered. Female flowers mauve, mostly extra-axillary or sometimes solitary, axillary generally 2, opposite each other, larger than the male flowers. Fruits olive green, ovoid or globose 3-4 cm across; 1-8-seeded berries. Pulp yellow, soft and sweet. Seeds compressed, oblong, shiny, often banded. The generic name is derived from the Greek ódiosô (divine), and ópyrosô (fruit), referring to the excellent fruit of the genus. The specific name is Greek and means ódark woodô.

Its common name is derived from the coast of southeastern India, Coromandel; locally it is known as *temburini* or by its Hindi name *tendu*. It is used in making an Indian cigarette

product known as beedi in wrapping the tobacco together to be smoked. Bark of East Indian Ebony is hard and dry, burns with spark and sound.

**Uses**: *Diospyros melanoxylon* leaf is considered to be the most suitable wrapper on account of the ease with which it can be rolled and its wide availability. Leaves of many other plants like *Butea monosperma, Shorea robusta* etc. also find use as Bidi wrappers in different parts of the country but the texture, flavour and workability of *Diospyros* leaves are unmatchable. The wide-scale use of *D. melanoxylon* leaves in Bidi industry is mainly based on their enormous production, agreeable flavour, flexibility, resistance to decay and capacity to retain fire. The broad morphological characters on which leaves, are selected and categorized for Bidi making are size, thickness of leaves, texture, relative thickness of midrib and lateral veins.

Forest trees suffer with a large number of fungal pathogens. Efforts have been made by Bakshi (1976), Bilgrami *et al*. (1979,1981), Jamalludin *et al*., (2001), Dadwal and Jamaluddin (2001) Arya & Arya (2002) to report new leaf spot diseases of forest trees. Considering the huge losses to forest biomass and ultimate reduction in yield an effort is required to find out effective control measures.

Fungi growing on leaves in a parasitic fashion are termed as foliicolous fungi. These fungi are either obligate parasites (biotrophs) or necrotrophs. The fungi cause diseases which can be identified by disease symptoms. Fungi may cause large scale mortality in the nursery or they could seriously affect plantations by reducing the biomass or loss of valuable germplasm collection. The diseases caused by powdery and downy mildews & anthracnose result in severe losses of foliage. Further, if the plants are like Tea (*Camellia sinensis* (L.)

Kuntze) or Timru (*Diospyros melanoxylon* Roxb.) the infection on leaves means production of toxins which will be ultimately consumed by human beings in form of tea or bidi leaves.

Fungi rank second only to insects as a cause of plant diseases, which result in heavy loss of plant products. Pathogenic fungi alone cause nearly 20% reduction in the yield of major food and cash crops. (Agrios, 2000). One third global agricultural production is reportedly destroyed each year due to plant diseases. Variety of control measures presently are in use. In physical methods, use of sunlight and UV radiations etc. are included, while the most commonly known means of controlling fungal diseases in fields and green houses and sometimes in storage is through the use of chemical compounds that are toxic to fungi. No doubt the use of chemicals has been found very effective in controlling fungal diseases but some major problems threaten to limit the continued use of fungicides. Firstly some fungi have developed resistance to chemicals. This necessitates use of higher dosage or the development of new chemicals to replace those to which fungi are resistant. Secondly some fungicides are not readily biodegradable and tend to persist for years in the environment. This leads to third problem, the detrimental effects of chemicals on organisms other than target fungi, (Brady, 1984). Because of these problems associated with the use of chemicals, researchers are now trying to use environmentally safe alternative methods of fungal control.

The search for simple biodegradable bioactive compounds of plant origin against fungi has been the target of interest for ecologically safe products. (Soundharrajan *et al.,* 2003)

Aqueous extracts of many allelopathic plants are known to exhibit antifungal effect. Allelochemicals reduce the germination of spores and mycelial growth of pathogenic fungi. Many research workers have tried to find safe and economical control of plant diseases by using extracts of different plant parts. (Bhowmick and Chaudhary, 1982; Vir and Sharma,

1985; Jeyrajan *et al.,* 1988; Swada *et al.,* 1971; Singh *et al.,* 1980; Sumbali and Mehrotra, 1981; Loke, 1990; Kazmi *et al.,* 1993.)

**Fungi already reported from *T. grandis* include**

*Acorocybe hansfordii*

*Auricularia polutricha*

*Calderiomyce*

*Cephalosporium curtipes*

*Cercospora tectonae*

*Chaconia tectonae*

*Circinotrichium pseudocladium*

*Didymobotrichium pubescens*

*Fusarium* sp

*Ganoderma applanatum*

*Grammothele effuse – reflexa*

*Hypomyces haematococcus*

*Irpex flavus*

*Microxyphium fagi*

*Myrangium tectonae*

*Nectaria haematococcus*

*Olivea tectonae*

*Pestalotia* sp.

*Phyllosticta tectonae*

*Podospora nanaopodalis*

*Polyporus adustus*

*Prathoda saparva*

*Sarcinella* sp.

*Sphaceloma tectonae*

*Synnematium jonesii*

*Tilachlidium pinnatum*

*Uncinula tectonae*

*Veronaea tectonae*

***Terminalia arjuna* Roxb.**

*Colletotrichium arjunae*

*Fomes durissimus*

*Pestalotiopsis disseminata*

*Sphaceloma terminaliae*

*Uredo termenalia*

*Xylaria trichopoda*

***Diospyros melanoxylon*** Roxb.

*Aecidium rhytismodieum*

*Aecidium miliare*

*Cercospora kaki*

*Pseudocercospora kelleri*

*Sarcinella gorakhpurensis*

*Stereum lobatum*

***Madhuca indica* L.**

*Cylindroncladium scorparium*

*Pestalotia dichaeta*

*Pestalotia sp.*

*Phyllachora madhuca*

*Phyllachora madhucae*

*Polystictus steinbelianus*

*Scopela echinulata*

*Sarcinella sp.*

*Sphaceloma madhucae*

***Bambusa arundinacea* Nees**

*Amauroderma rugosus*

*Anthostomella bambusae*

*Botrydiplodia theobromae*

*Cerodothis aurea*

*Craterellus cornucopiodes*

*Dacryopinax spathularia*

*Daedalea flavida*

*Daedalea quercina*

*Flammula dilepis*

*Fomes durus*

*Fomes hypoplstus*

*Guepinia ramosa*

*Hexagonia apiaria*

*Irpex flavus*

*Merulius similis*

*Phyllachora* sp.

*Polyporus friabilis*

*Polystictus steinheilianus*

*Polyporus anthelimintícus*

*Polyporus rubidus*

*Polystictus oblectans*

*Polystictus perennis*

*Poria diversipora*

*Schizophyllum commune*

*Sporomiella intermedia*

*Thelephora palmata*

*Tramella fucifromis*

*Trametes persoonii*

*Tremellodon gelatinosum*

**Plants used for Biocontrol**

Plants are the richest source of renewable bioactive organic chemicals. The total number of plant based chemicals may exceed 4,00,000 of these 10,000 are secondary metabolites, whose major role in the plants is reportedly defensive (Swain, 1977).

The screening of plant extracts for antimicrobial activity has shown that a great number of these plants contain active compounds. The presence of antibacterial, antifungal, and other biological activities has been demonstrated in extracts of different plant species used in traditional medicine practices. (Hashem, 2011)

Basic researches for over more than forty years in the fields of biological and biochemical have made it possible to envisaged not only how new pesticides may be synthesized but also a completely new approach for the protection of plants using secondary

plant products, which may be toxic to a specific pest yet harmless to man. There has been a renewed interest in botanical pesticides because of several distinct advantages (1) Pesticidal plants are generally much safer than conventionally used synthetic pesticides. These pesticides will not cause harm in nature. (2) Plant based pesticides will be renewal in nature and would be economical. (3) Some plants have more than one chemical as an active principle responsible for their biological properties. These may either be selected for one particular biological effect or may have diverse biological effects (Singh, 1993).

Efforts are being made these days to shift from the conventional use of chemicals to the use of eco-friendly botanicals for the management of plant parasitic nematodes. Organic amendments are not only safe to use but also have the capacity to improve soil structure and fertility (Trivedi, 2002).

**1. *Alangium salviifolium* (Linn.f.) Wang Alangiaceae**

A deciduous, rambling shrub or a tree, up to 10m in height with a maximum girth of 1.2 m widely distributed over the plains and foothills, throughout the greater part of India.

Bark pale yellow brown, aromatic, rough with shallow cracks, exfoliating in sub ï corky scales, leaves alternate, variable in shape, oblong or elliptic ï oblong, acuminate, base rounded or acute, glabrous above and sparsely pubescent beneath, up to 15 cm long ï petioled flowers white fragrant, in axilary fascicles; drupe ellipsoid, black, crowned by persistent calyx edible; seeds large enclosed in red mucilaginous, sweet but rather astringent pulp.

Roots have been used in Ayurvedic medicine. The root bark is very bitter, and is reputed as acure for skin diseases. It is credited with anthelmintic, purgative and emetic properties. It contains an alkaloid which when administered in small doses causes a transient fall in blood pressure followed by a sustained rise, depression of the heart, and irregular respiration. The bark exhibits anti ï tubercular activity, and is active against gram ï positive organisms.

The leaves are applied as poultice in rheumatism. They contain the alkaloids, alangimarckine ($C_{29}H_{37}N_3O_3$ mp $186^0$), ankorine, deoxytubulosine, 3ô ï epitubulosine has been isolated. The leaves also contain stigmasta ï 5,22,25 ï trien 3ß ï ol, ß- sistosterol, friedelin and N ï benzoyl ï L ï phenylalaninol (m p 169). The presence of myristic acid, three triterpenoids, viz. triterpene A ($C_{30}H_{50}O_2$, m p 248 ï $50^0$) isoalangidiol ($C_{30}H_{52}O_2$ m p 224 ï $63^0$) and alangidiol ($C_{30}H_{52}O_2$, m p 262 ï $63^0$).

The alkaloidal extract of these leaves showed mild adrenolytic, non ï specific antispasmodic, hypotensive and anti ï cholinesterase activity. A quartenery base isolated from the water ï soluble fraction of the alcoholic extract produces a fall in carotid blood pressure of anaesthized dogs. An alcoholic extracts of the leaves showed hypoglycaemic activity in albino rats.

**2. *Alisicarpus alsinoides* (L.) DC.**

Var. nummulariformis (DC.)Baker

Prostrate or suberect, glabrous or sparsely hairy herbs, often with radially spreading branches. Leaves 1 ï 4.5 x 0.9 ï 3.5 cm, ovate ï oblong, elliptic ï oblong or nearly

orbicular, glabrous above, sparsely hairy beneath. Flowers purple or violet, in 3 ï 7 cm long. Terminal and axillary, lax or compact racemes. Pods 1.3 ï 2.5 cm ellipsoid ï oblong, glabrous, brown, smooth.

**3. *Butea monosperma* (Lam.) Taub.**

A deciduous tree with somewhat crooked trunk up to 15 m in height and 1.6 ï 2.0 m in girth; commonly found throughout India except in arid regions. Bark bluish grey or light brown; leaves long petioled, 3 ï foliolate, leaflets coriaceous. Broadle obovate from a cuneate or deltoid base, glabrescent above, denselt finely silky below, flower buds dark brown, flowers bright orange red, sometimes yellow, in 15 cm long racemes on bear branches, pods pendulous, silky tomentose 1- - 13 cm long containing 1 seed at its apex: seed flate, reniform 3.3 - 3.8 cm x 2.2 ï 2.5 cm. The tree is silviculturally important as it is one the most commonest on the plains of India and capable of thriving where most species will not grow. It grows in water logged situations on black cotton soils, even on saline, alkaline and swampy badly drained soils and on barren lands.

The bark is reported to possesô astringent, bitter, pungent, alterative, aphrodisiac, and anthelmintic properties. It is useful in tumours bleeding piles and ulcers. The decoction is prescribed in cold, cough, fever, various forms of haemorrage, menstrual disorders and in the preparation and elixirs. An alcoholic extract of the bark is reported to inhibit the activity of *Escherichia coli*. The roots are useful in elephantiasis and in curing night blindness and other defects of sights. They are also reported to cause temporary sterility in women.

The green leaves are commonly lopped for fodder; the yield of milk in buffaloes feed with *Butea* leaves, is reported to improve. Their digestibility is comparable to that of straw: their caloric content is reported to be 3.761 cal/g dry weight. The leaves are also reported to

contain alkaloids. They are credited with astringent, tonic, diuretic and have aphrodisiac properties. They are used to cure boils, pimples and tumourous haemorrhoids and are internally given in flatulent colic, worms and piles. The leaves are extensively used for platers, cups, native umbrellas and for wrapping. They are also used as bidi wrappers and as manure.

The flowers yield brilliant but very fugitive yellow dye. It is contained in the sap and may be obtained in the form of decoction or an infusion from dried flowers. The addition of alum or an alkaline substance deepens the colour to orange and also makes it less fugitive. The decoction is used to dye cotton fabrics, sola articles and wooden carpets and to control white ants in the field. The flowers are reported to posseses astringent, diuretic, aphrodisiac and tonic properties. They are used to reduce swellings for bruises and sprains. They are also effective in leprosy, leucorrhoea and gout.

**4. *Calotropis procera* (Ait.) Ait.f. Asclepediaceae**

A small erect and compact shrub covered with cottony tomentum, up to 5.4 m in height, found growing wild throughout India. Bark soft, corky leaves sub sessile, broadly ovate, ovate ï oblong, mucronate, cottony pebscent when young, flowers white, purple spotted or pink, with erect petals scented in long pedunculated cottony, umbellate cymes which become glabrous; follicles sub ï globose, ellipsoid or ovoid, recurved 7.5 ï 10.0 cm x 5.0 ï 7.5 cm; seeds broadly ovate, acute, flattened narrowly margined, light brown, coma 3.2 cm, comprising a tuft of silky hairs. It grows mainly on coarse, sandy and alkaline soils. The growth is luxuriant on waste and fallow lands, roadside on the ruins of building, sea shores, river banks etc.

The latex contains the cardiac glycosides, calotropin, uscharin, calotoxin, calactin and uscharidin. Calotropin is the common aglycone of all the glycosides. Calotropin has marked anti ï blood coagulating activity used for treating coronary thrombosis. *C. procera* contains voruschin, proceroside two genins, uzarigenin and syriogenin, Ŭ and ɓ ï amyrin and ɓ ï sitoserol.

Leaves possess antifungal properties. An aqueous extract of the leaves inhibit the larva hatching of root ï knot nematode. Leaves contain ascorbic acid (241 ï 411 mg/kg dry weight). The leaf yield oï pyrocatechuic acid and an alkaloid. The guinea worms are controlled. Fresh terminal leaf buds given internally for three days on empty stomach before sunrise were reported to cure migraine completely. The tender fresh leaves are often in the indigenous system of medicine to cure fits and convulsions in children. The extracts of the leaves which have been smeared with oil and rock salt is poured into ear for earache. Leaves are bandaged on painful rheumatic joints, swellings, sores, and wounds. A powder of leaf is dusted on wounds and ulcers to inhibit excessive granulation. Alcoholic and aqueous and petroleum ether extracts of leaves have shown anti implantation activity. A decoction of leaves along with soap is an effective remedy against white ants and aphids. Powdered leaf show insecticidal property. Young shoot and roots have been reported to be used as tooth brush and to cure toothache.

**5. *Cymbopogon martini* (Roxb) Wats. Poaceae**

*Cymbopogon martinii* is a species of grass in the lemon grass genus best known by the common name palmarosa. Other common names include Indian geranium and rosha or rosha grass. This perennial grass is native to southeast Asia, especially India, and it is cultivated for its oil. The essential oil of this plant, which contains the active compound geraniol, is valued for its scent and for a number of traditional medicinal and household uses. Palmarosa oil has

been shown to be an effective insect repellent when applied to stored grain and beans, an antihelmintic against nematodes, and an antifungal and mosquito repellent. Palmarosa oil, which has a scent similar to roses, is added to soaps and cosmetics.

**6. *Cynodon dactylon* Rich.ex Pers. Poaceae**

The plant is also used in biliousness, vomiting, burning sensation, hallucinations, fever, menorrhagia, leucorrhoea, chronic diarrhoeae, dysentery, catarrhal opthalmia, epistaxis, retention of urine. Its extract has significant application in dropsy, syphilis, piles and chronic gleet.

An aqueous extract of leaves containing significant amounts of amino acids shows anticonvulsant activity. The chloroform extract of the leaves exhibited potent antimicrobial activity against Gram positive and Gram negative bacteria, the activity is due to aromatic acids. Arundorin, furfural, furfural alcohol, B- ionone,2- (4ô ï hydroxyl ï phenyl) prpionic and 3 ï methoxy ï 4ô ï hydroxybenzoic acids) phytol, B sitoserol ï D ï glucoside, stigmasterol acetate and a phagostimulant, phytore.

**7. *Datura stramonium* Linn Solanaceae**

The leaves contain the flavonoids, chrysin, liquitigenin, naringenin, kaempferol, quercetin, and the withanollde, withastramonolide. Capscidiol one of the major sespeterpine, phytoalexin, is isolated from the herb in response to infection to pathogens. It has phytotoxic and fungitoxic properties. It also inhibits pectinolytic enzymes *in vitro* and affects the molecular structure of isolated membrane of parasitic fungus *Phytopthora capsici* Lean. It exhibited muscle relaxation in vitro. The ingestion of seeds causes altered perception of environment visual hallucinations, mydriasis (dilation of eye pupil) and tachycardia. High level of ingestion may cause depression of the central nervous system. A teaspoonful of leaf

juice is given with warm milk to expel the intestinal worms particularly tape worms. The decoction of the leaves is reported to effectively control wheat rust on detached leaves. The ethanolic extracts of the plant exhibited antifungal activity against rice pathogens *Pyricularia oryzae*, *Rhizocotonia solani*, *Fusarium moniliforme,* and *Curvularia lunata*. The leaf extracts exhibited antifeedeant and insecticidal activities. Soil application of seed and leaf powder reduced the number of primary galls *Meloidogyne incognita*. The species is a common weed of various crop. The fungal pathogen *Alternaria alternata* was found capable of killing one week old seedlings when it was sprayed on them.

**8. *Grevillea robusta* R. Br. Proteaceae**

A native of Australia it is evergreen tree with a long conical crown reported to attain a height of about 150 ft in its native habitat but growing to a moderate size in India. Leaves alternate, 6 ï 12 in. long, fern like, deeply pinnate, dark green above silvery below, flowers orange ï coloured, solitary or several together, borne in racemes, 3 -4 in. long on short leafless branches of old wood, fruit an oblique

Coriaceous follicle, containing 1 or 2 seeds. The tree is grown nearly throughout India at elevations of 2000 ï 6000 ft. and produces itself naturally from seeds. It is resistant to drought and to frost, but is rather brittle and should not be grown in situations exposed to high winds. The tree with its fern ï like foliage is ornamental when young, but becomes ragged and unsightly as it ages. It is cultivated as a shade tree in tea and coffee plantations and is commonly planted in gardens and avenues. The tree comes to bloom from March to May.

The leaves of the tree are valued as green manure in coffee and tea plantations. The tree is not generally lopped, but the copious litter of dropping leaves is forked or ploughed in.

Analysis of leaves gave following moisture, 50.9; organic matter 45.9; ash 3.2, nitrogen 0.53; calcium (CaO), 1.30; potassium ($K_2O$) 0.42; and phosphorus ($P_2O_5$) 0.06%. Leaves contain quebrachitol (0.4%) and arbutin. The former a polylcohol) which has properties similar to those of mannitol, sorbitol and inositol, can be used in preparation of lacquers.

**9. *Eclipta alba* (L.) Hassk. Asteraceae**

The plant exhibit anti inflammatory activity. Its decoction constituents one of the ingredients of ayurvedic preparation RENONE which is highly effective in rheumatoid arthritis. The herb contains wedelolactone and demethyl wedelolactone possessing potent antihepatotoxic properties. The plant is an active ingredient of many herbal formulations prescribed for liver ailments. It is one of the ingredients of the ayurvedic formulations HEPATOGARD which is reported to reverse the biochemical and histopathological changes in the liver induced by paracetamol. The herb is also one of the ingredients of a compound formulation STIMIMULIVE which show significant protection against hepatotoxicity of antitubercular drugs. Besides hepatoprotection significant improvement in the appetite and body weight in patience of tuberculosis has also been observed. Ethanolic extracts of aerial parts of the plant neutralized the lethal activity of the venom of S. American rattle snake *in vitro* and *in vivo* in rats. Three compounds isolated from the plants i.e. wedalolactone, sistoserol and stigmasterol were probable responsible for the effect. Alcoholic extract of the hern has antiviral activity against Ranikhet disease. Plant juice cures skin infection. Bringhraj oil obtained from the plant is applied to scalp before night time in imsomnia. The herb is also used as an ingredient of tooth paste and shampoos. It also contains an alkolid ecliptin. The plant is a good source of thiophene derivatives which are active against nematodes. The petroleum ether extract of aerial parts contain terhienyl aldehyde, ecliptal $C_{13}$ $H_8S_3O$, mp 146 $^oC$). Besides stigmasterol

and ß- sitoserol. The aerial parts also contain 2 ï an ï geloyloxy methylene ï 5ôï (but-3 ï en-1-ynyl)- dithyopene. The roots are very rich in thiopene acetylenes. They contain the di ï thiophene derivatives.

*E. alba* is reported to be effective in the treatment of peptic ulcers. Immunoactive property has also been observed against surface antigen of hepatitis B virus. Methanolic extract of the whole plant from Japan showed the presence of 6 ï oleananetriterpene glycosides, eclalbasaponin. They were characterized as echinocystic acids, glycosides. The plant is used as forage for cattles. The shoots are rich in crude protein (10 %) and can be used as livestock feed.

**10. *Heterophragma adenophyllum* Seen. Bignoniaceae**

The plant is used in skin diseases. It showed antiviral and antihypertensive activities. The leaves contain ursolic acid, olenoic acid and ß-amyrine. The anticancer benzoquinones, lepachol, lepachon, dehydro Ŭï lepachone, dehydro ï iso- Ŭlepachone, dehydrotectol and tectol have been reported from the root and heartwood.

**11. *Pluchea lanceolata* (DC.) Oliv. & Hiern Asteraceae**

The plant is a stout herb growing 0.33-2 meters in height. The stem is grooved, rough and very hairy. The leaves are elliptical, large, 3-6 cm long and 2-3 cm broad, and have long petioles. The fruits, slender acheneôs, 0.4 cm long, bearded with 0.75 cm long pappus hairs. The flowers are yellow, many in heads, 0.5-1 cm in diameter. The fresh root is brown and becomes grayish on drying. The fresh roots resemble in aroma of camphor.

The plant is used for the inflammations and bronchitis, psoriasis, cough and piles. It is also used as antipyretic, analgesic, laxative and nerve tonic. The decoction of plant is used to prevent the swellings of joint in arthritis, rheumatism and neurological diseases. The roots are antipyretic, bitter, laxative and thermogenic and are used for allaying the pain caused by the sting of scorpions. Plant extract is used as a cooling agent in summer. The leaves are aperient and used as a laxative, analgesic and antipyretic.

It is the highly praised panacea for cough, hiccup and bronchial asthma, to reduce the excessive body fats. Puskaramula restrains the itching sensation and oozing in the skin diseases and thus facilitates the wound healing. It is pacifying to the brain and helps in strengthening it in mental debility.

On extraction of the plant with hexane and isolation, the compounds obtained are dihydroisoalan tolactone, isoalantolactone and alantolactone. From the roots, sitosterol, octadecanoic acid and D-mannitol have been isolated also. Two biologically active new sesquiterpene lactones., inunal and isoalloalantolactone are isolated. Alantolactone, isoalantolactone and dihydroisoa ï lantolactone isolated from roots. A germacranolide ï inunolide ï from root oil. Also alloalantolactone isolated from roots and characterized. Two new sesquiterpene lactones inunal and isolloalantolactone ï isolated and characterized.

**12. *Terminalia arjuna* (Roxb.) Wight & Arn. Combretaceae**

A large evergreen tree, with a spreading crown and drooping branches, common in most parts of India and also planted in many parts for shade and ornamental value. Stems rarely long or straight, generally buttressed and often fluted, bark very thick, grey or pinkish green, smooth, exfoliating in large, thin, irregular sheets; leaves sub ï opposite, oblong or elliptic,

coriaceous, usually10 ï 15 cm. long occasionally 25 cm, chordate short, acute or obtuse at the apex; flowers in panicled spikes; fruits2.5 ï 5.0 cm long, nearly glabrous, ovoid or oblong, with 5 -7 hard, winged angles.

The tree is common throughout the greater part of the Indian Peninsula along rivers, streams, ravines and dry water courses.

The powdered bark seemed to give relief in symptomic complaint of hypertension; it apparently had a diuretic and a general tonic effect in cases of the liver.

Leaves are feed to tasar silkworms. They contain crude protein, 10.10, crude fibre, 7.78, reducing sugars, 4.30 total sugars, 5.75; starch, 11.09, minerals 7.09%. The juice of fresh leaves is used in earache.

**13. *Tridax procumbens* Linn. Asteraceae**

The leaves are cooked as vegetables: they are also eaten by cattles. Analysis of the leaf gave (dry basis) crude protein 26.3, crude fibre 17.0, ether extract 1.8, sol. Carbohydrate 39.0, ash 15.9, $K_2O$ 8.4, CaO 4.6, $P_2O_5$ 1.1, and MgO 1.7 %. Fumaric acid is present in the leaves. The presence of ß- sitoserol and tannin has also been reported in the plant. The leaves are reported to be employed in bronchial catarrh, dysentery and diarrhoea and for restoring hair. The leaf juice possesses antiseptic, insecticidal and parasiticidal properties: It is used to check hemorrhage from cuts, bruises, and wounds. Petroleum ether extracts of the floral heads is toxic to webbing cloth moth and larvae of black carpet beetle. Flowe contains luteolin, glucoluteolin, quercetin and iso quercetin. The pollen may cause allergy in some people. The herb has become pest in many parts.

**14. *Vogelia indica* Gibs. ex Wight** **Plumbaginaceae**

Shrub or herbs. Stems striate or reduced to a caudex. Leaves simple, alternate or basal, sessile or petiolate but petiole usually indistinct from blade; stipules absent; leaf blade entire or rarely pinnately lobed, with chalk glands on both surfaces. Inflorescences terminal or axillary , unbranched or branched, spicate , spicate-racemose, subcapitate , capitate, or paniculate, all composed of 1--10 helicoid cymes;, 1--5-flowered; bracts. Flowers bisexual, actinomorphic, sessile or very shortly pedicellate. Calyx persistent, hypogynous, tubular to funnelform, 5-ribbed, 5-lobed. Corolla hypogynous, petals connate but sometimes only at base, lobes or segments 5 and twisted. Stamens opposite corolla lobes, hypogynous or inserted at corolla base; anthers 2-locular, dehiscing longitudinally. Pistil 1. Ovary superior, 1-locular. Styles 5, free or connate. Stigmas 5. Ovule 1, pendulous from a basal funicle. Capsules usually enclosed within calyx. Seeds 1 per capsule; embryo straight, surrounded by thin starchy endosperm.

**15. *Withania somnifera* (L.) Dunal** **Solanaceae**

An erect, evergreen, tomemtose shrub, 30 ï 150 cm high, found throughout the drier parts of India in waste places and on bunds, also cultivated to a limited extent for the medicinal roots. Ashwagandha is mentioned as an important drug in the ancient ayurvedic literature. It consisted of the roots which were prescribed for hiccups, female disorders, cough, rheumatism and drops. Aswangandha is useful in the treatment of inflammatory conditions, ulcers and scabies when applied locally.

The leaves of the plant from different habitats contain different withanoides ï a group of $C_{28}$ steroids characterized by a 6 ï membered lactone ring in the 9 ï carbon atom side chain, a

differing I substitution patterns. Withaferin-A is the most important of the withanolides isolated so far, to which the curative properties of the leaves are attributed.

**1.1 List of Plants used for biocontrol study**

| Sr. no. | Leaf extract of Plants | Family | Active components |
|---|---|---|---|
| 1. | *Adathoda vasica* Nees | Acanthaceae | Vasicine, Vasicinone |
| 2. | *Alangium salvifolium* (Linn.f.) Wang. | Alangiaceae | Alangimarckine, ankorine, campesterol, episterol, alangidiol and isoalangidiol. |
| 3. | *Alysicarpus vaginalis* (L.) DC | Fabaceae | |
| 4. | *Butea monosperma* (Lam.) Taub | Fabaceae | |
| 5. | *Calotropis procera* (Aiton)Wt. | Asclepediaceae | Asclepin and mudarin |
| 6. | *Cymbopogon martini* | Poaceae | Citronellol, geraniol, neral |
| 7. | *Cynodon dactylon* Rich. ex Pers. | Poaceae | |
| 8. | *Dalbergia sissoo* Roxb. | Fabaceae | |
| 9. | *Datura metel* L. | Solanaceae | Tropane alkaloids |
| 10. | *Eclipta alba* (L.) Hassk | Asteraceae | Demethylwedelolactone, polypeptides, polyacetylenes, thiophene-derivatives, triterpenes and flavonoids |
| 11. | *Grevillea robusta* A. Cunn | Proteaceae | |
| 12. | *Heterophragma adenophyllum* (Wall. ex G. Don) Seem. ex Benth. & Hook. f. | Bignoniaceae | Ursolic acid, oleanic acid, ɓ - amyrin |
| 13. | *Pluchea lanceolata* (DC.) Oliv. & Hiern | Asteraceae | Quercetin, Isohamnetin |
| 14. | *Polyalthia longifolia* Sonn. | Annonaceae | |
| 15. | *Terminalia arjuna* (Roxb.) Wight & Arn. | Combretceae | |

| | | | |
|---|---|---|---|
| 16. | *Tridax procumbens* L. | Astereaceae | ɓ-sitosterol-3-O-ɓ-D-xylopyranoside |
| 17. | *Vogelia indica* ex.Wt. | Plumbaginaceae | |
| 18. | *Withania somnifera* (L.) Dunal | Solanaceae | Withaferin-A, withanolides |

## Objectives

- Survey of different forest areas to find out the occurrence of leaf spot diseases in selected tree species.
- Isolation of fungi from the diseases leaves and purification of the cultures.
- Pathogenicity test of isolated fungi.
- To study the Phyllosphere mycoflora of *Madhuca indica* and *Diospyros melanoxylon.*
- To study the cultural characters of certain leaf spot fungi.

- To control and noteworthy diseases by application of botanical pesticides.

## METHODOLOGY

### 1. SURVEY

A survey was undertaken in Jambughoda WLS, Ratanmahal WLS, Shoolpaneshwar WLS, Pavagadh forest area during January 2007 ï December 2009.

Diseases symptoms as leaf spot in forest trees was observed and samples were collected in clean polythene bags.

### 2. Isolation of Fungi

The infected leaves of plants were collected and washed thoroughly with running water. The infected portions were surface sterilized with 0.1% mercuric chloride for 1 min and after this treatment the tissue sections are transferred to dishes containing sterile distill water and wash

thoroughly to free them from the chemicals. In some cases the central core of infected plant tissue, cut with a sterilized pair of scissors or a knife sterilized by momentary dipping in 90 percent alcohol and then flaming for a few seconds. The fungi were cultured and maintained on Potato Dextrose Agar Medium. PDA medium amended with 250εg Streptomycin sulphate per ml.

The organisms thus isolated from the diseased tissues was then be purified by single tip or single colony/ spore method. These plates were incubated at 25±1°C for 7days. Once fungal colonies were formed in the agar plates, each colony was transferred to a new agar slant to obtain a pure culture.

**3. Identification of fungus based on morphological characters**

During field survey the Materials were collected in clean polythene bags from different locations and brought to the laboratory. The identification of cultures was done based on morphological characters of conidia/ spore and final confirmation was done from IARI, New Delhi and Agharkar Research Institute, Pune.

**4. Diseased symptoms**

Survey was conducted in and nearby forests of Vadodara to find out the diseased symptoms in leaves. The progress of Foliicolous spots was monitored and isolation was done from infect leaves. The artificial inoculation in healthy leaves confirmed the Kochôs postulates. The physiological studies were undertaken to know the cultural behavior of some of the pathogens and Ecofriendly management was tried.

## 5. Pathogenicity test

Healthy leaves of plants were inoculated with fungal culture *in vivo* condition after surface sterilizing them with ethyl alcohol and then covering them with plastic bags tied along with wet adsorbent cotton tied near the leaf base. If the plants are susceptible to the pathogen, then the symptoms appeared after a few days. Plants were monitored for the development of disease symptoms and pathogen was reisolated from the leaf after seven days to confirm the pathogenicity according to Kochôs Postulates.

## 6. Bio – control of foliicolous fungi by (Poisoned Food Technique)

The healthy leaves were collected and washed well and dried in oven at 60 $^{0}$C for 48h. The dried leaves were powdered and stored in plastic bags. Twenty grams of leaf powder was extracted in a Soxhlet extractor with 200 ml methanol for 8 hours. The extract was concentrated then the residue was treated with 20 % of methanol. It was added to dry residue and water soluble compounds were filtered out.

Effect of Methanolic and aqueous leaf extracts were obtained by Soxhlet Extraction method of 24 plants. It was tested on 4 different foliicolous fungi. The leaf extracts were mixed with appropriate volume of medium (PDA) to obtain concentrations ranging from 2.0 to 10.0% in the final volume of 100 ml of medium. This 100 ml medium was dispensed into 90 mm petri plates with triplicates. **(**Nene and Thapliyal, 1979)

Fungal isolates of selected fungi were placed in the centre of each plate. Control sets were also prepared without plant extract. The plates were incubated at 25 °C ±2°C and

growth of colony was measured after 7 days of inoculation. The radial growth of mycelium was measured at two points along the diameter of the plate and the mean of these two readings was taken as the diameter of the colony. The growth of the colony in control sets was compared with that of various treatments and the difference was converted into percent inhibition by following formula

$$\text{Percent inhibition} = \frac{\text{Diameter of control set - diameter of treated set}}{\text{Diameter of control set}} \times 100$$

**CULTURAL STUDIES**

Single spore cultures of the organisms were prepared by subculturing. The cultures were made bacteria free by the method described by Brown (1924). They were maintained on 2%

agar slants of modified Asthana and Hawkersô medium óAô (which was further selected for detailed nutritional studies with the constituents

D ï glucose - 10 g

$KNO_3$ - 3.5 g

$KH_2 PO_4$ - 1.75 g

$MgSO_4. 7H_2O$ - 0.75 g

Distilled water ï 1000ml

Borosil glassware and pure reagents supplied by Qualigens and SRL were used throughout the present investigation.

For cultural studies Petri dishes of (90mm internal diameter, containing 20 ml agar) were inoculated with a piece of mycelium at the edge kept in diffused daylight at room temperature (20-25$^oC$) and examined at 7 days intervals. Separate slides were prepared for fungi and mounted in lactic acid with cotton blue.

**LACTOPHENOL – COTTON BLUE STAIN**

Phenol : 20ml

Lactic acid : 20 ml

Glycerol : 40 ml

The above mentioned chemicals were mixed and heated at 70 $^0C$ and then 5 ml of 1 % aqueous cotton blue was added.

For Physiological studies 25 ml of the liquid basal medium was taken in 150 ml Erlenmeyer conical flasks. Unless other wise stated the culture media were autoclaved at 15 lbs psi for 30 min whenever the medium contained complex substances liable to decomposition or denaturilazation, fractional sterilization was done which involved exposure to steam for 30

min on three successive days. With the help of agar disc method (Garrett 1936) 10 days old culture were used for inoculating the flasks containing different media. Inoculated flasks where incubated at 25 + 2 $^{0}$C for 15 days. At the end of incubation period change in pH of the medium and degree of sporulation of the organisms was recorded.

In order to access the growth of organisms their fungal mats were harvested at the end of incubation period on previously dried and weighed Whatman filter paper no: 1. The filter paper were again dried in a electric oven at 60 $^{o}$ C for 72 h and then they were cooled in a dessicator atleast for 48h and finally weighed. The difference between the final an initial weight of the filter paper indicated the dry weight of the fungal mats. Dry weights of the mycelial mat and degree of sporulation were considered as measure of response of the organisms to different treatment. Each set of treatment run in triplicates and only the average dry weight was always taken as a standard value for comparison of growth. The dry weight results were statistically analyzed and standard error (S.E) was calculated by the formula:

$$S.E. = \sqrt{\frac{\text{Mean square of the error}}{\text{No of replicates}}}$$

And Critical difference (C.D.) by the formula:

$$C.D. = S.E. \times t \times \sqrt{2}$$ where t represented probability at 5% level

Dry weights of mycelial mats were graded into Good, moderate and poor. The general mean (G.M.) of the experiment + C.D. at 5 % level has been considered moderate. The dry weights higher or lower than the moderate have been designated as good or poor respectively.

Inoculated flasks were incubated at 25± 2$^{o}$C for 5, 10 and 15 days. At the end of incubation period, change in pH of the medium was determined. In order to assess the growth of organisms, their fungal mats were harvested at the end of incubation period on previously dried and weighed Whatman filter paper No. 1. the filter papers were again dried in an electric oven at 60$^{o}$C for 48h and then they were cooled in a desiccators at least for 2 h and finally weighed to calculate the growth of foliicolous fungi by the following formula

$$\% \text{ Growth} = \frac{\text{Initial Dry weight ï final dry weight}}{\text{Initial dry weight}} \times 100$$

The difference between the initial and final weights of the filter paper indicated the dry weight of fungal mat. Dry weights of the mycelia mat were considered as measure of response of the organisms to different treatments. Each set of the treatment was run in triplicates and only the average dry weight was always taken as standard value for comparison of growth. The dry weight results were statistically analyzed by using the MS office Excel software and the significant values were taken for study.

Dry weight of mycelial mat was graded into good, moderated and poor. The general mean (G.M) of the experiment ± SD with Annova has been considered moderate. The dry weights higher or lower than the moderate have been designated as good or poor respectively.

**(A) Selection of suitable culture media**

The following culture media were employed:

**(a) Natural Medium**

1. **Host – decoction medium**: 200g of the host tissue was cut into small pieces and boiled for an hour in a steamer. It was then filtered through a cloth and total volume was raised to 1000 ml.

**(b) Semi Synthetic Media**

**2**. **Potato Dextrose medium:** 200g of potato was peeled and sliced into small pieces. It was boiled in an autoclave for 40 min in 500 ml of distilled water and then filtered through a cloth. Twenty grams of dextrose was added and total volume was raised to 1000ml.

**(c) Synthetic Media**

**3. Asthana and Hawker's Medium 'A'**

| | |
|---|---|
| D ï glucose | - 5 g |
| $KNO_3$ | ï 3.5g |
| $KH_2PO_4$ | ï 1.75g |
| Mg $SO_4$ $7H_2O$ | ï 0.75g |
| Distilled water | ï 1000ml |

**4. Modified Asthana and Hawker's Medium 'A'**

| | |
|---|---|
| D ï glucose | -10g |
| $KNO_3$ | ï 3.5g |
| $KH_2PO_4$ | ï 1.75g |
| Mg $SO_4$ $7H_2O$ | ï 0.75g |
| Distilled water | ï 1000ml |

**5. Czapek Dox's Medium**

| | |
|---|---|
| Sucrose | ï 30g |
| Na $NO_3$ | ï 2g |
| $K_2HPO_4$ | ï 1g |
| Mg $SO_4$ $7H_2O$ | ï 0.5g |
| KCl | ï 0.5g |
| $FeSO_4$ $7H_2O$ | ï 0.01g |
| Distilled water | ï 1000ml |

**6. Coon's Medium**

| | |
|---|---|
| Maltose | - 3.5 g |
| L ï aspargine | - 0.25 g |
| $KH_2PO_4$ | - 1.25 g |
| $MgSO_4.7H_2O$ | - 0.50 g |
| Distilled water | - 1000 ml |

**7. Richard's medium**

| | |
|---|---|
| Sucrose | - 50 g |
| $KNO_3$ | - 10 g |
| $KH_2PO_4$ | - 5 g |
| $MgSO_4.7H_2O$ | - 2.5 g |
| $FeCl_3$ | - 0.02 g |
| Distilled water | - 1000 ml |

**8. Czapek's Medium**

| | |
|---|---|
| Sucrose | - 30 g |
| $NaNO_3$ | - 2 g |
| $KH_2PO_4$ | - 1 g |
| KCl | - 0.5 g |
| $FeSO_4$ .7 $H_2O$ | - 0.01 g |
| $MgSO_4.7H_2O$ | - 2.5 g |
| Distilled water | - 1000 ml |

**9. Elliot's Medium**

| | |
|---|---|
| D-glucose | - 5 g |
| L-aspargine | - 1.0 g |
| $KH_2PO_4$ | - 1.36 g |
| $Na_2$ $CO_3$ | - 1.06 g |
| $MgSO_4.7H_2O$ | - 0.50 g |
| Distilled water | - 1000 ml |

**(B) Effect of suitable Hydrogen ion concentration**

To select suitable hydrogen ion concentrations for better growth of foliicolous fungi, the following initial hydrogen ion concentrations of 2, 4, 6, 8 and 10 were adjusted. The pH of

the Modified Asthana and Hawkerôs medium was adjusted by using 1N HCl or 1N NaOH solutions. After adjustment of suitable hydrogen ion concentration 25 ml of the medium was transferred to 150 ml Conical flasks (Borosil grade). These flasks were autoclaved and inoculated with previously grown culture of decay fungi. After inoculation the flasks were incubated for 15 days. After completion of incubation period, each test fungus was filtered by using Whatman filter paper no 1. The filtrate was used to determine final pH. The filter papers were dried in oven for 48h at 60$^{o}$C and weighed to calculate the growth of foliicolous fungi. The pH of the filtrate was determined with the help of pH meter.

**(C) Selection of suitable temperature**

To select a suitable temperature for the growth of foliicolous fungi, the temperatures like 0,5,10,15,20,25,30,35 and 40$^{o}$C were used. The same procedure was followed as above mentioned in suitable growth and hydrogen ion concentration tests except that flasks were incubated at different temperatures in BOD incubator.

**(D) Effect of carbon sources on foliicolous fungi**

To study the effect of carbon sources on growth of foliicolous fungi amount of individual substance in the basal medium was calculated, and a quantity equivalent to that was singly substituted in the basal medium by replacing the original corresponding substance. The amount of polysaccharides was similar to the amount of glucose present in the basal medium. The medium devoid of glucose was served as control for carbon. Ten different carbon sources *i.e*. Sucrose, Raffinose, D - Arabinose, L ï Arabinose, Xylose, Fructose, Maltose, Mannitol, D- Galactose, Starch, Rhamnose were studied. After completion of incubation period the fungi were filtered with Whatman filter paper No.1 and dried for 48 h at 60 ºC in

oven. The dried filter papers were weighed to calculate the growth of each test fungi. The filtrate was used to determine the final pH.

**(E) Effect of Nitrogen on growth of foliicolous fungi**

The basal medium óAô was used for studying the effect of different nitrogen sources on the mycelial growth and sporulation of the - fungi.

**Modified Asthana and Hawker's medium**

| | |
|---|---|
| D ï glucose | -10.0g |
| $KNO_3$ | ï 3.5g |
| $KH_2PO_4$ | ï 1.75g |
| Mg $SO_4$ $7H_2O$ | ï 0.75g |

The quantity of various nitrogen sources was adjusted by replacing $KNO_3$ so as to give the same amount of nitrogen as furnished by 3.5g $KNO_3$ in the basal medium.

The basal medium supplemented with eight nitrogen sources was used for growth of test fungi. The basal medium supplemented with Potassium nitrate, Sodium nitrate, Ammonium acetate, Ammonium oxalate, Ammonium sulphate, Ammonium nitrate, Calcium nitrate, Peptone were used for growth of these fungi, which acted as nitrogen sources. Flasks containing 25 ml of basal medium were autoclaved at 121ºC temperature for 20 min, inoculated with test fungi and incubated for 15days. After completion of incubation period, each test fungus was filtered by using Whatman filter paper no 1. The filtrate was used to determine final pH. The filter papers were dried in oven at 60 $^0$C for 48 h and then kept in desiccator before weighing to calculate the growth of foliicolous fungi.

**(F) Utilization of the sugars by foliicolous fungi**

Utilization of different mono di and trisaccharides were studied. Paper chromatography was used to find out preferential utilization of Sucrose, Raffinose and Rhamnose for this purpose. The quantity of various sugars was similar to that used in experiment dealing with carbon requirements. Dry weights of mycelial mat and pH of the medium were recorded after incubation period of 5, 10 and 15 days and filtrates were analyzed daily to detect the presence of various sugars during their utilization. Drops of known volume (0.05 ml of sample was taken with the help of 1/100 ml pipette every second day and were placed on the chromatograms by micropipette at a position located for this purpose. The running solvent was n-butanol-acetic acid- water (4:1:5) v/v). In order to separate glucose and galactose the running solvent was n-butanol (100ml) aniline (0.91 ml), pthallic acid (1.6g), distilled water (10ml) was used as spraying reagent for the detection of sugars. Chromatograms were developed after drying at room temperature and by heating in an electric oven at $100^{o}C$ for 90 sec. The Rf values were calculated by the following formula:

$$Rf = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

**(G) Effect of vitamins by foliicolous fungi**

Vitamins are organic molecules required in small amount and not used as a source of either energy or structure materials of protoplasm. Fungi like other organisms require minute amounts of these specific organic compounds for their normal development. It is believed

that they are needed for metabolic reactions and functions as coenzymes or constituent parts of coenzymes.

Fungi in their ability to synthesize their vitamin requirements occupy a position in between the totally independent higher plants and completely dependent animals. Schopfer (1943) has distinguished two groups of fungi, i.e. (a) auxoautotrophs (b) auxoheterotrophs. The auxoautotrophs fungi are capable to synthesize all their vitamin requirements and the auxoheterotrohs include those fungi either completely or partially lack biosynthetic capacity for vitamin production. It has been suggested that different degree of vitamin deficiency in fungi operate through a complicated synthetic mechanism (Lilly and Barnett, 1948).

Our current knowledge of vitamin requirements of fungi indicates that they generally need only water soluble vitamins (Bilgrami and Verma, 1978). Although some of the organisms are reported to attain the same mycelial output even on a vitamin free medium yet usually an exogenous supply of vitamins accelerates the rate of growth of fungi. In the present investigation an attempt has been made to study the effect of some vitamins on the growth and sporulation of the four pathogens under study.

Following four concentrations (in µg/l) of the vitamins were used in order to select the most suitable concentration for growth and sporulation:

| **Vitamins** | **conc. of vitamins (µg/l)** | | | |
|---|---|---|---|---|
| Thiamine (vit. $B_1$) | 50 | 100 | 150 | 200 |
| Pyridoxine(vit. $B_6$) | 50 | 100 | 150 | 200 |

| | | | | |
|---|---|---|---|---|
| Riboflavin (vit. $B_2$) | 25 | 50 | 75 | 100 |
| Ascorbic acid | 25 | 50 | 75 | 100 |
| Nicotinic acid | 25 | 50 | 75 | 100 |
| Folic acid | 10 | 20 | 30 | 40 |
| Biotin | 5 | 10 | 15 | 20 |
| Cyanocoblamin (vit.$B_{12}$) | 10 | 20 | 30 | 40 |

## 1. SURVEY

### Jambughoda Wildlife Sanctuary

**Jambughoda WLS** is located in Halol and Jambughoda talukas of Panchmahals district and Sankheda taluka of Vadodara district having extent of 130.38 sq. km. Terrain of the area is undulating to hilly. As per Champion & Seth, 1968), the forest cover is constituted by dry teak forest (5A/C1b), southern dry mixed deciduous forest (5A/C3), *Butea* forest (5/E5), southern dry tropical riverine forest (5/1S1), dry deciduous scrub (5/DS1) and secondary dry deciduous forest (5/2S1). Teak forest occupies major part of the Sanctuary. *Tectona grandis* (Sag), *Terminalia crenulata* (Sadad),. *Dalbergia latifolia* (Sisham), *Acacia catechu* (Khair), *Diospyros melanoxylon* (Tendu/Timru), *Madhuca indica* (Mahuda), *Anogeissus latifolia* (Dhav), *Lagerstroemea parviflora*, *Aegle marmelos* (Bili), *Butea monosperma* (Palas/Khakharo), *Mitragayna parviflora* (Kalam), *Zizyphus* sp. (Bor), *Lannea coromandelica* (Modad) and *Wrightia tinctoria* (Dudhalo) are important tree species in the area.

**Ratanmahal WLS (Plate – I, fig. A,B)**

A survey was undertaken in Ratanmahal Wildlife Sanctuary (RWLS) between November 2007 ï to 2009, December and specimens were collected. RWLS is an area of 55.65 $km^2$ consisting of dry deciduous forest.

The total existing sanctuary area lies between the river Panam and Orsang. The 11 villages of Ratanmahal forest are situated at the southernmost part of Limkheda taluka of Dahod district of Gujarat state. Ratanmahal lies nearly 35 km south-east from Devgadh Baria, the head quarter of Baria taluka. It is situated between 74º 37ô to 74º 11ô E Longitude and between 22º 32ô to 22º 35ôN Lat. The forest of the area was part of Kanjeta state. It is bounded by Jhabua district of Madhya Pradesh on its south-eastern side and Devgadh Baria on north-western side. The climate is sub-tropical arid, which turns damp and humid during monsoon. Rainfall ranges between 957 to 2101mm.

Teak, *Anoegessus latifolia, Terminalia* spp. *Diospyros melanoxylon, Emblica officinalis, Buchnania lanzan, Butea monosperma,* etc are important trees of the area. Total of 543 species of plants are recorded in the sanctuary.

**Shoolpaneshwar Wild Life Sanctuary (WLS) (Plate – I, Fig. B,C)**

This sanctuary has vast, undulating terrain, ever-pervading greenery, tall inspiring canopy, deep awesome valleys, soberly silent rocks, gentle youthful streams, majestic waterfalls, breathtaking landscapes, culminating at the congregation of Vindhyan-Satpura hill ranges. The sanctuary was first created in 1982 over an area of 150.87 sq. km. As "Dumkhal Sanctuary"- an important home for sloth bears. Subsequently, in 1987 and 1989, the area of

the sanctuary was enlarged to 607.71sq. Km. and it was renamed as "**Shoolpaneshwar Sanctuary".** The sanctuary derives its name from a historic temple of Lord Shiva, which once existed in this region on the banks of river Narmada. The temple is now submerged due to the Sardar Sarovar Reservoir. However, a new Shoolpaneshwar temple has since been built near Rajpipla. The word "Shoolpaneshwar" refers to Lord Shiva portrayed as having "Shool" or "Trishul" in his hand i.e. `Paniô

The forest area rated as one of the best and thickest in the state, is spread over an area, which includes a major watershed feeding two major reservoirs with the Rajpipla hills as backdrop. The flora of the ecosystem represents semi-evergreen to moist deciduous forest. There are more than 543 species of flowering plants like *Diopsyros melanoxylon, Emblica officanalis, Acacia catechu, Terminalia* spp. There are vast patches of bamboo plantations often referred to as bamboo-brakes.

2. **List of fungi isolated from leaves of five plants**

| | |
|---|---|
| **1) *Tectona grandis*** | - *Fusarium pallidoroseum* |
| | - *Thielevia subthermophila* |
| | - *Alternaria alternata* |
| | - *Phomopsis tectonae* |
| | - *Lasiodiplodia theobromae* |
| ***2) Terminalia arjuna*** | - *Pestalotiopsis disseminata* |
| | - *Gloeosporium gleoesporoides* |
| ***3) Bambusa arundinacea*** | - *Drechslera rostrata* |

- *Melanconiopsis microspora*

- *Curvularia prasadi*

- *Colletotrichium capsici*

- *Pestalotiopsis maculans*

***4) Madhuca indica*** - *Fusarium roseum*

*5)* ***Diospyros melanoxylon*** - *Gliocladium virens*

- *Cladosporium cladosporoides*

**3. Identification of fungi based on morphological characters**

Members of the *Botryosphaeriaceae* (*Botryosphaeriales*, *Dothideomycetes*, *Ascomycota*) are cosmopolitan and occur on a wide range of monocotyledonous, dicotyledonous and gymnosperm hosts (von Arx & Müller 1954, Barr 1987). Based on 28S rDNA sequence data Crous et al. (2006) showed that *Botryosphaeria* is polyphyletic and they divided it into several genera distinguishable by conidial morphology and phylogenetic data.

*Botryosphaeria* was thus restricted to species with *Fusicoccum* anamorphs. However, the clade containing *Diplodia/Lasiodiplodia* could not be fully resolved. In a multigene genealogy Phillips et al. (2008) resolved and separated this clade into six genera including *Diplodia*, *Lasiodiplodia*, *Neodeightonia*, *Barriopsis*, *Phaeobotryon* and *Phaeobotryosphaeria*.

*Lasiodiplodia* species are common, especially in tropical and subtropical regions where they cause a variety of diseases (Punithalingam 1980). According to Sutton (1980) the genus is based on *Lasiodiplodia theobromae*. The main features that distinguish this genus from other closely related genera are the presence of pycnidial paraphyses and longitudinal

striations on mature conidia. Thus far 20 species have been described and they are differentiated on the basis of conidial and paraphyses morphology.

***Lasiodiplodia theobromae* (Pat.) Griffon and Maubl**

Culture of *L. theobromae* was isolated from naturally infected leaf of *Tectona grandis* from Arboretum of the M.S.University of Baroda campus.

*Lasiodiplodia theobromae* Pat. is a well known patheogen causing both field and storage diseases of different crops, fruits and plantation trees, (Khurana and Singh, 1972; Talukdar 1974; Singh *et al.,* 1977 and Iilag and Marfi, 1977). It is an important pathogen of mango fruit, soft rot of papaya, guava, litchi, stem end rot of mango, die back of lemon plants.

Mycelium immersed or superficial, branched, septate, dark choclate brown conidomata, pycinidia euatromatic, immersed or superficial, separate or aggregated and confluent, globose, carbonous, dark brown, uni ï or multilocular, wall of dark brown, thick walled texture angularis, paler and thinner towards the conidiogenous region, often with true; conidiospore absent: condiogeneous cells holoblastic, determinate, discrete, cylindrical, hyaline, smooth, with no percurrent or sympodial proliferation, formed from cells lining the inner pycnidial walls; condia acrogenous, hyaline when young later becoming dark brown, medianly euseptate, thick walled, ellipsoid, base truncate, with longitudinal striations from apex to base: paryphyses hyaline, cylindrical, septate.

***Phomopsis tectonae***

The fungus was isolated from severely infected leaf of *Tectona grandis* from Kadipani area, Chota Udepur near Vadodara. The spot were circular, grayish brown and of varied size. The fungal species has been reported earlier on Teak plant by (Tiwari *et al.,* 1981).

*Phomopsis* Sacc. Pycnidia dark, ostiolate, immersed erumpent, nearly globose; conidiophores simple, conidia hyaline, 1 ï celled, of two types, ovoid to fusoid conidia; and filiform, curved or bent stylopores; parasitic, causing spots on various plant parts.

The genus *Pestalotiopsis* Steyart is a hetergenous group of Coelomycetous fungi consisting of 205 described species that are differentiated primarily on conidial characteristics such as size, septation, pigmentation and presence or absence of appendages. *Pestalotiopsis* is characterized by spores having mostly four ï euseptate and pigmented median cells with two to four apical appendages arising as tubular extensions from the apical cell and centric basal appendage (Jeewon *et al.,* 2002).

Pirone (1978) reported that 12 different species of *Pestalotiopsis* caused leaf spots, needle blight, tip blight and gray blight on a range of hardy ornamentals including *Camelia (P. guepinii* (Desm.) Stey.) Gardenia (*P. langloisii* Guba). Yew (*Taxus*) (*P. funera* (Desm) Stey.) and *Rhododendron* (*P. macrotricha* Kleb)

***Pestalotiopsis disseminata* (Thüm.) Steyaert, (1949)**

*Pestalotiopsis* species are anmorphic members of the family Amphisphariaceae (Kang *et al.,* 1999) and they are usually found in tropical and subtropical plants theroughout the world (Jeewon *et al.,* 2004; Tejesvi *et al.,* 2007, 2008). *Pestalotiopsis* species have gained much attention and importance in recent years as they produce many important secondary metabolites (Strobel, 2002; Harper *et al.,* 2003; Kumar *et al.,* 2004). The symptoms of the

disease are the appearance of small, oval and discolour lesions which are irregularly scattered on the leaves. The brown or grey spots develop irregularly.

Colonies compact or effuse, buff, grayish brown, blackish brown or black; mycelium immersed, branch, septate, hyaline to pale brown: conidiomata acervular septate or confluent, formed of brown or thin walled or texture angularis, dehiscence irregular, conidiophores hyaline, branched and septate at the base and above, cylindrical or ligniform, formed from the upper cells or the pesudo parenchyma: conidiogenous cells holoblastic, annellidic, indeterminant, integrated, cylindrical, hyaline, smooth, with several percurrent proliferation: condia fusiform, straight or slightly curved, 4 ï euseptate, base simple or rarely with branched appendage, apical cell conic, hyaline, with two or more apical, simple or branched, spathulate or espathulate appendages, median cells brown, some time versicolour, thick walled, smooth or verreculose.

***Pestalotiopsis maculans* (Corda) Nagraj**

*Pestalotiopsis* species are of considerable interest to researches and pharmacists due to their ability to synthesize a wide range of economically important bioactive molecules (Strobel, 2002; Tomita; 2003; Ding *et al.,* 2009; Liu *et al*., 2009).

The species was reported causing necrotic leaf spot in *Arbutus unedo* and *Ceratonia siliqua* in Spain (Trapero *et al.,* 2003). This is the first report of *Pestalotiopsis maculans* infecting leaves of *Bambusa arundinacea.*

Acervular conidiomata up to 200µm in diameter. All isolates had 5 celled smooth conidia, apical and basal cells were hyaline, while the three median cells were brown; the upper two

were darker than the lower one. Conidia were 22 to 30µm (mean length) and 5 to 9µm averaging 17µm long.

## 5. Pathogenicity test

Isolated fungi were artificially infected on host plant and re-isolation from symptoms confirmed Kochôs postulate **(Plate – IV).**

## 6. Biocontrol of Foliicolous fungi

India is the largest consumer of pesticides in the world. Pesticides which include insecticides, fungicides, herbicides, rodenticides and fumigants, are undoubtedly the largest group of toxic chemicals that are introduced profusely into the environment. They are defined as any substance or mixture of substances used for preventing, destroying, repelling or mitigating the pest. Most of the chemicals products fall within four main categories viz. organochloride insecticides, organophosphate insecticides, carbamate insecticides and pyrethroid. Pesticides have an inmate capacity to cause damage to the biological system, which may involve human health or environment. The most dramatic of such effects on human are accidental acute poisoning (Choudhary and Sinha).

Synthetic fungicides are currently used as the primary means for the control of plant diseases. However, the alternative control methods are needed because of the negative public perceptions about the use of synthetic chemicals, resistance to fungicides among fungal pathogens, and high development cost of new chemicals. (Lee *et al.,* 2007)

Some fungicides are not readily biodegradable and tend to persist for years in the environment. This leads to third problem, the detrimental effects of chemicals on organisms

other than target fungi. Because of these problems associated with the use of chemicals, researches are now trying to use environmentally safe alternative methods of fungal control.

**Ecofriendly approach to control fungal pathogens**

The commonly used synthetic fungicides have been found to display side effects in form of carcinogenicity, teratogenecity and pollutive effects. Uses of less harmful and true eco ï friendly products of plant origin are replacing the routine fungicides (Fawcett and Spencer 1970, Khanna and Chandra 1972, Dixit *et al.,* 1983, Arya and Mathew 1990, Arya *et al.,* 1995). Efforts are on to find out substitutes for chlorine containing, pentachlorophenol, ethylene dioxide, Gammexane and Dieldrin like pesticides. Use of synthetic pesticide is increasing day by day to meet the challenges of agriculture sector. Modern scientific developments are in no way less than concern with the health of common man.

The Botanical pesticides like pyrethrum, rotenone, ryania and nicotine, but thereafter, these botanicals were relegated to insignificant position in pest control. Pyrehthrum is extracted from flowers of *Chrysenthemum cinerieolium* and rotenone is derived from rhizomes of *Derris* and *Lonchorpus*. It has been promising source of biopesticide. Neem owes its toxic attributes azadirachtin, nimbin, salannin, meliantriol etc. Neem seed kernels are richest source of meliacins and contain 0.2 ï 0.3 % azadirachtin and 30 ï 40% oil. Though neem leaves and seeds contain azadirachtin, bark also contains this yet in smaller quantities. George (1999) reported Swallow root *(Decalepis hamiltonii*) of family Asclepediaceae causing protection of food grains against insect infestation. Rice borer (*Sitophilius oryzae*) and Red rust of beetle (*Tribolium*) were controlled by the application of Swallowrot. Inhibition of growth was observed on garlic extract (Tansy and Appleton 1975). Electron microscopic studies revealed thickening in cell wall in *Rhizoctonia solani*, wheras,

*Colletotrichum lindemuthianum* revealed a singular accumulation of osmiophill bodies immediately under the cell membrane when subjected to suspension of micronized garlic powder in distilled water (Bianchi *et al.,* 1997).

**Sources of Natural fungicides**

The secondary metabolites of plants are a vast repository of biologically active compounds. (Wilkins and Board 1989) reported 1400 plants as potential compounds of antimicrobial agents with many different classes of compounds, and several other metabolites from new plant species are being identified every year (Aqil and Ahmad, 2003; Eksteen *et al*., 2001; Qasim and Blan, 1996; Ushiki *et al.,* 1996). A detailed description of the plant- derived antifungal metabolites representing different classes of compounds was provided earlier. (Grayer and Harborne, 1994; Nychas, 1995). Majority of the identified natural fungicides are terpenes, phenolic compounds or nitrogen ï containing secondary products such as alkaloids. Extracts of *Azadiracthta indica, Lantana camara, Lawsonia inermis, Datura* spp., *Acacia* spp. *Trachyspermum ammi etc*. Widely used as natural fungicides. These are several increasing reports on the potent antagonistic activity of extracts from many other several other plant spp. ( Afolayan *et al.,* 2002; Dhaliwal, 2002; Letessier *et al*;., 2001; Pinto *et al.,* 1998; Singh and Tripathi, 1999)

Antifungal spectrum and stability of natural fungicides is dependent on the chemical nature of their constituents. Antifungal activity of aqueous extracts *of Padus aviam, Populus tremata* and *Chelidonium majus* against *Puccinia tritica* correlated with the high phenolic content and peroxidise activity. Fungicide potential of extracts from different parts of *Heracleum sibiricum* was in correlation with the phenolic compounds. (Karavaev *et al.,* 2002). Leaf extracts evaluated, owing to its high content of phenols and flavonoids (Parimelazhagan, 2001).

Essential oils, the complex mixture of volatile compounds, mainly monoterpenes ($C_{10}$) and sesquiterpenes ($C_{15}$), and their oxygenated derivatives such as alcohols, aldehydes, ketones, acids and esters (Wijesekara *et al.,* 1997), are a major group of natural fungicides. Multiple components rather than a single component, were responsible for fungicidal activity of essential oils. Majority of the essential oils were broad spectrum antifungal. However, the composition of these active components is affected by the genotype, geographical location, environment and agronomic conditions and even with diurnal rythm.

To control fungal pathogens of fruit crops Arya (2010) suggested use of natural fungicides like plant extracts, essential oils, gel and latex etc. Arya *et al.,* (2005) found fruit peelings (at 25 % conc. For *Myrothecium roridum* and Chaetomium ganglegarum) and seeds (at 25% against *Phoma multirostrata* and *Eurotium chevalieri*) of bitter gourd (*Momordica charantia* L. and Cucurbitaceae) effective against 4 fungi. The effect may be due to presence of alkaloid momordicine (0.038%) and some saponines in the fruit (Sabnis and Daniel, 1990) and Elaterin a (Cucurbitacin) present in seeds and fruit wall.

**Mode of action of Natural fungicides**

Though the chemical nature of several natural fungicides is available, very few attempts have been made to determine the mechanisms operating to control the fungal pathogens. Based on he available findings we can conclude that any one or more than one of the following mechanisms are responsible to restrict (fungistatic) or kill fungicidal) the phytopathogeneic fungal agents.

**A) Inhibition of fungal Metabolic pathways**

Chemical fistulosin (Octadecyl 3 ï hydroxyvindole) isolated from the roots of *Allium fistulosum*, inhibits the protein synthesis of *Fusarium oxysporum* (Phay *et al.,* 1999). Eugenol

(4 ï allyl ï 2 methoxy phenol), a major component of several medicinal and aromatic plants, inhibits the involved in free radical scavenging, lipid peroxidation and maintenance of redox potential, which together reduce the aflatoxigenecity of the fungus (Jayshree and Subrmanyam, 1999).

**B) Alteration in cell wall composition and structure**

The cell wall protects the fungi against external agents including antifungal metabolites. Many antifungal concentration target at cell wall composition and affects the integrity of cells resulting in fungal death.

**C) Changes in Membrane Permeability**

Membranes act as barrier between the cell and its external environment and also separate various organelles of the cell. Natural fungicides, particularly essential oils and their monoterpenoid components affect the structure and function (Knobloch *et al.,* 1989). This happens due to inhibition of membrane enzymatic reactions such as respiratory electron transport, proton transport and coupled phosphorylation steps (Knobloch *et al.,* 1986). Essential oils can degenerate hyphal tips and promote cytoplasmic retraction (de Bilerbeck *et al.,* 2001)

**D) Alterations in the Hyphal structure**

Treatments with natural fungicides result in microscopically detectable and often macroscopically visible changes in the hyphal structure. The hyphal deformations are mainly due to altered or lysed cell wall, and vacuolization or evacuation of the cytoplasm. Trypsin and chmotrypsin inhibitors from cabbage foliage cause leakage of intracellular contents of *Botrytis cinera and Fusarium solani.* Kaemoferol ï 3 ï O-b-D- apiofuranosyl -12) ï b-D-glucopyranoside, a flavonol diglycoside from the leaves of *Phytolacca americana*, lyse the

cell walls diverse pathogenic fungi such as *B. cinera, Magnaporthe grisea, Penicillium italicum, Diaporthe actinidiae, Botryosphaeria dothidea* and *Colletotrichum gloeosporiodes* (Bae *et al.,* 1997).

**E) Inhibition of Fungal Cell Wall degrading enzymes**

Pathogenic fungi produce cell wall degrading fungi produce cell wall degrading enzymes that degrade the plant cell wall polymers and facilitate the pathogen penetration and further colonization. Production of (CWDE) cell wall degrading enzymes is of significance in the pathogenisis of necrotrophic fungal pathogens, and is of minor significance in case of biotrophic pathogens. Important CWDE involved in the pathogenesis of necrotrohic fungi is polygalacturonases, pectinlyase, pectimethylesterase,ß ï 1,4 ï glucanase and celulase. The virulence of several necrotrohioc is often related to the differences in their production of CWDE (Carder *et al.*, 1987).

Extracts of *Allium cepa* and *A. porrum* inhibits the production of polygalacturonase by *Sclerotinia scleroternum, B. cinerea, Fusarium moniliforme, Phoma terrastris, P. lycopersici, D. Bryoniae, Sclerotium cepivorum* and *Rhizoctonia bataticola* mediated by the heat labile and protease inhibitor in sensitive factors (Flavaron *et al.,* 1993). Aqueous extracts of *Ocimum sanctum* inhibits the production of pectinolytic and cellulolytic enzymes of *Rhizopus arrhizus* and *Botryodiplodia theobromae* (Patil *et al.,* 1992). Putrescine reverses the inhibitory effect of *O. sanctum* extract suggesting its effect on fungal fungal ornithin decarboxylases pathway. Fruit and flower extracts of *Datura innoxia* inhibits the *in vitro* production of endo and exo pectinolytic and cellulotytic enzyme of *Colletotrichum capsici*. (Chitra *et al.,* 2001). Purified chestnut cystatin strongly affects the protease activity of *B. cinerea.* However unlike biocontrol agents (Elad and Kapt, 1999, Kapat *et al.,* 1998) the

inhibitory action of natural fungicides on fungal CWDE in the infection courts has not studied and needs further investigation

In the present study 8 types of leaf extracts were used against four foliicolous fungi, *Lasiodiplodia theobromae, Pestalotiopsis disseminata, Pestalotiopsis maculans, Phomopsis tectonae.* Methanolic fractions exhibited more promising results suppressing the fungal growth. The periodic data regarding fungal growth, exposed to various concentrations of methanolic extracts of *Alangium salviifolium, Alisicarpus vaginalis, Butea monosperma, Cymbpogon martini, Dalbergia sisso, Pluchea lanceolata, Vogelia indica, Withania somnifera* are present in below table.

**Table 3.1. Percentage inhibition of *Lasiodiplodia theobromae* at different concentration of leaf extracts**

| Sr. no | Plant Selected | Methanolic extract | | |
|---|---|---|---|---|
| | | 1 ml | 5 ml | 10 ml |
| 1. | *Alangium salviifolium* | 12 ±1.52 | 36 ±3.05 | 80 ± 1.52 |
| 2. | *Alysicarpus vaginalis* | 8 ±2.0 | 12 ±2.06 | 43 ±2.64 |
| 3. | *Butea monosperma* | 5 ±1.52 | 12 ±0.57 | 14 ±4.16 |
| 4. | *Cymbopogon martini* | 10 ±3.5 | 40 ±3.05 | 94 ±1.52 |
| 5. | *Dalbergia sisso* | 1.25 ±1.52 | 7.5 ±2.0 | 50 ±1.52 |
| 6. | *Pluchea lanceolata* | 16 ±2.08 | 28 ±2.08 | 65 ±3.15 |
| 7. | *Vogelia indica* | 7 ±2.08 | 5 ±2.0 | 2 ±0.57 |
| 8. | *Withania somnifera* | 6 ±3.05 | 49 ±2.51 | 79 ±4.0 |

* indicates each compound values are based on three replicates
Results were significant at P Ò0.05 level by one way ANOVA

*L. theobromae* showed maximum inhibition by *C. martini methanolic extract,* which showed 94% inhibition at 10% concentration. *B. monosperma* and *V. indica enhances growth at lower concentration, W. somnifera* extract depicted 79% inhibition at 10 ml methanolic extract *P. lanceolata* inhibition increase with increase in methanolic concentration. *V. indica* leaf extract showed very poor inhibition compare to other plants extract.

**Table 3.2. Percentage inhibition of *Pestalotiopsis disseminata* at different concentration of leaf extracts**

| **Sr. no** | **Plant Selected** | **Methanolic extract** | | |
|---|---|---|---|---|
| | | **1 %*** | **5% *** | **10%*** |
| 1. | *Alangium salviifolium* | 15 ±3.05 | 35 ±2.08 | 68 ±1.52 |
| 2. | *Alysicarpus vaginalis* | 15 ±1.52 | 22 ±2.51 | 31 ±2.30 |
| 3. | *Butea monosperma* | 13 ±1.56 | 5 ±2.68 | 0 ±0.0 |
| 4. | *Cymbpogon martini* | 12 ±2.30 | 65 ±2.51 | 92 ±1.52 |
| 5. | *Dalbergia sisso* | 3 ±1.0 | 16 ±2.68 | 24 ±2.0 |
| 6. | *Pluchea lanceolata* | -12 ±5.29 | 11 ±2.08 | 42 ±3.05 |
| 7. | *Vogelia indica* | -3 ±1.52 | -11 ±2.08 | -14 ±1.52 |
| 8. | *Withania somnifera* | 21 ±1.52 | 51 ±4.0 | 76 ±3.0 |

* indicates each compound values are based on three replicates
Results were significant at P Ò0.05 level by one way ANOVA

**Table 3.3. Percentage inhibition of *Pestalotiopsis maculans* at different concentration of leaf extracts**

| Sr. no | Plant Selected | Methanolic extract | | |
|---|---|---|---|---|
| | | 1% * | 5% * | 10% * |
| 1. | *Alangium salviifolium* | 22 ±4.58 | 34 ±5.29 | 37 ±3.05 |
| 2. | *Alysicarpus vaginalis* | 14 ±4.58 | 19 ±4.61 | 34 ±4.04 |
| 3. | *Butea monosperma* | 8 ±2.51 | 18 ±2.0 | 42 ±1.56 |
| 4. | *Cymbpogon martini* | 16 ±3.05 | 42 ±1.52 | 90 ±2.0 |
| 5. | *Dalbergia sisso* | 14 ±1.0 | 26 ±1.0 | 49 ±1.73 |
| 6. | *Pluchea lanceolata* | -15 ±1.73 | 7 ±2.64 | 55 ±3.78 |
| 7. | *Vogelia indica* | -13 | -23 | -31 |

| | | | | |
|---|---|---|---|---|
| | | ±2.0 | ±1.15 | ±1.0 |
| 8. | *Withania somnifera* | 40<br>±4.0 | 62<br>±1.52 | 68<br>±1.0 |

* indicates each compound values are based on three replicates
Results were significant at P Ò0.05 level by one way ANOVA

From the above table no.3, *C. martini* depicted remarkable inhibition at 10% followed by *W. somnifera* and *P. lanceolata*. However at 1% *P. lanceolata* enhanced growth of the fungus which was similar to *V. indica* where at all concentration growth of fungal colony was higher compare to control. This indicates *P. lanceolata* leaves have growth enhancing compounds.

*A. vaginalis* and *B. monosperma* showed inhibition at higher concentration.

**Table: 3.4 Percentage inhibition of *Phomopsis tectonae* at different concentration of leaf Extracts**

| **Sr. no** | **Plant Selected** | **Methanolic extract** | | |
|---|---|---|---|---|
| | | **1% *** | **5% *** | **10% *** |
| 1. | *Alangium salviifolium* | 30<br>±0.57 | 63<br>±2.0 | 76<br>±2.51 |
| 2. | *Alysicarpus vaginalis* | 18<br>±1.52 | 30<br>±1.0 | 40<br>±3.51 |
| 3. | *Butea monosperma* | 5<br>±2.30 | 24<br>±2.0 | 53<br>±2.0 |
| 4. | *Cymbopogon martini* | 21<br>±4.0 | 100<br>±0.0 | 100<br>±0.0 |
| 5. | *Dalbergia sisso* | 33<br>±2.0 | 53<br>±1.52 | 62<br>±1.15 |
| 6. | *Pluchea lanceolata* | 10 | 26 | 54 |

| | | | | |
|---|---|---|---|---|
| | | ±3.05 | ±2.08 | ±1 |
| 7. | *Vogelia indica* | -21<br>±2.0 | -48<br>±1.0 | -58<br>±3.05 |
| 8. | *Withania somnifera* | 63<br>±2.0 | 67<br>±0.57 | 83<br>±1.0 |

* indicates each compound values are based on three replicates
Results were significant at P Ò0.05 level by one way ANOVA

It is evident from table 3.1 significant inhibition was observed in *Cymbopogon martini*. The inhibitory effect may be because of Palmorosa oil. The essential oil inhibits the spore germination in fungal growth. Chemically oil contains geraniol. The other plant whose leaf extract was found inhibitory was *Withania somnifera*. The leaves of *W. somnifera* contained withanolides. Withaferin A is reported to have antibiotic and antitumor activities. The leaves of *Alangium salvifolium* contain alkaloids. The leaves also contain tri terpenes. *Alangium* was found effective in *Phomopsis tectonae* and *Lasiodiplodia theobromae.*

The presence of antibacterial substances in the higher plants is well established (Srinivasan, 2001). Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug (Didry *et al.*, 1998). Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure.

The results of this study clearly reflect that *Cymbopogon martini* plant has the potential to induce toxic effect on mycelial growth and proliferation of fungi. The relative intensity of this effect however varies with the species involved, as well as the concentrations of the extract employed.

Earlier (Bajwa *et al.,* 2008) have reported inhibitory effect by methanolic shoot extracts.

The variation in antifungal activity of the extracts in different solvents may be attributed to the different chemical nature of the solvents. It is likely that different types of chemical nature of the solvents. It is likely that different types of chemical were dissolved in different solvent that resulted in variable activity of the extracts of same part of the plant in different solvents.

## CULTURAL STUDIES

Behavior of a fungus/pathogen depends upon its nutritional response. Phytopathogenic organisms express a similarity in broader behavior for their basic nutritional needs, yet they maintain their individuality for the choice of specific substances (Cochrane, 1958). It is well established that phytopathogens show greater diversities in their ability to utilize the same element from different nutrient media (natural, semi-synthetic, synthetic culture media). These culture media may contain essential elements needed for proper sporulation of *Lasiodiplodia theobromae, Pestalotiopsis disseminata, Pestalotiopsis maculans, Phomopsis tectonae,* but their performance may be different at different pH and temperature. Experiments were, therefore conducted to determine the effect of pH, temperature, different medias as these factors may contribute significant information in formulating the strategies for control measure for these phytopathogens.

## a) Selection of suitable Culture media

First attempt to obtain laboratory cultures of fungi was made by the great Italian botanist, Micheli (1967-1737). He could succeed in growing three different molds viz., *Mucor, Aspergillus* and *Botryis,* on freshly cut surface of melon, quince and pear. Bulliard (1791) followed Micheliôs lead and obtained cultures of *Mucor* on a paste prepared from moistened breads. Use of such substrates for artificial culture of fungi continued till Pasteur (1860) during his studies on alcoholic fermentation, used what might be considered as an approximation of a chemically defined medium. However, it was Raulin (1869) one of the Pasteurôs disciples, who devised the first synthetic medium for fungi, during nutritional studies of the common mold, *Aspergillus niger.*

**Table 3.5: Mycelial dry wt, final pH of four foliicolous fungi on different media after 15 days of incubation.**

| Sr. No. | Media | *Pestalotiopsis disseminata* | | *Pestalotiopsis maculans* | | *Phomopsis tectonae* | | *Lasiodiplodia theobromae* | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dry wt. (g)* | Final pH* | Dry wt.(g)* | Final pH* | Dry wt.(g)* | Final pH* | Dry wt.(g)* | Final pH* |
| 1. | Coonôs | 0.06 ±0.005 | 7.26 | 0.09 ±0.016 | 7.30 | 0.13 ± 0.03 | 7.26 | 0.07 ±0.06 | 7.27 |
| 2. | Asthana & Hawkerôs | 0.13 ±0.02 | 7.24 | 0.18 ±0.021 | 7.27 | 0.14 ±0.023 | 7.26 | 0.09 ±0.012 | 7.25 |
| 3. | Modified Asthana & Hawkerôs | 0.14 ±0.009 | 6.90 | 0.08 ±0.021 | 7.05 | 0.21 ±0.01 | 7.02 | 0.13 ±0.023 | 7.00 |
| 4. | Elliotôs | 0.10 ±0.003 | 6.9 | 0.05 ±0.007 | 7.2 | 0.11 ±0.01 | 6.9 | 0.07 ±0.003 | 6.6 |
| 5. | Richardôs | 0.45 ±0.005 | 7.2 | 0.45 ±0.065 | 7.25 | 0.57 ±0.040 | 7.25 | 0.35 ±0.034 | 7.24 |
| 6. | PDA | 0.25 ±0.018 | 6.8 | 0.12 ±0.01 | 6.8 | 0.26 ±0.005 | 6.9 | 0.28 ±0.028 | 6.8 |
| 7. | Czapekôs | 0.26 ±0.02 | 7.1 | 0.31 ±0.023 | 7.01 | 0.21 ±0.015 | 7.0 | 0.42 ±0.002 | 7.0 |
| 8. | DOX | 0.19 | 7.00 | 0.39 | 7.05 | 0.22 | 7.00 | 0.18 | 7.00 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ±0.02 | | ±0.03 | | ±0.005 | | ±0.005 | |
| 9. | Host Decoction | 0.58 ±0.046 | 6.8 | 0.35 ±0.036 | 6.8 | 0.24 ±0.025 | 7.31 | 0.28 ±0.15 | 6.0 |

* indicates each compound values are based on three replicates
Results were significant at P Ò0.05 level by one way ANOVA

Observation from Table no:1 revealed that best growth of *P. disseminata* was obtained on Host decoction followed by Richardôs, Czapekôs, PDA, DOXôs, Modified Asthana & Hawkerôs, Asthana & Hawkerôs, Elliotôs and Coonôs medium. Statistical analysis revealed best growth on *T. arjuna* leaf decoction, Richardôs, Czapeckôs while it was poor on all other mediums.

The growth of *P. maculans* was maximum on Richardôs followed by DOX, Czapekôs and was poor on all the others. Richardôs medium supported maximum growth of *P. tectonae* followed by PDA, *T. grandis* decoction, DOX, Czapekôs medium while poor growth was accomplished on rest all mediums.

*Lasiodiplodia theobromae* achieved maximum yield on Czapekôs followed by Richardôs after 15 days incubation, moderate growth was observed on PDA, *T. grandis* decoction followed by modified Asthana & Hawkerôs medium and was poor on rest others. The excellent sporulation was observed on Modified Asthana & Hawkerôs and PDA medium while in rest others it varied from moderate to poor.

Alam *et al.,* (2001) reported that highest mycelia growth and sporulation of *L. theobromae* was observed on PDA. Kumar and Singh (2000) also stated that *L. theobromae* grew well in Potato Dextrose Medium. Xu *et al.,* (1984) and Maheswari *et al.,* (1999) reported in their findings about PDA the best source for sporulation of *L. theobromae.* PDA and Host decoction media could not be considered for selection for basal media due to their

changeable nature and unknown composition and it is not possible to keep the concentration of the constituentôs constant throughout the studies. (Arya, 1985)

Though modified Asthana & Hawkerôs medium did not support excellent growth statistically yet it supported sufficient growth and excellent sporulation of all the fungi under study. Further the medium is easy to handle with regard to the expected need for modifications and substitutions of its constituents. It was therefore selected to use modified Asthana and Hawkerôs medium for all subsequent cultural studies.

**Classification**

Some of the common criteria for classifying media are their chemical composition, physical state and their empirical use. In fact, every medium is designed for a definite use and hence its physical and chemical characteristics must conform to its application and function. According to their use, media may be categorized into the following types:

1. **Routine media:** These media are with certain complex raw materials of plant or animal origin such as yeast extract, malt extract, peptone etc., and are employed for routine cultivation and maintenance of a wide variety of fungi.
2. **Enriched media:** These media are prepared by supplementing the routine laboratory media with some specific substances to meet the nutritional requirements of more fastidious organisms and are employed for their cultivation.
3. **Selective media:** These media facilitate the isolation of a particular group of organisms or species from mixed inoculums. Such media contains substances which inhibit all except the desired organisms.
4. **Differential media:** Supplemented with certain reagents of chemicals, these media aid in differentiating between various kinds of organisms on the basis of visible differences in

their growth patterns. However, such type of media is used more often in bacteriological laboratories.

5. **Assay media:** This type of medium is specifically employed for the assay of vitamins, amino acids, antibiotics, disinfectants, etc. and are of definite composition.
6. **Biochemical media:** Such media are generally used for the differentiation of microorganisms on the basis of their biochemical activities, and are helpful in the study of their metabolic processes.

According to the chemical composition media are classified into the following types:

1. **Natural media:** A natural media comprises entirely complex natural products or unknown composition. The raw materials of a natural medium may be of plant or animal origin, and some of the common ingredients employed for this purpose include extracts of plant and animal tissues *e.g*. fruits, vegetables, egg, milk, blood, body fluids, yeast, malt and manure extracts, etc. Obviously, the chemical composition and concentration of a natural medium is not well defined. On account of their complex nature, these media are able to support a variety of organisms, and hence are quite useful for routine laboratory cultures of fungi. Brefeld (1881), who was one of the pioneers in the field of fungal culture was not much impressed by the utility of some natural media he used, that he considered atleast one of them, *viz*. manure extract, of universal applicability for culture of fungi. Other advantages of natural media are their low cost and easier method of preparation. However, these media have certain limitations too. Due to their complex nature, their chemical composition and concentration can not be controlled. This limits their use to routine culture of fungi only, as investigations pertaining to fungal nutrition and metabolism can hardly be carried out on such media.
2. **Semisynthetic media:** These media are so designed that some of their constituents are of known chemical composition, while others are derived from some natural sources

with unknown composition. The chemical make up of a semisynthetic medium is, thus, only partly known. Consequently, on a limited amount of control may be exercised on the composition and concentration of a semi synthetic medium, by making necessary changes in the chemically known faction. Semi synthetic media have also limited application in the physiological studies on fungi, and can best serve as a routine medium. Potato dextrose agar is one of such accepted and popular media. Lilly and Barnett (1951) consider all agar solidified media as semi synthetic ones, because their extract chemical makeup is partly obscured by the addition of agar agar.

3. **Synthetic media:** These are chemically defined media of known composition and concentration, and are exclusively composed of pure chemical substances. However absolute purity of the ingredients is seldom achieved, although substances of only analytical reagent quality are used for such purposes. On account of their known composition as well as being in the solution, these media are quite useful for nutritional and metabolic studies of fungi. The composition of these media may be amended as per requirement and as such they may be simple or complex in make up. A simple synthetic medium contains a single carbon and energy source, a nitrogen source, generally as ammonium salt, some sulphur and phosphorus sources and various minerals. All these ingredients are dissolved in a buffered aqueous base. However, for more fastidious organisms, a complex synthetic medium is designed by incorporating some additional factors such as vitamins, amino-acids, purines, pyrimidines, *etc,* or by employing a multitude of carbon and nitrogen sources together.

## (b) Selection of suitable pH

Following 5 initial pH values were adjusted 2, 4, 6, 8, 10. The results obtained for four different fungi after 15 days incubation.

It is evident from the **table: 3.6** that *P. disseminata* grew between pH 2 to 10. Its growth was good at pH 6.0 followed by pH 4. Final pH drifted towards neutral side. The growth was poor at 2, 8 and 10. *P. maculans* showed optimum growth at pH 6.0 and moderate at 8, 10 and poor at pH 2 and 4.0

*P. tectonae* and *L. theobromae* both fungi exhibited optimum pH for growth at the pH 6.0 and poor growth in other pH values. *L. theobromae* was able to grow within a wide range of pH from 4.0 to 8.0. The result indicated that from slightly acidic pH to neutral pH the growth of the organisms was possible. These results were in agreement with (Saha *et al.,* 2008).

**Table 3.6: Average dry wt. (g) of four foliicolous fungi at different pH values**

| Sr. No. | Initial pH | *Pestalotiopsis disseminata* | | *Pestalotiopsis maculans* | | *Phomopsis tectonae* | | *Lasiodiplodia theobromae* | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dry | Final | Dry | Final | Dry | Final | Dry | Final |

| | | wt.(g)* | pH* | wt.(g)* | pH* | wt.* | pH* | wt.(g)* | pH* |
|---|---|---|---|---|---|---|---|---|---|
| 1. | 2 | 0.02 ± 0.003 | 3.27 | 0.04 ±0.021 | 3.24 | 0.026 ± 0.011 | 3.22 | 0.02 ± 0 | 3.26 |
| 2. | 4 | 0.09 ± 0.01 | 6.33 | 0.04 ± 0 | 6.25 | 0.053 ± 0.015 | 6.14 | 0.03 ± 0.007 | 6.1 |
| 3. | 6 | 0.108 ± 0.017 | 7.15 | 0.22 ± 0.037 | 7.55 | 0.190 ±0.16 | 7.26 | 0.25 ± 0.077 | 7.05 |
| 4. | 8 | 0.06 ± 0.007 | 7.17 | 0.13 ± 0.049 | 7.13 | 0.070 ± 0.025 | 7.06 | 0.06 ± 0.020 | 7.33 |
| 5. | 10 | 0.05 ±0.02 | 9.8 | 0.06 ± 0.03 | 9.8 | 0.050 ± 0.0007 | 9.83 | 0.02 ± 0.014 | 9.83 |

* indicates each compound values are based on three replicates
Results were significant at P Ò0.05 level by one way ANOVA

**Table 3.7 Mycelial dry wt. and final pH of four different foliicolous fungi at different temperatures**

| Sr. No. | Temp ( $^0$C ) | *Pestalotiopsis disseminata* | | *Pestalotiopsis maculans* | | *Phomopsis tectonae* | | *Lasiodiplodia theobromae* | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dry wt.(g)* | Final pH* | Dry wt.(g)* | Final pH* | Dry wt.(g)* | Final pH* | Dry wt.(g)* | Final pH* |
| 1. | 2 | 0 | 5.6 | 0 | 5.6 | 0 | 5.6 | 0 | 5.6 |
| 2 | 5 | 0.020 ± 0.012 | 5.6 | 0.025 ± 0.004 | 5.60 | 0.019 ± 0.005 | 5.6 | 0.028 ±0.005 | 5.1 |
| 3. | 10 | 0.026 ± 0.002 | 5.5 | 0.023 ± 0.004 | 5.5 | 0.031 ± 0.007 | 5.4 | 0.030 ± 0 | 5.6 |
| 4. | 15 | 0.078 ± 0.001 | 5.9 | 0.042 ± 0.036 | 6.0 | 0.181 ± 0.028 | 6.0 | 0.75 ± 0.011 | 6.1 |
| 5. | 20 | 0.112 ± 0.045 | 6.3 | 0.087 ± 0.015 | 5.9 | 0.348 ± 0.005 | 6.3 | 0.86 ± 0.015 | 6.9 |
| 6. | 25 | 0.052 ± 0.010 | 6.4 | 0.100 ± 0.029 | 6.3 | 0.148 ± 0.003 | 6.4 | 0.180 ± 0.053 | 7.0 |
| 7. | 30 | 0.048 ± 0.009 | 7.0 | 0.80 ± 0.006 | 6.3 | 0.109 ± 0.021 | 6.7 | 0.06 ± 0.015 | 7.0 |
| 8. | 35 | 0.051 ± 0.016 | 5.3 | 0.060 ± 0.013 | 5.1 | 0.050 ± 0.009 | 5.1 | 0.027 ± 0.009 | 5.3 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 9. | 40 | 0.01 ± 0 | 6.0 | 0.020 ± 0.03 | 6.0 | 0 | 6.3 | 0.020 ± 0.02 | 6.3 |

* indicates each compound values are based on three replicates
Results were significant at P Ò0.05 level by one way ANOVA

The influence of different temperatures *i.e.* 2, 5, 10, 15, 20, 25, 30, 35, 40$^0$C, has been investigated. Observation from Table: 3 shows all the four foliicolous fungi failed to grow at 2 $^0$C and 40 $^0$C except for *P. maculans* and *Lasiodiplodia theobroma*e showed poor growth. All the four fungi exhibited maximum growth at 20 $^0$C and 25 $^0$C except for *P. maculans* which showed maximum growth at 30 $^0$C. Sporulation was excellent at 20 $^0$C followed by 25 $^0$C and 30 $^0$C. Sporulation was moderate at 15 $^0$C and 35 $^0$C. While at temperatures at 10 $^0$C and 35 $^0$C showed poor sporulation. According to (Saha *et al.,* 2008) *L .therobromae* was capable of growing at temperatures that range between 8 ï 36 $^0$C. Best growth was observed at 28 $^0$C and no growth was observed at 40 $^0$C which was similar to our results. In other study Eng *et al.,* (2003) reported similar observations when he studied the effect of temperature on growth characteristics of *Botrydiplodia theobromae.*

According to Lisa *et al.,* (2006) optimum temperature for growth of 16 isolates of *Pestalotiopsis* sp. was between 22$^0$C and 28$^0$C, however, certain isolates exhibited a slower growth rate within the optimum temperature ranged

Since the result indicated good growth and excellent sporulation of all four foliicolous fungi at 20 $^0$C subsequent experiments were carried out at this temperature.

## d) Effect of different Carbon source

Living organisms are known to utilize about forty elements, among which carbon plays the key role. As a component of both structural and functional cell ï constituents, carbon comprises about fifty per cent of the total mycelia dry weight in fungi. A multitude of organic constituent of fungal cell, like carbohydrates, proteins, nucleic acids, enzymes etc. are all made up of carbon. Practically , all the important compounds of cell ï wall, like cellulose, chitin, and pectin substances contain carbon in varying form and concentration, and thus provide the structural frame ï work of the fungal cell. In their functional role, carbon compounds are still more significant, because fungi, being chemotrophs, obtain all their energy ï requirements from catabolic degradation of one or other carbon containing ingredients of the cell.

Fungi exhibit carbon heterotrophy and obtain their carbon requirement from various organic sources. Although in a few cases utilization of inorganic carbon in the form of $CO_2$ has also been reported but not as the sole source. A variety of organic compounds are utilized

by fungi, and the nature of organisms largely determines the range of substrate. A massive literature has accumulated on carbon ï nutrition of fungi. It has been helpful in deriving certain general conclusions. For example monosaccharides have generally been reported to be more easily utilize sources than the oligo or the polysaccharides.

Though interesting inferences have been derived on the basis of laboratory experiments, yet a wide gap still exists between the facts and the conclusions. This is mainly because the nature of carbon compounds available under natural conditions are sometimes quite different from the one on which the fungi have to feed under controlled laboratory conditions. The crude forms in which various compounds exist in nature are generally not so readily utilized under the cultural conditions. This obviously reflects that the efficiency of the organism is much more put to challenge under natural conditions than under the laboratory set up. The mere fact that there is a proof of their efficiency to derive food from different natural substrates.

**Table 3.8: Effect of different Carbon sources on growth of 4 foliicolous fungi after 15 days .**

| Sr. No | Carbon Sources | *Pestalotiopsis disseminata* | | *Pestalotiopsis maculans* | | *Phomopsis tectonae* | | *Lasiodiplodia theobromae* | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dry wt. (g) | Final pH | Dry wt. (g) | Final pH | Dry wt. (g) | Final pH | Dry wt. (g) | Final pH |
| 1. | Rhamnose | 0.138 ±0.081 | 7.00 | 0.114 ± 0.020 | 6.80 | 0.116 ±0.03 | 6.50 | 0.125 ±0.056 | 7.00 |
| 2. | Xylose | 0.121 ± 0.052 | 6.90 | 0.084 ± 0.029 | 6.20 | 0.060 ± 0.034 | 6.3 | 0.114 ± 0.049 | 7.03 |
| 3. | D-Arabinose | 0.067 ± 0.005 | 6.50 | 0.060 ± 0.043 | 5.63 | 0.033 ± 0.003 | 5.66 | 0.094 ± 0.058 | 7.00 |
| 4. | L ï Arabinose | 0.102 ± 0.008 | 7.90 | 0.095 ± 0.064 | 6.86 | 0.069 ± 0.010 | 6.40 | 0.133 ± 0.036 | 6.90 |
| 5. | D - Glucose | 0.14 ±0.68 | 6.90 | 0.15 ±0.07 | 7.05 | 0.21 ±0.030 | 7.02 | 0.13 ±0.009 | 7.00 |
| 6. | D ï Fructose | 0.102 ± 0.030 | 7.10 | 0.159 ± 0.036 | 6.43 | 0.118 ± 0.030 | 6.20 | 0.102 ± 0.015 | 6.96 |
| 7. | D- Galactose | 0.098 ± 0.054 | 6.90 | 0.140 ± 0.017 | 6.46 | 0.123 ± 0.101 | 6.26 | 0.132 ± 0.057 | 7.23 |
| 8. | Maltose | 0.142 ± 0.06 | 7.46 | 0.148 ± 0.009 | 6.56 | 0.136 ± 0.059 | 6.30 | 0.139 ± 0.053 | 6.50 |
| 9. | Sucrose | 0.141 ±0.04 | 7.00 | 0.143 ±0.07 | 7.00 | 0.104 ± 0.09 | 6.50 | 0.134 ± 0.062 | 6.80 |
| 10. | Mannitol | 0.096 ± 0.002 | 8.00 | 0.080 ± 0.05 | 6.53 | 0.122 ± 0.096 | 6.50 | 0.107 ± 0.035 | 6.90 |
| 11. | Raffinose | 0.189 ± 0.018 | 7.50 | 0.144 ±0.04 | 7.00 | 0.154 ±0.051 | 6.50 | 0.180 ±0.03 | 6.30 |
| 12. | Starch | 0.113 ± 0.030 | 8.26 | 0.129 ± 0.049 | 6.93 | 0.139 ± 0.023 | 7.03 | 0.087 ± 0.011 | 7.43 |
| 13. | Control | 0.052 ± 0.010 | 6.4 | 0.100 ± 0.029 | 6.3 | 0.095 ± 0.003 | 6.4 | 0.080 ± 0.053 | 7.0 |

* indicates each compound values are based on three replicates
Results were significant at P Ò0.05 level by one way ANOVA

Mycelial growth was observed to be much higher in presence of all the carbon sources tested compared to control, which did not contain any carbon compound. (Table 3.8).On the basis of above table all the four phytopathogenic fungi showed maximum growth on Maltose followed by D- Fructose, starch. *P. disseminata* and *Lasiodiplodia theobromae* showed good

growth on L- Arabinose sugar while *P. maculans* and *P. tectonae* indicated moderate growth on this sugar.

Media having D-arabinose as carbon source recorded minimum mycelial growth for all four fungus. According to (Saha *et al*., 2008), (Jash *et al.,* 2003) sucrose was the best carbon source for the growth of *Alternaria zinniae* and mannitol produced least growth.

D ï arabinose showed moderate growth on *P. disseminata*, *P. maculans* and *Lasiodiplodia theobromae* while it showed poor sporulation and growth on *P. tectonae.*

Varied degree of growth was observed in Xylose by four fungus. Excellent growth was attained by *P. disseminata* and *Laiodilpodia theobromae* while *P. maculans* and *P. tectonae* showed moderate growth.

**Utilization of Monosaccarides**

These compounds are also popularly referred as simple sugars and are sweet in taste and soluble in water. They have general formula Cn ($H_2O$)n. They possess a free aldehyde ï CHO or a ketone (-CO-) group, beside the primary $CH_2OH$ and secondary CHOH alcohol groups. In a monosaccharide carbon unit, the aldehyde and primary alcohol groups are attached on the two extremities, while the ketone group is located on the second carbon atom. Classification of these sugars is based on the number of carbon atoms present and the functional group involved. As per the formal criteria a monosaccharides may be a triose if the chain has three carbon atoms a tetrose with a chain of four carbon atoms a pentose -5 carbon and hexoses ï with 6 carbon atoms *etc.*

**Disaccharides**

If the alcohol employed in a glycosidic linkage is component of another sugar molecule, the product is a di ï saccharide. Structures of some common examples of disaccharides like Maltose, cellobiose, trehlose, lactose melibiose and sucrose are described below.

**Maltose – (Rf. 0.35)** Two glucose Ŭï 1 ï 4, glucoside linkage. From the structure of Maltose it is obvious that one of the sugar units still possess a free hemi acetal form, therefore maltose in a solution will comprise three different forms of molecule. *viz.* Ŭï ɓ ï and aldehyde, in a state of equilibrium. This disscaride is obtained as a intermediate product during the digestion of starch to glucose.

**Sucrose – (Rf. 0.33)** Glucose + Fructose: Ŭ ï D ï Glucopyranosyl ï 1 ï 2 ï ɓ ï D ï fructofuranoside). In sucrose molecule both the carbonyl groups are involved in the formation of glycoside linkage therefore, only one form of sucrose exists. Sucrose is designated as a non reducing dissacharide because if absence of any free aldehyde group in its molecule. It is not able to reduce Benedictôs solution.

**Oligosaccharides**

It consists of 2 ï 10 monosaccharide moieties and upon hydrolysis yields monasacaride however sometimes di-saccarides are also treated as oligosaccahrides besides the naturally occurring tri saccharides and tetra saccharides etc. Raffinose, Gentianose etc. are the examples of trisaccharides while stachyose is a tetra saccharides which yields after hydrolyses glucose, fructose and two molecules of galactose.

**Polysaccharides**

These are compounds of polymeric structure containing a large number of monosaccharide units if all these units are of the same sugar. The polysaccharide is designated as

homopolysaccharide, while those comprising of two or more different types of sugar units are called as hetro - polysaccharide units. Common examples of naturally occurring polysaccharides are cellulose, starch, pectin, glycogen, etc.

**Starch**

It is a compound of high molecular weight and is a polymer of D ï glucose. It is generally found as a storage compound in plants and is stored as insoluble grains. Starch grains consist of two different polysaccharides amylase and amylopectin having distinct properties.

## e) Effect of different Nitrogen sources

The role of nitrogen in fungal physiology has received considerable attention of the mycologists during the last three decades. The account has been well illustrated by Foster (1949), Hawker (1950), Lilly and Barnett (1951) and Cochrane (1958). Due to vague and contradictory biochemical data it is difficult to computerize the findings under a single orbit. Contradictions in the conclusions arise mainly because every nitrogen source used in the culture medium undergoes complex transformations, which vary with the nature of the organisms and the experimental set up. Like carbon sources, nitrogen is also used for both functional as well as structural purposes by fungi. The form of nitrogen has a profound effect on metabolism of micro ï organisms. Literature is full with conflicting claims regarding the comparative superiority of a particular form or source of nitrogen over the other. Specificity for the choice of nitrogen is more pronounced in some and less in other organisms. Occasional attempts have been made to classify the fungi on the basis of their nitrogen requirements. The classification which needs attention is that of Robbins (1937) who grouped fungi, into four categories on basis of their capacity to utilize nitrate, ammonium, organic and elemental nitrogen.

**Table: 3.9. Effect of different Nitrogen Compounds on growth of four different foliicolous fungi**

| Sr. No. | Nitrogen Source | *Pestalotiopsis disseminata* | | *Pestalotiopsis maculans* | | *Phomopsis tectonae* | | *Lasiodiplodia theobromae* | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dry wt.(g)* | Final pH* | Dry wt.(g)* | Final pH* | Dry Wt.(g)* | Final pH* | Dry Wt. (g)* | Final pH* |
| 1. | Potassium nitrate | 0.159 ± 0.09 | 6.36 | 0.179 ± 0.27 | 6.00 | 0.082 ± 0.10 | 5.03 | 0.088 ± 0.13 | 6.16 |
| 2 | Sodium nitrate | 0.160 ± 0.13 | 6.40 | 0.163 ± 0.07 | 6.13 | 0.137 ± 0.02 | 6.63 | 0.155 ± 0.19 | 7.76 |
| 3. | Ammonium acetate | 0.107 ± 0.06 | 5.73 | 0.161 ± 0.05 | 6.50 | 0.149 ± 0.14 | 5.3 | 0.102 ± 0.06 | 5.26 |
| 4. | Ammonium oxalate | 0.153 ± 0.24 | 7.53 | 0.120 ± 0.17 | 6.80 | 0.169 ± 0.24 | 5.1 | 0.123 ± 0.25 | 5.9 |
| 5. | Ammonium sulphate | 0.164 ± 0.19 | 4.36 | 0.157 ± 0.27 | 4.43 | 0.180 ± 0.13 | 4.06 | 0.102 ± 0.02 | 3.23 |
| 6. | Ammonium nitrate | 0.156 ±0.16 | 4.53 | 0.134 ± 0.27 | 2.66 | 0.080 ± 0.12 | 3.8 | 0.183 ± 0.08 | 3.26 |
| 7. | Calcium nitrate | 0.134 ± 0.10 | 5.36 | 0.162 ± 0.19 | 5.4 | 0.162 ± 0.24 | 6.9 | 0.140 ± 0.25 | 6.5 |
| 8. | Peptone | 0.194 ± 0.26 | 5.63 | 0.193 ± 0.23 | 5.73 | 0.070 ± 0.16 | 5.6 | 0.154 ± 0.25 | 5.6 |
| 9. | Control | 0.052 ± 0.010 | 6.4 | 0.100 ± 0.029 | 6.3 | 0.148 ± 0.003 | 6.4 | 0.112 ± 0.053 | 7.0 |

* indicates each compound values are based on three replicates
Results were significant at P Ò0.05 level by one way ANOVA

Among the eight nitrogen sources tested, maximum growth of *P. disseminata* and *P. maculans* was observed on Peptone, whereas, *P. tectonae* showed poor growth on this source, *L. theobromae* showed maximum growth in Ammonium nitrate. Our results are similar to that of Holb and Chauhan, (2005) who showed that Peptone was the best source that produced quickest growth of *Monilia polystroma.*

*P. maculans* and *L. theobromae* showed similar growth on Ammonium oxalate, P. *tectonae* and *P. disseminata* revealed similar growth in basal medium with Ammonium oxalate. Ammonium sulphate was better nitrogen source for all three fungus except *L. theobromae* which displayed moderate growth of the foliicolous fungi.

*P. disseminata* exhibited very poor growth in Sodium nitrate source compared to *P. maculans, P. tectonae, L. theobromae* which showed good growth after fungal mat harvesting. *P. maculans* and *P. tectonae* showed equal dry weight in Calcium nitrate source whereas in *P. disseminata, L. theobromae* the growth was moderate in Calcium nitrate source.

*P. disseminata* and *P. maculans* revealed excellent growth in Potassium nitrate after 15 days of incubation but this source did help P. tectonae and L. theobromae for better sporulation.

Excellent growth was shown in Ammonium nitrate by *P. disseminata* and *L. theobromae,* moderate growth was found in *P. maculans*, but *P. tectonae* showed poor growth on this source.

Ammonium acetate showed good growth in *P. maculans* and *Phomopsis tectonae,* but excellent growth was seen in *P. disseminata* and *L. theobromae*.

Besides the eight nitrogen sources little growth was noticed in control, which was devoid of nitrogen. This may be possible because of small amount of nitrogen carried along with inoculum.

**Utilization of Nitrogen sources**

**Nitrates**

In general, nitrates have been reported to be excellent sources for imperfect fungi and Ascomycetes (Lilly and Barnett, 1951; Hacskaylo *et al.,* 1954; Thind and Randhawa, 1957; Suryanarayanan1958; Misra and Mahmood, 1960; Agarwal *et al.,* 1968). Higher Basidiomytes are generally incapable of utilizing it, while some members of this group show feeble response.

Among the Phycomyctes, species of *Pythium* (Saksena *et al.,* 1952; Grover and Sindhu, 1965) showed a favorable response towards the nitrate nitrogen. Saproleginales (Bhargava, 1954; Reischer, 1951) Blastocladiales (Cantino, 1955) as well as two marine Phycomycetes) do not grow on nitrate nitrogen. Incapacity to use nitrate nitrogen is usually considered absolute in some of the fungi. However, there exists possibility that at least some of such forms might be capable of metabolizing nitrate nitrogen in later stages if initial growth is attained at the expense of some readily available nitrogen source. Cochrane (1950) observed that spores of *Streptomyces griseus* were incapable to grow on nitrate medium but a pre grown mycelium, after it is inoculated in a nitrate containing medium grew well. There are also some reports (Raper *et al.,* 1954; Pontecorvo, 1953; Sakaguchi and Ishitani mutation. Efficiency of different forms of nitrate is sometimes lost by mutation. Efficiency of different forms of nitrate varies for fungi. Tandon (1967) reported that sodium nitrate, calcium nitrate and magnesium nitrate were generally inferior to potassium nitrate for Fungi imperfecti. Difference in the value of various types of nitrates is obviously due to different cations involved in these compounds. There are several reports (Linderberg, 1944; Norkrans, 1959; Fergus 1952, Biilgrami, 1964; Tandon, 1967) to suggest that within a genus individual species differ for their nitrate utilization. The capacity to use nitrate by fungi actually depends upon their nitrate reductase activity.

Ammonium nitrate has also been found to have extensive application in fungal nutrition as nitrogen source. This substance is reported to be inferior to potassium nitrate for a large numer of imperfect fungi (Mix 1933; Durairaj, 1956; Suryanarayanan, 1958; Bilgrami , 1964 and Tandon, 1967). A pronounced fall in pH of ammonium ï nitrate medium during the growth of fungi is common (Isaac, 1949; Haskins and Weston, 1950; Pelletier and Keit, 1954; Srivastava, 1955; Narsimha, 1969). This is indirect evidence about the preferential utilization of ammonium ion. Analytical studies with *Scopulariopsis brevicaulis*

## (G) Vitamin requirements of fungi

Our current knowledge of vitamin requirements of fungi indicates that they generally need only water soluble vitamins of B-complex series, including thiamine ($B_1$), riboflavin ($B_2$), pyridoxine ($B_6$), niacin (nicotinic acid), panthothenic acid, biotin (H), folic acid group, inositol, p-aminobenzoic acid and cyanocobalmin ($B_{12}$). None of the fat soluble vitamins like A, D, E and K have so far been found to be synthesized by fungi and it appears that they do not require these growth factors. However, a number of growth factor requirements of fungi are still poorly understood and therefore any extreme and hasty conclusion in this regard needs caution. Moreover, several fungi are known to respond with stimulated growth to various natural materials, which may contain unknown growth factors, because in many such cases identical growth response could not be induced by addition of specific purified vitamins or other nutrients.

**Thiamine (Vitamin $B_1$)**

**Structure:** Thiamine molecule consist of two moieties, viz. (i) 2, 5-dimethyl 6-amino pyrimidine (simply referred as pyrimidine) and (ii) 4-methyl-5-hydroxyethyl thiazole commonly called as thiazole. These two components can be chemically or biologically made

to couple leading to the synthesis of thiamine. Its chemical structure as well as those of its two components is represented below.

Information on the synthesis, occurrence as well as history of this vitamin is available from Williams and Stries (1938), Rosenberg (1942) and Schopfer (1943).

**Metabolic role**: In the form of thiamine pyrophosphate (TPP), this vitamin is long known to perform the functions of coenzyme catalyzing the decarboxylation of Ŭ-keto-glutaric acid etc. Role of TPP in the pyruvate decarboxylation in fungi is evident from accumulation of pyruvate in thiamine deficient cultures (Haag, 1940; Writh and Nord, 1942; Friend and Goodwin, 1945).as well as enhanced ethanol production in its presence (Dammann *et al.,* 1938; Schopfer and Guilloud, 1945). TPP is also a coenzyme in transketolation reactions of pentose-phosphate pathway (Jensen, 1954) and helps in the transfer of the glycoaldehyde moiety to the aldose. Thiamine has also been reported to promote cytochrome synthesis in *Ustilago sphaerogena* (Grimm and Allen, 1954) and prevents oxalate accumulation (Nagate *et al.,* 1954).

**Fungal requirements:** Thiamine is required by the largest number of fungi, and possibly on this account, it was the first vitamin to be demonstrated as essential for *Phycomyces blakesleanus* (Schopfer, 1934; Burgeff, 1934). Subsequent studies on thiamine requirement of fungi have shown that majority of fungi belonging to diverse taxa are auxoheterotrophic for this vitamin. Among Phycomycetes, the number of thiamine deficient species is not very large, but considerable. Most of the species of *Phytopthora* (Robbins, 1938) and *Phycomyces* (Leonian and Lilly, 1938; Robbins, 1938b; Robbins and Kavanagh, 1938a) as well as *Mucor ramannianus* (Muller and Schopfer 1937; Muller 1941), *Allomyces kniepii* (Quantz, 1934), *Blakeslea trispora* (Leonian and Lilly, 1938) and *Blastocladiella emersonii* (Barner and Cantino, 1952) require thiamine. On the contrary, species of *Mortierella* (Robbins and

Kavanagh, 1938 b) and *Rhizopus* (Schopfer, 1935) are compleely auxoautotrophic barring a few exceptions only.

Thiamine requirement of Ascomycetes has not been investigated very much and has mostly been confined to yeasts. Available reports suggests that yeasts as well as some other, Ascomycetes show multiple vitamin requirements including that of thiamine, *e.g. Saccharomyces*, *Kloeckera brevis, Zygosaccharomyces japonicas* (Burkholder et al., 1944), *Rhodotorula aurantiaca* (Robbins and Ma 1944*), Ermothecium ashbyii* (Schopfer amd Guilloud, 1945), *Chaetomium* (Lilly and Barnett 1949), *Trichophyton* (Robbins and Ma 1945), *Glomerella* (Srinivasan and Vijayalakshmi, 1960), *Sordaria* (Fields amd Maniotis, 1963), etc. Thiamine heterotrophy is most common among Basidiomycetes. Most of the species investigated under the following genera were found to be thiamine deficient; *Boletus* (Melin and Nyman, 1940, 1941; Melina and Norkrans, 1942), *Clitocybe* (Lindenberg, 1946 a), *Coprinus* (L. Fries, 1945, 1955), *Exobasidium* (Sundstrom, 1960), *Marasmius* (Lindenberg, 1944), *Mycena* (Fries, 1949), *Peniophora* (Fries, 1950), *Polyporous* (Fries, 1938; Noecker, 1938), *Tricholoma* (Norkrans, 1950), *Lactarius* (Jayko *et al*., 1962) etc. Sadasivan and Subramanium (1954) have listed several fungi, which are either partially or totally deficient for thiamine. Many of the imperfect fungi have also been reported to be thiamine requiring. Some important one includes species of *Phyllosticta* (Bilgrami, 1963; Tandon, 1967), *Gloeosporium* spp., *Colletotrichum papaya* and *Pestalotia mangiferae* (Tandon, 1967), *Pestalotia pauciseta* and *Botryodiplodia theobromae* (Prasad, 1966), *Sclerotium rolfsii* (Sahani, 1967), some strains of *Colletotrichum* (Singh, 1973) and *Cercospora cruenta* (Janadaik and Kapoor, 1972).it has been observed that different fungal species differ in their mode of thiamine requirements. While few fungi require the intact thiamine molecule for their optimum growth, majority of them can do equally well or even better (Norkrans, 1950) when the two components of the this vitamin are supplied separately

in equimolar concentrations. Many fungi are even capable of doing away with one or the other component of the vitamin, which obviously indicate that such organisms have not only the capacity to synthesize the other moiety of this vitamin but they are also able to bring about a coupling of the two moieties and synthesize thiamine, because none of the moieties is individually active as vitamin. Available reports (Robbins and Kavanagh, 1942, 1944; Cochrane, 1958) suggest that ability to synthesize pyrimidine moiety is less common which is indicated by the requirement of pyrimidine by a large number of fungi. Thiazole, on many of the fungi is capable to synthesize this component of the vitamin. Biosynthesis of thiamine has often been supposed to be simple and direct condensation phenomenon of its two components, viz. pyrimidine and thiazole. However, evidences though indirect, have been adduced suggesting an indirect pathway of its biosynthesis (Harris, 1956), which may be schematized as below:

Pyrimidine + thiazole precursor ⟶ thiamine- like intermediate ⟶ thiamine

In contrast to its general role as a growth promoting factor, certain fungal species respond to thiamine with growth inhibition or they destroy or inactivate atleast a part of this vitamin. Inhibition of growth due to addition of thiamine has generally been recorded in fungi auxoautotrophic for this vitamin, including species of *Ciborinia* (Lilly and Barnett, 1948 b), *Fusarium* (Writh and Nord , 1942; Elliott, 1949; Esposito et al., 1962, Mathur *et al.,* 1964) and *Rhizopus* (Schopfer, 1935; Robbins and Kavanagh, 1938), *Colletotrichum lindemuthianum* (Mathur *et al.,* 1950). Such response has however, been suggested (Schopfer and Guilloud, 1945) to be an effect of accelerated production and accumulation of ethyl alcohol due to increased availability of thiamine, because thiamine pyrophosphate (cocarboxylase) is the coenzyme in pyruvate decarboxylation. Inactivation or destruction of this vitamin has been recorded in two different fungi viz. *Phycomyces blakesleeanus* and *Sclerotium rolsfii*. Both these fungi are heterotrophic for thiamine and are able to utilize both

intact thiamine as well as its two moieties. Irrespective of the form in which this vitamin is supplied to these fungi, a part of thiazole is either destroyed or inactivated through the activity of an enzyme. The temperature relation of this enzyme may possibly explain the observation that thiamine is more active as a growth regulator at lower temperatures (Robbins and Kavanagh, 1944).

**Riboflavin (Vitamin $B_2$)**

**Structure:** Riboflavin has the empirical formula $C_{17}H_{20}N_4O_6$ and chemical name 6, 7-dimethyl-9-(1-D-ribityl)-isoalloxazine.

Riboflavin was first identified in 1935 by Kuhn and Karrer as prosthetic group of an enzyme isolated from yeast by Warburg and Christian in the year 1932. This enzyme could oxidize NADPH and had riboflavin 5ô-phosphate (flavin mononucleotide, PMN) as its prosthetic group. Subsequently yet another riboflavin derivative, *viz.* flavin adenine dinucleotide (FAD) was found to act as the coenzyme. Many riboflavin containing enzymes are now known. Bothe these active forms of riboflavin viz. PMN and FAD are produced by phosphorylation reactions with ATP under the influence of specific enzymes as shown below:

$$\text{Riboflavin} + \text{ATP} \xrightarrow[Mg^{++}]{\text{flavokinase}} \text{flavin mononucleotide} + \text{ADP}$$

$$\text{Flavin mononucleotide} + \text{ATP} \xrightarrow[Mg^{++}]{\text{flavin nucleotide pyrophosphorylase}} \text{flavin adenine dinucleotide} + \text{PPi}$$

**Methanolic role:** Riboflavin is now known to comprise the prosthetic group of a multitude of oxidizing enzymes, known collectively as flavin enzymes and thus plays a fundamental role in metabolism. The flavoproteins (flavin containing enzymes) perform the important function

of deoxidizing the reduced NADH or NADPH, and thus ensure the cell, an interrupted availability of these coenzymes in oxidized form ($NAD^+$ and $NADP^+$) , which in turn are essential for the functioning of the dehydrogenises they belong to. The FMN or FAD, which get reduced in the process, are reoxidized by one of the cytochrome enzymes, which are heme-proteins. Some of the flavoprotein dehydrosgenases are, however, unable to negotiate directly with the cytochrome chain, and a specific enzyme, *viz.* electron transferring flavoprotein, mediates in such cases by accepting and donating electrons from the former the latter. A few flavoproteins may even be reoxidized directly by $O_2$ and are autoxidizable. These enzymes are designated as aerobic dehydrogenases. *Penicillium notatum* and *P. resticulosum* are known to produce a glucose oxidase, which is an aerobic dehydrogenase. The D- and L-amino acid oxidases are also flavin containing aerobic dehydrogenases.

Riboflavin derivatives constitute the prosthetic groups of several other enzymes alo, which include non autoxidizable dehydrogenases like succinic dehydrogenase, and the cytochrome-linked lactic dehydrogenase. Some of the flavoproteins are also metaloroteins, containing molybdenum, iron, copper *etc.* A molybdoflavoprotein catalyzes the reduction of nitrate, whereas a copper containing flavoprotein acts as nitrate reductase.

No other role is known for riboflavin either in fungi or any other biological system, except for some indirect effect on synthesis of compounds like carotenoids (Zalokar, 1954, 1955), nicotic acid (Dalgliesh, 1955) *etc.*

**Fungal requirements:** Fungi apperar to be more or less autoauxotrophic for riboflavin, as there is until now only a singular report of riboflavin-heterotrophy among fungi. Jennison *et al* (1955) reported that *Poria vaillantii* requires an external source of riboflavin. Otherwise riboflavin is known to be synthesized by many yeasts and other related species, and a large number of filamentous fungi. *Ashbya gossypii*, *Ermothecium ashbyii* and *Candida* spp. are

profilic producers of riboflavin and are commercially harnessed for this vitamin. However some riboflavin requiring mutants of *Neurospora* and *Aspergillus* have been isolated and a requirement for this vitamin has been shown in slime molds, *viz Dictyostelium* spp., (Sussman, 1956) and several bacterial species particularly lactobacilli. This has aroused fresh interested in the fungal requirements of this vitamin which need further attention.

**Pyredoxine:**

**Structure:** Pyredoxine was isolated from liver cells in the year 1938 and was synthesized a year later in 1939. Subsequently it was observed that two of its closely allied derivatives viz. pyridoxal and pyridoxamine were also or even more active as vitamin. All these three compounds are together referred to as vitamin $B_6$, as they differ only slightly in their structure, i.e., in the presence of either a primary alcohol or an aldehyde or a primary amine group in their molecule

**Metabolic role:** Many metabolic transformations of amino acids, like decarboxylation, transmination, synthesis of tryptophan etc. require pyridoxal phosphate as the coenzyme. It is, therefore, suggested that pyridoxine might not be acting directly as vitamin; rather it might be functioning as a precursor of pyridoxal, which after phosphorylation by ATP, yields the coenzyme pyridoxal phosphate.

In *Neurospora crassa* pyridoxal phosphate has been shown to participate in a variety of enzymatic reactions (Yanofsky, 1932; Umbreit *et al.*, 1947; Strauss, 1951; Reissing, 1952) including reduction of nitrite (Silver andMcElory, 1954). Evidences obtained from animal cells indicate that pyridoxal or its phosphate esteralso plays a fundamental role in active transport of amino acids and metel ions across cell membranes, serving as carrier.

**Fungal Requirements:** Several fungi belonging to Ascomycetes and Fungi imperfecti have been reported to require this vitamin as a growth factor but the list of such organisms is far more concise than that for thiamine. Pyridoxine requirement for the fungi was first demonstrated for *Saccharommyces cerevisiae* (Schultz *et al*., 1938) and was soon extended for several species of yeast (Schultz et al., 1939; Eakin and Williams, 1939; Burkholder, 1943; Snell and Rannefeld, 1945). Among the filamentous fungi pyridoxine-heterotrophy has been reported in *Ophistoma* spp. (Fries, 1942, 1943; Robbins and Ma, 1942 b, c), *Trichophyton discoides* (Robbins *et al.,* 1942), *Ceratocystis pilifera* (Leaphart, 1956), *Leptographium* spp. (Leaphart, 1956), *Colletotrichum capsici* (Mishra and Mahmood, 1961) and *C. gloeosporoides* (Prasad, 1966) *etc.*

The lone report of a pyredoxnine requiring basidiomycete concerns Ustilago maydis which utilizes vitamin $B_6$ and exhibits enhanced synthesis of indoleacetic acid from tryptophan (Alighisi *et al*., 1946). The three constituents of vitamin $B_6$ *viz*. pyredoxin, pyredoxal and pyridoxamine appear to be of almost similar value to fungi (Snell and Rannefeld, 1945; Melnick *et al.,* 1945) although further investigations on this aspect may be more revealing, particularly because some of the bacteria utilize them differently. Also *Saccharomyces cerevisiae* attains best growth on pyridoxine and some pyredoxine specific mutants of *Ophiostoma multiannulatum* have been reported (Wikberg, 1959). Pyredoxineless mutants of *Neurospora crassa* and *N. sitophila* have also been obtained, but their requirement for this vitamin is reported to be conditioned by various factors like presence or absence of thiamine (Stokes *et al*. 1943; Tatum and Bell, 1946), pH of the media (Strauss, 1951), etc.

**Nicotinic Acid (Niacin):**

**Structure:** Nicotinic acid, an oxidation product of nicotine has long been identified as a part of the phosphopyridine coenzymes NAD and NADP. In fact, its metabolic role through these

coenzymes was anticipated well before its nutritional significance was authentically established. It is believed that the active form of nicotinic acid is nicotinamide, although different organisms exhibit varying capacity to transform nicotinic acid into its amide, and also the enzyme catalyzing such transformation has not yet been isolated. The structure of nicotinic acid is as shown below:

**Metabolic role:** As component of NAD and NADP the nicotinamide, which is the active biological derivative of this vitamin, participates in essentially all the oxidation reduction reactions occurring within the living cells. Also it is due to the nicotinamide, which the coenzymes NAD and NADP are capable of being reversibly oxidized and reduced and thereby serve as oxidizing and /or reducing agents. No other metabolic role has been assigned to this vitamin.

**Fungal requirement:** Niacin heterotrophy has been frequently reported both in yeasts and filamentous fungi, and the deficiency appears to be more common among, the former. A number of yeasts, including *Torula, Mycotorula*, *Candida Kloeckera* as well as *Saccharomyces* have been reported to niacin deficient (Burkholder, 1943; Burkholder *et al*., 1944; Wright, 1943; Miyashita *et al.,* 1958). Rogosa (1943) found that all the 114 strains of yeasts that he studied, were niacin deficient. Leonian and Lilly, (1942) reported that *Saccharomyces cerevisiae* exhibited strainal differences with regard to their requirement for this vitamin. The fact that the filamemtous fungi were niacin deficient was discovered rather late. Cantino (1948) reported *Blastocladia pringsheimii* as completely deficient for niacin. Since then some more phycomycetes fungi, including *Blastocladia ramose* (Crasemann, 1957), *Phlyctorhiza variabilis* (Rothwell, 1956) etc. have been added to the list. Some other filamentous fungi reported to require this vitamin either belong to Ascomycetes, *e.g.* Venturia inaequalis (Fothergill and Ashcroft, 1955), Trichphyton equinum (Georg, 1949 a), Glomerella cingulata (Struble and Keitt, 1950); or to imperfect fungi, e.g. *Microsp orum*

*audouini* (Area Leao and Cury, 1950). In basidiomycetes, however, niacin deficient fungi are yet to be recorded, although *Pholiota aurea* is able to grow with niacin as the only growth factor (Bach, 1956) and niacin less mutants may be isolated from niacin independent population of *Polyporous abietinus.*

Niacin less mutants are rather easy to induce, and in fact induced or spontaneous mutants for this trait have been isolated in various fungi, including *Ophiostoma multiannulatum* (Fries, 1948), *Glomerella cingulata* (Andes and Keitt, 1950), *Neurospora crassa* and *Aspergillus niger* the last two being very much helpful in studies relating to the pathway for niacin biosynthesis.

**Biotin**

Structure: Biotin also belongs to the B ï complex family of vitamins. Isolated from egg yolk, Kogl and Tonnis (1936) recognized its growth promoting activities and named it as Biotin. Earlier it was designated as co ï enzyme R, because it was found necessary for growth and respiration of bacterium *Rhizobium*. Williams et al., 1940) reported it as a essential growth factor for certain yeast. Structure of this vitamin was established by Du Viegneaud *et al.,* 1942a and its synthesis was achieved by Harris et al., (1943) Biotin molecule comprises a single tetra hydro thiophene ring with a side chain of 4 ï methyl groups. Although in some related compounds the number of methyl groups may be less (Norbiotin with 3) or more than (homobioton with 5).

**Metabolic role**

Data obtained from studies with Biotin indicate that this vitamin has some definite role to perform in a variety of cellular process in fungi, although much remains to be understood regarding the manner in which Biotin participates in this reaction. Generally this vitamin has

been associated with reactions involving fixation of $CO_2$ into large organic molecules. Gyorgy 1954 suggested that Biotin controls the synthesis of aspartic acid either during carboxylation of pyruvic acid or during conversion of oxaloacetic acid to aspartate.

$$\text{Phosphophenol pyruvic acid} + CO_2 \xrightarrow[\text{Carboxylase}]{\text{Phosphopyruvate}} \text{Oxaoacetic acid} \rightarrow \text{Aspartic acid}$$

**Fungal requirement**

So far Biotin requirement among fungi appears to be next only to thiamine and is more common among the yeast than the filamentous form. However auxo heterotrophic specie belonging to all the major taxonomic groups of mycelial forms is already on the record. It has been observed that Biotin requiring fungi exhibit some characteristic response to other growth factors. Generally Biotin deficiency is accompanied by thiamine heterotrophy. Those exhibiting such behavior include *Helminthosporium solani* (Singh 1973), *Gleoesporium musarum, G. papaya* and *Colletotrichum papaya* (Tandon 1967), *Pestalotia pauciseta* (Prasad, 1966) *Colletotrichum gleosporoides* (Singh and Prasad, 1967), *Phyllosticta bauhiniae, P. caricapapayae* and *P. pendanicola* (Bilgrami, 1963). However Biotin occurs naturally as Biocytin (ļ -N-Biotnyl ï 1 ï lysine). In many biological material which also has been reported to be equally active for various fungi, including Neurospora carassa and a Biotin less mutant Penicillium chrysogenium (Wright *et al.,* 1952). Isariacretacea (Taber and Vining 1959) and *Saccharomyces carlsbergensis* (Wrigth *et al.,* 1951).

**Folic acid**

The nutritional factor was first obtained from the leaves of spinach and was according designated as ñFolic acidò (L. folium). Its structure was however elucidated from a sample obtained from a liver and is as shown below

The molecule consists of three different compounds viz. Glutamic acid, p ï aminobenzoic acid and a substituted pterin. Pterin and p- aminobenzoic acid are combindingly called pteroic acid. Folic acid is known to occur in different biological material in variety of forms, with variations in its component as well as in mode of their linkage. Some of the folic acid species contain more than one glutamic acid molecule for example 3 in pteroyltriglutamic acid and 7 in pteroylhepraglutamic acid. Various other folic acids like Biopterin, rhizopterin leucovorin are also known.

**Metabolic role**

Metabolic reactions involving various amino acids yield the so called 1 ï carbon fragment which constituents a pool of reactive C -1 intermediate. This 1 ï carbon units are found in the form of formyl derivates of folic acid which acts as co ï enzymes in reactions involving transfer of 1 carbon compound during the synthesis of various cell constituents. For example $N^5$, $N^{10}$ ï methelene tetra hydo folic acid is known to act as the co ï enzyme in glycin and serine inter conversions. Under the catalytic influence of various derivatives of tetra hydro folic acid. 1 ï carbon units play a significant metabolic role and contribute to biosynthesis of creating, methyl nicotine amide, histidine and purine.

Role of folic acid in these biosynthetic reactions in fungi was demonstrated by Cutts and Rainbow 1950 and Nymn and Fries 1962 who reported that a mixture of amino acids and purines could substitute a requirement of p ï amino benzoic acid in some fungi.

**Fungal requirement**

Heterotrophy for folic acid as such does not seem to prevail among the fungi and unlike bacteria, fungi generally seems to auxo autotrophic for this growth factor. However the ability of synthesis folic acid is conditioned among a few fungi by availability of the folic

acid precursor p ï amino benzoic acid (PABA). This is because of few fungi including Rhodotorula (Robbins and MA 1944: Hasegawa and Banno 1959: Nymn and Fries 1962: Ahearn *et al*., 1962), a strain of *Saccharomyces cerevisiae* (Rainbow 1948) and *Blastocladia pringsheimii* (Crasemann 1957) have been found to be deficient for PABA and thus they require a extraneous supply of PABA for the synthesis of Folic acid. PABA deficient mutants have also being artificially induced in many fungi (Fries 1945: Bonner 1946: Giles 1946: Iquchi 1952: Pontecorvo *et al.,* 1953).

**Table: 3.10 (a) Effect of different vitamins on growth of 4 foliicolous fungi after 15 days incubation**

| Vitamin - B | * Conc. µg/l | *Pestalotiopsis disseminata* | | *Pestalotiopsis maculans* | | *Phomopsis tectonae* | | *Lasiodiplodia theobromae* | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dry wt. (g)* | Final pH* | Dry wt. (g)* | Final pH* | Dry wt. (g)* | Final pH* | Dry wt. (g)* | Final pH* |
| **Thiamine Vit $B_1$** | 50 | 0.094 ± 0.025 | 6.86 | 0.044 ± 0.024 | 6.1 | 0.091 ± 0.024 | 6.9 | 0.056 ± 0.046 | 6.93 |
| | 100 | 0.079 ± 0.014 | 6.83 | 0.046 ± 0.039 | 6.06 | 0.074 ± 0.039 | 6.9 | 0.067 ± 0.020 | 7.3 |
| | 150 | 0.097 ± 0.051 | 7.96 | 0.042 ± 0.031 | 6.06 | 0.08 ± 0.031 | 6.7 | 0.068 ± 0.022 | 6.83 |
| | 200 | 0.055 ± 0.033 | 7.83 | 0.042 ± 0.030 | 6.1 | 0.076 ± 0.030 | 7.0 | 0.065 ± 0.013 | 6.66 |
| **Pyredoxin Vit $B_6$** | 50 | 0.137 ± 0.026 | 7.06 | 0.240 ± 0.139 | 6.0 | 0.122 ± 0.096 | 5.86 | 0.112 ± 0.074 | 7.0 |
| | 100 | 0.139 ± 0.023 | 7.1 | 0.207 ± 0.060 | 6.96 | 0.085 ± 0.035 | 6.23 | 0.121 ± 0.026 | 7.0 |
| | 150 | 0.165 ± 0.010 | 7.03 | 0.186 ± 0.043 | 6.86 | 0.073 ± 0.040 | 6.2 | 0.123 ± 0.025 | 7.03 |
| | 200 | 0.164 ± 0.035 | 7.1 | 0.118 ± 0.068 | 6.80 | 0.091 ± 0.045 | 5.56 | 0.11 ± 0.023 | 7.1 |
| **Riboflavin Vit $B_2$** | 25 | 0.186 ± 0.037 | 7.16 | 0.184 ± 0.030 | 7.23 | 0.138 ± 0.053 | 6.9 | 0.122 ±0.021 | 7.9 |
| | 50 | 0.243 ± 0.141 | 7.1 | 0.150 ± 0.052 | 7.53 | 0.166 ± 0.096 | 7.20 | 0.108 ± 0.020 | 7.86 |
| | 75 | 0.144 ± 0.026 | 7.13 | 0.19 ± 0.054 | 7.8 | 0.145 ± 0.050 | 7.43 | 0.089 ± 0.018 | 8.0 |
| | 100 | 0.154 ± 0.060 | 6.9 | 0.172 ± 0.045 | 7.1 | 0.112 ± 0.039 | 7.2 | 0.46 ± 0.009 | 7.76 |
| **Cyano-coblamine Vit $B_{12}$** | 10 | 0.015 ± 0.006 | 4.8 | 0.108 ± 0.003 | 4.83 | 0.066 ± 0.030 | 5.03 | 0.065 ± 0.019 | 5.0 |
| | 20 | 0.017 ± 0.001 | 4.8 | 0.062 ± 0.024 | 4.83 | 0.070 ± 0.013 | 4.69 | 0.057 ± 0.023 | 4.9 |
| | 30 | 0.025 ± 0.009 | 4.8 | 0.047 ± 0.002 | 3.1 | 0.089 ± 0.023 | 5.0 | 0.030 ± 0.017 | 4.93 |
| | 40 | 0.046 ± 0.011 | 4.86 | 0.069 ± 0.023 | 4.2 | 0.113 ± 0.045 | 4.93 | 0.035 ± 0.023 | 4.93 |

* indicates each compound values are based on three replicates

Results were significant at P Ò0.05 level by one way ANOVA

**Table: 3.10 (b) Effect of different vitamins on growth of 4 foliicolous fungi after 15 days incubation**

| | * Conc. µg/l | *Pestalotiopsis disseminata* | | *Pestalotiopsis maculans* | | *Phomopsis tectonae* | | *Lasiodiplodia theobromae* | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dry wt. (g)* | Final pH* | Dry wt. (g)* | Final pH* | Dry wt. (g)* | Final pH* | Dry wt. (g)* | Final pH* |
| **Nicotinic acid (Niacin)** | 25 | 0.140 ± 0.062 | 7.06 | 0.221 ± 0.055 | 7.0 | 0.096 ± 0.028 | 6.26 | 0.121 ± 0.036 | 6.53 |
| | 50 | 0.123 ± 0.008 | 7.0 | 0.132 ± 0.031 | 7.1 | 0.06 ± 0.021 | 6.1 | 0.120 ± 0.020 | 6.5 |
| | 75 | 0.153 ± 0.034 | 7.03 | 0.167 ± 0.058 | 7.1 | 0.07 ± 0.024 | 6.03 | 0.082 ± 0.047 | 6.26 |
| | 100 | 0.121 ± 0.026 | 7.33 | 0.164 ± 0.009 | 7.2 | 0.056 ± 0.027 | 5.96 | 0.136 ± 0.023 | 6.3 |
| **Ascorbic acid** | 25 | 0.127 ± 0.009 | 7.5 | 0.166 ±0.044 | 6.5 | 0.136 ± 0.055 | 6.5 | 0.073 ± 0.064 | 7.53 |
| | 50 | 0.131 ± 0.033 | 7.56 | 0.153 ± 0.013 | 6.9 | 0.104 ± 0.013 | 6.73 | 0.085 ± 0.027 | 7.5 |
| | 75 | 0.119 ± 0.033 | 7.53 | 0.131 ± 0.014 | 6.7 | 0.108 ± 0.014 | 6.53 | 0.11 ± 0.036 | 7.53 |
| | 100 | 0.100 ± 0.012 | 7.53 | 0.158 ± 0.093 | 6.85 | 0.108 ± 0.093 | 6.50 | 0.097 ± 0.034 | 7.53 |
| **Folic acid** | 10 | 0.151 ± 0.010 | 7.53 | 0.198 ± 0.019 | 7.50 | 0.12 ± 0.050 | 6.83 | 0.173 ± 0.065 | 8.0 |
| | 20 | 0.218 ± 0.085 | 7.53 | 0.194 ± 0.030 | 7.53 | 0.129 ± 0.017 | 6.73 | 0.163 ± 0.023 | 8.1 |
| | 30 | 0.203 ± 0.041 | 7.56 | 0.234 ± 0.047 | 7.6 | 0.126 ± 0.028 | 6.6 | 0.156 ± 0.030 | 8.0 |
| | 40 | 0.190 ± 0.043 | 7.63 | 0.222 ± 0.037 | 7.56 | 0.159 ± 0.020 | 6.53 | 0.141 ± 0.019 | 8.0 |
| **Biotin Vit H** | 5 | 0.127 ± 0.024 | 7.5 | 0.161 ± 0.38 | 6.76 | 0.090 ± 0.024 | 6.3 | 0.112 ± 0.010 | 8.03 |
| | 10 | 0.094 ± 0.010 | 7.26 | 0.163 ± 0.019 | 6.66 | 0.123 ± 0.009 | 6.3 | 0.106 ± 0.011 | 8.2 |
| | 15 | 0.140 ± 0.068 | 7.3 | 0.168 ± 0.023 | 6.53 | 0.094 ± 0.023 | 6.3 | 0.104 ± 0.015 | 8.06 |
| | 20 | 0.121 ± 0.015 | 7.46 | 0.194 ± 0.027 | 6.83 | 0.108 ± 0.027 | 6.23 | 0.123 ± 0.034 | 8.06 |
| **Control** | | 0.14 | 7.0 | 0.08 | 7.0 | 0.21 | 6.9 | 0.13 | 7.0 |

* indicates each compound values are based on three replicates

Results were significant at P Ò0.05 level by one way ANOVA

It is evident from Table no.7 that Thiamine inhibited the growth of all four fungi as compared to control this was in agreement with (Esposito *et al.,* 1961) who found out that Thiamine inhibited the growth of *Fusarium roseum* by stimulating the formation of ehtanol. Reports of thiamine inhibition of growth are also common (Elliot, 1949; Robbins and Kavanagh, 1938; Wirth and Nord, 1942), (Shoper and Guilloud 1945) attributed the thiamine inhibition of *Rhizopus* to ethanol accumulation. Unlike these Arya (1996) reported enhanced growth of four *Phomopsis* spp. on this vitamin. Growth of *Phomopsis viticola* and *P. psidii* was better in medium containing Pyridoxine (Arya, 1996) Partial deficiency of this vitamin was observed by Srivastava (1966) for guava and mango isolates of *Lasiodiplodia theobromae.* With increase in concentration of Pyredoxine growth of *Pestalotiopsis disseminata* and *Lasiodiplodia theobromae* was stimulated, whereas growth of *Pestalotiopsis maculans*, *Phomopsis tectonae* was suppressed with increase in vitamin concentration.

Mycelial growth of two species of *Pestalotiopsis* increased with increasing concentration of Nicotinic acid. However, growth reduced at 100 ppm in *Pestalotiopsis* and *Phomopsis tectonae* similar to *Phomopsis pedilanthi* studied by Arya (1996). *Pestalotiopsis maculans* and Lasiodiplodia theobromae grew best in Pyredoxine while *P. disseminata* and *Phomopsis tectonae* on Riboflavin (vit. $B_{12}$)

**Table 3.11: Effect of different vitamins on growth of four foliicolous fungi after 15 days incubation**

| Vitamins | *Pestalotiopsis disseminata* | | *Pestalotiopsis maculans* | | *Phomopsis tectonae* | | *Lasiodiplodia theobromae* | |
|---|---|---|---|---|---|---|---|---|
| | **Dry Wt. (g)*** | **Final pH*** | **Dry Wt. (g)*** | **Final pH*** | **Dry Wt. (g)*** | **Final pH*** | **Dry Wt. (g)*** | **Final pH*** |
| All Vitamins | 0.125 ± 0.016 | 6.79 | 0.101 ± 0.014 | 6.2 | 0.078 ± 0.025 | 6.3 | 0.068 ± 0.27 | 6.55 |
| (-)Thiamine | 0.066 ± 0.013 | 7.0 | 0.112 ± 0.019 | 6.4 | 0.058 ± 0.007 | 6.15 | 0.082 ± 0.013 | 7.2 |
| (-) Pyredoxin | 0.119 ± 0.055 | 6.95 | 0.166 ± 0.039 | 6.63 | 0.083 ± 0.035 | 6.96 | 0.113 ± 0.055 | 7.18 |
| -Riboflavin | 0.105 ± 0.025 | 6.98 | 0.134 ± 0.029 | 6.45 | 0.084 ± 0.012 | 6.2 | 0.074 ± 0.050 | 6.03 |
| (-) Nicotinic acid | 0.094 ± 0.025 | 7.0 | 0.104 ± 0.013 | 7.0 | 0.055 ± 0.012 | 7.1 | 0.083 ± 0.019 | 7.0 |
| (-)Ascorbic acid | 0.168 ± 0.056 | 7.03 | 0.332 ± 0.28 | 6.92 | 0.07 ± 0.068 | 6.11 | 0.126 ± 0.036 | 7.06 |
| (-) Folic acid | 0.138 ± 0.052 | 7.0 | 0.100 ± 0.016 | 6.5 | 0.059 ± 0.020 | 6.23 | 0.137 ± 0.013 | 7.0 |
| (-) Biotin | 0.105 ± 0.026 | 7.0 | 0.149 ± 0.036 | 7.2 | 0.070 ± 0.029 | 7.36 | 0.095 ± 0.039 | 7.36 |
| (-) Cyano coblamine | 0.162 ± 0.061 | 6.95 | 0.218 ± 0.016 | 6.6 | 0.121 ± 0.47 | 6.0 | 0.133 ± 0.051 | 7.0 |
| Control No vitamin | 0.140 | 7.0 | 0.085 | 7.0 | 0.211 | 6.9 | 0.130 | 7.0 |

* indicates each compound values are based on three replicates
Results were significant at P Ò0.05 level by one way ANOVA

An experiment was performed to study the exogenous growth factor requirements of the four foliicolous fungi by removing a single vitamin from the synthetic basal medium óAô singly and comparing with all vitamins added in complete basal medium. Addition of all vitamins to the basal medium suppressed growth of *P. disseminata, P. tectonae* and *L. theobromae* compare to control where no vitamin was added. Removal of Ascorbic acid enhanced the growth of all organisms except *P. tectonae.*

**Table 3.12: Utilization of different sugars incorporated in modified Asthana and Hawker's medium 'A' by four different fungi**

| Fungi | | | **Rhamnose** | **Sucrose** | **Raffinose** |
|---|---|---|---|---|---|
| | | **Days** | **Dry wt.(g)** | **Dry wt.(g)** | **Dry wt.(g)** |
| ***Pestalotiopsis disseminata*** | | 5 | 0.066 ± 0.019 | 0.074 ± 0.047 | 0.084 ± 0.002 |
| | | 10 | 0.110 ± 0.045 | 0.116 ± 0.014 | 0.113 ± 0.010 |
| | | 15 | 0.138 ± 0.002 | 0.141 ± 0.023 | 0.189 ± 0.038 |
| Presence in days | Sucrose/ raffinose | | | | |
| | Glucose | | | | |
| | Fructose | | | | |
| | Galactose | | | | |
| ***Pestalotiopsis maculans*** | | 5 | 0.043 ± 0.005 | 0.104 ± 0.031 | 0.082 ± 0.014 |
| | | 10 | 0.058 ± 0.007 | 0.121 ± 0.049 | 0.129 ± 0.059 |
| | | 15 | 0.114 ± 0.045 | 0.143 ± 0.011 | 0.144 ± 0.014 |
| Presence in days | Sucrose/ raffinose | | | | |
| | Glucose | | | | |
| | Fructose | | | | |
| | Galactose | | | | |
| ***Phomopsis tectonae*** | | 5 | 0.039 ± 0.021 | 0.048 ± 0.014 | 0.075 ± 0.005 |
| | | 10 | 0.081 ± 0.009 | 0.079 ± 0.013 | 0.123 ± 0.040 |
| | | 15 | 0.116 ± 0.041 | 0.104 ± 0.029 | 0.154 ± 0.029 |
| Presence in days | Sucrose/ raffinose | | | | |
| | Glucose | | | | |
| | Fructose | | | | |
| | Galactose | | | | |
| ***Lasiodiplodia theobromae*** | | 5 | 0.049 ± 0.001 | 0.080 ± 0.017 | 0.090 ± 0.012 |
| | | 10 | 0.093 ± 0.010 | 0.108 ± 0.015 | 0.152 ± 0.002 |
| | | 15 | 0.125 ± 0.053 | 0.134 ± 0.024 | 0.180 ± 0.040 |
| Presence in days | Sucrose/ raffinose | | | | |
| | Glucose | | | | |
| | Fructose | | | | |
| | Galactose | | | | |

* indicates each compound values are based on three replicates

Results were significant at P Ò0.05 level by one way ANOVA

**Rhamnose**

Monosaccharides play an important role in the carbohydrate metabolism of fungi. Most of the complex sugars are broken down into simple sugars before they are utilized by fungi. L ï rhamnose is a pentose sugar. It supports growth of large number of fungi

**Sucrose (Plate – V)**

Sucrose with Rf 0.4 is a common disaccharide. It is found in a large number of plants. Scientists have shown that fungi can hydrolyzed sucrose into glucose and fructose and thus it is assimilated through a hydrolytic pathway.

However few fungi like *Myrothecium verucaria* (Mendelôs, 1954) were able to consume this sugar through a known hydrolytic pathway. Chromatographic studies revealed that sucrose, glucose and fructose were present upto 6 days in case of *Lasiodiplodia theobromae.* Glucose was completely utilized within 10 days, while fructose in 12 days by L. theobromae.

In *P. disseminata* sucrose was present upto 4 days, glucose up to 10 days and sucrose up to 12 days, while in case of *P. maculans* sucrose was present up to 4 days and glucose and fructose upto 10 days. Scientists have found that absence of glucose or fructose may be due to simultaneous utilized by fungi growing in culture.

**Raffinose**

Raffinose is a trisaccharide found associated with many higher plants. Raffinose is usually found to occur in beet root and cotton seeds. A molecule of Raffinose is composed of 3 monosaccharides; glucose, fructose and galactose. It is evident from table 3.12 that raffinose was utilized by all the four pathogenic fungi. After 15 days the growth was less in case of

*Pestalotiopsis maculans*. Raffinose was broken into simpler compounds within 2 - 4 days. Presence of fructose was recorded upto 10 days in case of *P. maculans* and 12 days in case of *P. disseminata* and *L. theobromae*. It could not be utilized by completely by *P. tectonae* in 15 days.

## References


Abdel-Fattah, H. M., Moubasher, A. H. and Abdel-Hafez, S. I. (1977) Fungus flora of root and leaf surface of broad bean cultivated in Oases, Egypt. *Naturalia Monspeliensia Ser. Bot.* 27: 167-177.

Abdel-Hafez, S. I. (1981) Phyllosphere and phylloplane fungi of wheat cultivated in Saudi Arabia. *Mycopathologia* 75: 33-38.

Abdel-Hafez, S. I. (1984) Rhizosphere and phyllosphere fungi of four fern plants growing in Saudi Arabia. *Mycopathologia* 85: 45-52.

Abdel-Hafez, S. I. (1985) Leaf surface fungi of *Argemone mexicana* growing in Saudi Arabia. *Cryptogamie, Mycol*. 6: 69-78.

Abdel-Hafez, S. I., El-Said, A. and Gherabawy, Y. A. (1995) Mycoflora of leaf surface stem, bagasse and juice of adult sugarcane (*Saccharum officinarum* L.) plant and cellulolytic ability in Egypt. Bull. Fac. Sci., Assiut Univ. 24: 113- 130.

Abdel-Hafez, S. I., El-Said, A. H. M. and Gherabawy, Y. A. (1995) Seasonal variations of air, leaf and stem surfaces of sugarcane and amylolytic ability in Egypt. Bull. Fac. Sci., Assiut Univ. 24: 153-179.

Abdel-Kader M. I., Moubasher, A. H. and Abdel-Mallek, A. Y. (1984) Selective effect of the systemic insecticide phosphamidon on soil-surface and leaf surface fungi. *Mycopathologia* 84: 151-158.

Afolayan A.J., Grierson D.S., Kamizi L, Madamombe I., Masika P.J. (2002) *In vitro* antifungal activity of some South African medicinal plants. *South African J. Bot*. 68: 72-76.

Agarwal AK, Chauhan S. (1988) A new species of the genus *Pestalotiopsis* from Indian soil. *Indian Phytopathol* 41:625ï 627

Agrios G.N. (2000) Significance of plant diseases. In: *Plant Pathology*. Academic Press, London pp. 25-37.

Ahmad V.U., Fizza K.Z., Khan MA, Farooqui T.A. (1991) *Phytochemistry*, 30: 689.

Akai S. (1951) On the anatomy of the necrotic lesions on the leaves and stems of the plants, ñMem. Coll. Kyoto Univ. 61: 1 ï 30.

Akrofi A.Y. and Amoah F.M. (2009) *Pestalotia* spp. causes leaf spot of *Vitellaria paradoxa* in Ghana, *African Journal of Agricultural Research* 4:4, pp 330 ï 333.

Alam, M.S. Begum M. F., Sarkar, M.R. Islam M.R. (2001) Effect of temperature, light, and meida on growth, sporulation, formation of pigments and pycnidia of *Botrydiplodia theobromae Pat. Pak. J. Biol. Sci.* 4: 1223 ï 1227

Anonymous (1989) The Ayurvedic Pharmacopoeia of India. Ministry of Health and Family Welfare, Department of Health, Govt. of India, New Delhi,(3) pp. 163-165.
Anonymous (1999) State of forest Report FSI, Dehradun

Arya A. (1983) Phytopathological Techniques In: Arun Arya D.Phil. thesis, Allahabad University

Arya, C. & Arya A. (2002) Some new leaf spot diseases of Social Forestry trees ï I. *J. Mycol. Pl. Pathol,* 32 ( 2): 260-262.

Arya A., Bedi S.J. Jasrai Y.T., Patel V.S. (2005) Ecofriendly approach to control fungal bioderiogens.In: *Urban Pollution issues and solutions* pub. By Nidhi pub. New Delhi.pp. 50 ï 56.

Bae E.Y. Shin E.J., Lee D.H. Hoh Y.J., Kin J,H. (1997) Antifungal Kaempferol-3-O-ɓ-D-apiofuranosy 1-(1,2)-ɓ-D-glucopyranoside from leaves of *Phytolacca americana* L. *Korean J. Plant Pathol.* 13: 371-376.

Bajwa R., Shafique S., Shazia S. (2008) Fungitoxicity of aqueous and organic solvent extracts of *Datura metel* against *Ascochyta rabiei, Mycopath*, 6: (1 and 2): pp. 17 ï 22

Bakshi B.K (1976) Forest Pathology Principles & practices in forestry. Pub. by F. R. I. Dehradun, pp.400.

Barnett E.L. (1971) Illustrated genera of fungi imperfect fungi, pub. By Burghes pub. Company. Pp. 225.

Beg A.Z., Ahmad I. (2002) In vitro fungitoxicity of the essential oil of *Syzygium aromaticum. World J. Microbiol. Biotechnol*. 18: 313-315.

Bhatti, M.H.R. (1988) Antifungal properties of plant leaf decoction against rust of wheat, *Pak. J. Bot.* 20: 259 ï 263

Bhowmick B. N. and Chaudhary B.K. (1982) Antifungal activity of leaf extracts of medicinal plants on *Alternaria alternata. Ind. Bot. Rep*. 1: 164 ï 165.

Bianchi A, Zambonelli A, DôAulerio A.Z., Bellesia F (1997) Ultrastructural studies of the effects of *Allium* sativum on phytopathogenic fungi i*n vitro. Plant Disease.* 81: 1241-1246.

Bilgrami K.S. and Verma R.N. (1978) Vitamins and growth factor in fungal nutrition. In: *Physiology of fungi* pp. 269 ï 293.

Bilgrami K.S. and Dubey H.C. ( ) Leaf spots and leaf blights In*: A textbook of Modern Plant Pathology* pp. 297 - 311

Bilgrami K.S. Jamalludin & Rizvi M.A (1979) *Fungi of India Part –I* Today and Tomorrows Printers& Pub., New Delhi, pp.467.

Bilgrami K.S. Jamalludin & Rizvi M.A (1981) *Fungi of India Part –II* Today and Tomorrows Printers & Pub., New Delhi, pp.128

Brady, N.C. (1984) The Nature and properties of soil. MacMillan Publishing Company, NewYork, pp. 528

Broxton P., Woodcock P.M., Gilbert P. (1983) A study of the antibacterial activity of some polyhexamethylene bigusnides towards *E. coli* ATCC 8739. *J. Appl. Bacteriol.* 54: 345-353.

Burgeff H. (1934) Pflanzliche Avitaminose undihre Behebung durch Titaininzufuhr Vorlaufige Blitteilung *Ber. Deut. Botan. Ges*. 52: 384-390.

Burkholder P.R. and Moyer D. (1944) Vitamin requirements of certain yeasts and bacteria, *J. Bacteriol.* 48: 385-391

Burkholder P.R. and Moyer M. (1943) Vitamin deficiencies of fifty yeasts and molds. *Bull. Torrey Botan. Club.* 70:372-377

Butler E.J. and Bisby G.R. (1960) The Fungi of India, ICAR, New Delhi.Pp.550.

Cantino E.C. and Horenstein E.A. (1955) The role of ketoglutarate and polyphenol oxidase in the synthesis of melanin during morphogenesis in *Blastocladiella emersonii. Physiol Pl.* 8:189 -221

Carder J.H., Hignett R.C., Swinbrune T.R. (1987) Relationship between the virulence of hop isolates of *Verticillium alboatrum* and their in *vitro* secretion of cell wall- degrading enzymes. *Physiol. Mol. Plant Pathol.* 31: 441-452.

Chen YX, Wei G, Chen WP (2002) New species of *Pestalotiopsis. Mycosystema* 21:316ï 323.

Chitra H., Gomathi V., Kannabiran B. (2001) Inhibitory effect of *Datura innoxia* on the enzymatic activities of the anthracnose fungus *Collectotrichum capsici in vitro*. *Indian Phytopath.* 54: 253-255.

Cochrane V.W. (1950) The metabolism of species of Streptomyces. III. The nitrate metabolism of *Streptomyces griseu Bull. Torrey. Bot. Club.* 77: 176-180.

Cochrane V.M. (1958) Physiology of Fungi, John Wiley and Sons, Inc, NewYork, pp.524.

Cowan M.M (1999) Plant products as antimicrobial agents. *Clin. Microbiol. Rev*. 12: 564 ï 582.

Dadwal, V.S. and Jamalludin (2004) *Ind. J. Tropical Biodiversity* 12 (1/2): 9-15

Dadwal, V.S.and Jamalludin (2001) A note on basal canker of teak in plantations . *Indian Forester* 127(3): 365-366.

Dalgliesh C.E. (1955) Metabolism of the aromatic amino acids. Advances in Protein Chem. 10:33-150.

Daniel M. (1991) Methods in plant chemistry & economic Botany. Pub. by Kalyani Pub.Ludhiana, pp.209.

Das R., Chutia M., Jha D.K. (2010) Factors affecting Sporulation of *Pestalotiopsis disseminata* causing grey blight disease of *Persea bombycina* Kost. the primary food plant muga silkworm, *Crop Protection*, 29: pp. 963 ï 968.

deBillerbeck V.G., Roques C.G., Bessiere J.M., Fonvielle J.L., Dargent R. (2001) Effects of *Cymbopogon nardus* (L.) W. Watson essential oil on the growth and morphogenesis of *Aspergillus niger. Can. J. Microbiol.* 47:9-17.

Deyrup S.T.,Swenson D.C, Gloer J.B., Wicklow DT (2006) Caryophyllene sesquiterpenoids from a fungicolous isolate of *Pestalotiopsis disseminata. J Nat Prod* 69:608ï 611

Dhaliwal H.S., Thind T.s., Mohan C, Chhabra B.R. (2002) Activity of some essential oils against Uncinula necator causing powdery mildew of grapevine. *Indian Phytopathol.* 55: 529-531.

Didry, N., Dubreuil, L., Trotin, F. and Pinkas, M. 1988. Antimicrobial activity of the aerial parts of *Drosera pellata* smith on oral bacteria. *J.Ethnopharmacol*., 60: 91-96

Ding G., Li Y., Fu S., Liu S., Wei, J. and Che Y. (2009) Ambuic acid and torreyanic acid derivatives from the endolichenic fungus *Pestalotiopsis* sp. *Journal of Natural Products* 72: 182 ï 186.

Elad Y, Kapat, A. (1999) The role of *Trichoderma harzianum* protease in the biocontrol of *Botrytis cinerea. Eur. J. Plant Pathol.* 105: 177-189.

El-Said A. H. M. (2001) Phyllosphere and Phylloplane fungi of Banana cultivated in Upper Egypt and their cellulolytic ability, *Mycobiology* 29(4): 210-217

Eng, F. Guiterrez R. and Favela E. (2003) A survey of temperature and pH effect on colonial growth of *Botrydiplodia theobromae* RC1 *Rev. Iberoam Micol.* 20: 172 ï 175.

Elliott E.S. (1949) Effect of vitamins on growth of some graminicolous species of *Helminthosporium* and *Fusarium, Proc. West. Va. Acad. Sci*. 20: 65-68.

Esposito R.G., Greenwood H. and Fletcher A.M. (1962) Growth factors requirements of six fungi associated with fruit decay, *J. Bacteriol.* 83: 250 ï 255.

Favaron F., Castiglioni C., diLenna P. (1993) Inhibition of some rot fungi polygalacturonases by *Allium cepa* L. and *Allium porrum* L. extracts. *J. Phytopathol.* 139: 201-206.

Fiori ACG, Schwan- Estrada KRF, Stangarlin J.R., Vida J.B., Scapin C.A., Cruz MES, Pascholati S.F. (2000) Antifungal activity of leaf extracts and essential oils of some medicinal plants against *Didymella bryoniae. J. Phytopath.* 148: 483-487.

Fries L. (1945) Ulber das Wachstum einiger Coprinus-Arten bei verschiedenen Wasserstoffionenkonzentrationen. A *Arkiv. Botan*. 32A: No. 10, 1-8.

Grayer R.J., Harbone J.B. (1994) A survey of antifungal compounds from higher plants, 1982-1993. *Phytochemistry* 37: 19-42.

Griffiths DA, Swart HJ (1974a) Conidial structure in two species of *Pestalotiopsis. Trans Br Mycol Soc* 62:295ï 304

Guba E.F. (1956) Monochaetia and *Pestalotia* vs. *Truncatella, Pestalotiopsis* and *Pestalotia*. Ann *Microb Enzymol Milan* 7:74ï 76

Guba E.F. (1961) Monograph of *Pestalotia* and *Monochaetia.* Harvard University Press, Cambridge.

Guimarães JB, Chambel L, Melzoch K, Pereira P, Tenreiro R. 2011 - *Cladosporium* sp. From phylloplane: a diversity evaluation on a Continental ecosystem. *Mycosphere* 2(3), 191-201.

Halfeld-Vieira BA, Nechet KA (2006) First report of *Pestalotiopsis macrochaeta* on *Carapa guianensis. Plant Pathol* 55:304

Harper J. K., Ford E.J., Strobel, G.A., Arif A., Grant D.M. Porco J., Tomer D.P. and Oneill K. (2003) Pestacin; a 1, 3 ï dihydro isobenzofuran from *Pestalotiopsis microspora* possessing antioxidant and antimycotic activities. *Tetrahedron,* 59: 2471 ï 2476.

Hashem M. (2011) Antifungal properties of crude extracts if five Egyptian medicinal plants against dermatophytes and emerging fungi. *Mycopathologia* DOI 10.1007/s11046-010-9390-6

Holb, I.J., Chauhan S.V.S. (2005) Effect of carbohydrate and nitrogen sources on the growth rates of *Monilia fructigena* and *M. polystroma isolates*. *J. Mycol Plant. Pathol*.35: pp 128 ï 131.

Homma Y, Takahashi H, Arimoto Y. (1992) Studies on mode of action of soyabean lecithin Part 3. Effects on the infection process of rise blast fungus, *Pyricularia oryzae. Ann. Phytopathol. Soc*. Japan. 58: 514-521.

Hopkins KE, McQuilken MP (2000) Characteristics of *Pestalotiopsis* associated with hardy ornamental plants in the UK. *Eur J Plant Pathol* 106:77ï 85

Horikawa T (1986) Yield loss of new tea shoots due to grey blight caused by *Pestalotia longiseta* Spegazzini. *Bull Shizuoka Tea Exp Stn* 12:1ï 8

Hussain, I., Nasir M.A. and Haque M. R. (1992) Effect of different plants extract on brown rust and yield of wheat. *J. Agri. Res.* 30: 127 ï 131.

Ilag L.L. and Marfil V.E. (1977) Diplodia pod rot of mungbean, *Philippine Agriculturist*, 61: 486 ï 491.

Jamalludin, Goswami M.G. & Oza B.M. (1989 ï 2001) Fungi *of India,* Scientific Publishers Jodhpur pp.350.

Jash S., Dutta S., Bandyopadhyay and Laha S.K. (2003) Effect of different cultural media, pH and carbon sources on growth and sporulation of *Alternaria zinnia* Pape causing leaf and flower blight of marigold, *Environ. Ecol.* 21: 321 ï 325.

Jennison M.W., Newcomb M.D. and Henderson R. (1955) Physiology of the wood-rotting Basidiomycetes. I. Growth and nutrition in submerged culture in synthetic media. *Mycologia.* 47: 275-304.

Jeewon R., Liew E.C., and Hyde K.D. (2004) Phylogenetic in relation to host association, *Fungal Diversity* 17: 39 ï 55.

Jeyarajan R., Ravidran G., Alagarsamy K., Eraivan K. Aiyanathan A. (1988) Effect of need products on growth and sporulation of fungal pathogens of rice. Paper presented at final workshop of IRRI-ADE-EWC project on botanical pest control in rice cropping systems. Int. Rice Re. Inst. Phillippines, pp. 12 ï 16.

Kand J. C., Hyde K.D. and Kong R.Y. (1999) Studies on Amphisphaeriales (sensu stricto) *Mycological Research* 103: 53 ï 64.

Kapat A., Zmand G., Elad Y. (1998) Effect of two isolates of *Trichoderma harzianum* on the activity of hydrolytic enzymes produced by *Botrytis cinerea. Physiol. Mol. Plant Pathol*. 52: 127-137.

Karavaev V.A., Solntsev M.K., Kuznestov A.M., Polyakova I.B., Frantsev V.V., Yurina E.V., Yurina T.V. (2002) Plant extracts as the source of physiologically active compounds suppressing the development of pathogenic fungi. *Plant Prot Sci*. 38: 200-204.

Kareem, S.O., Akpan, I. and Ojo O.P. (2008) Antimicrobial activities of *Calotropis procera* on selected pathogenic microorganisms, *African J. Biomedical Res*. Vol.11: pp. 105 ï 110.

Kasture A.V., Mahadik K.R., Wadodkar S.G. More H.N.(2004) Chromatography In: *Pharmaceutical analysis* Vol -2, pub ï Nirali rakashan, Pune

Keith L.M., Velasquez M.E., Zee F.T. (2006) Identification and characterization of *Pestalotiopsis* spp. Causing scab disease of guava, *Psidium guajava* in Hawaii. Plant Dis. 90: 16 ï 23.

Khan T.Z., Gill M.A., Nasir M.A. and Bukhari S.A. (1998) Antifungal properties of some plant extracts. *Pak. J. Phytopathol*. 10: 62 ï 65.

Lal A., Gour H.N. (2004) Natual fungicides for management of phyto-pathogenic fungi. In: *Annual Review of Plant Pathology* 3: pp. 331 ï 356.pub. Sceintfiv pub. Jodhpur.

Lee and Hyde (2001) Hong Kong mangroves and phylloplane fungi, *Mycologia,* pp. 595 ï 606.

Letessier M.P., Svoboda K.P., Walters D.R. (2001) Antifungal activity of the essential oil of hyssop (*Hyssopus officinalis*) *J. Phytopath.* 149: 673-678.

Linderberg G. (1946, a) Thiamin and growth of litter-decomposing Hymenomycetes, *Botan. Notiser.* 89-93.

Lisa M., Keith, Maile E., Velasquez and Zee F.T. (2006) Identification and characterization of *Pestalotiopsis* spp. Causing scab disease of guava, *Psidium guajava* in Hawaii. *Plant Dis.* 90: 16 ï 23.

Liu L., Liu S., Chen X., Guo L. and Che Y. (2009) Pestalofones A-E bioactive cyclohexane derivatives from the plant endophytic fungus *Pestalotiopsis fici. Bioorganic and Medicinal Chemistry* 15: 606 - 13

Lorito M., Broadway R.M., Hayes C.K., Woo SL, Noviello C, Williams D.L., Harman G.E. (1994) Protinase inhibitors from plants as a novel class fungicides. *Mol. Plant- Microbe Interact*. 7: 525-527.

Maqbool M.A. Hashmi A., Ghaffar A. (1988) Problem of root knot nematode in Pakistan and strategy for their control. Proc. U.S. Pakistan Intern. Workshop on Plant Nematol. pp.229 ï 240.

Mau J. Chen L., Hsieh P. C. (2001) Antimicrobial effect of extracts from Chinese chive cinnamom and *Corni fuctus. J. Agric. Food Chem*. 49: 183 ï 188.

Meena S.L. and Pandey R.P. (2004) A Reassessment of the phytodiversity of Gujarat state: Floristic composition and floristic analysis, vegetation, threatened and rare taxa and their conservation strategies, *J. Econ. Taxon. Bot*. Vol.4 pp. 867 ï 894.

Melin E. and Nyman B. (1940) Weitere Untersuchungen uber die Wirkung von Aneurin und Biotin auf das Wachstum von Wurzelpilzen. *Arch. Mikrobiol*. 11: 318-328.

Melin E. and Nyman B. (1941) Ulber das Wuchsstoffbediirfnis von *Boletus granulatus* (L.) Fr., *Arch. Mikrobiol*. 12: 254-259.

Mitra S.K. and Tandon R.N. (1970) nitrogen requirements of three species of *Pestalotia, Mycopathologia applicata,* (42)1-2, pp. 9 ï 16.

Nagamani A., Kunwar I.K., Manoharachary C. (2006) Handbook of Soil Fungi, pub by I. K. International, New Delhi. pp 477.

Nagaraja, T. G. (1991) Rhizosphere and phyllosphere studies in *Strychnos nux*-vomica Linn. *Ad. Plant Sci.* 4: 171-173.

Nagraj T.R. (1993) Coelomycetous Anamorphs with Appendage ï Bearing Conidia. Mycologue publications, Waterloo, Ontario, Canada.

Nene, Y. L. & Thapliyal, P. N. (1979) Fungicides in plant disease control, Oxford IBH Pub. New Delhi, pp.507.

Noecker N.L. (1938) Vitamin B in the nutrition of four species of wood-destroying fungi, *Amer. J. Botany*. 25: 345-348.

Norkrans B. (1950 b) Studies in growth and cellulolytic enzymes on *Tricholoma., Symbolae Botan. Upsalienses* 11 (1): 1-126

Nychas GJE. (1995) Natural antimicrobials from plants. In: *New methods of food preservation.* ed. Gould GW, 58-59. Blackie Academic, London, UK.

Pandey K.K, Pandey P.K & Rai M. (2005) Studies on molecular characterization, cultural
Parimelazhagan T. (2001) Botanical fungicide for the control of rice blast disease. *Bioved*. 12: 11-15.

Patacky N. (1998) Fungal Leaf Spot diseases of Shade and Ornamental Trees in the
pathogenic variability of *A. solani* isolates. Global Conference, Udaipur. pp 13

Patil R.K, Sharma A., Pathak V.N. (1992) Inhibition of polyamine biosynthesis in *Botryodiploda theobromae* Pat. and *Rhizopus arrhizus* Fisher by *Ocimum scantum* leaf extract. *Indian J. Mycol. Plant Pathol.* 22: 201-202.

Patil R.K., Patel K.D. Sharma A., Pathak V.N. (1992) Inhibitory effect of *Ocimum sanctum* extract on fruit rot fungi. *Indian J. Mycol. Plant Pathol.* 22: 199-200.

Pawar V.C. and Thaker V.S. (2006) *In vitro* efficacy of 75 essential oils against *Aspergillus niger, Mycoses* 49: 316 ï 323

Phillips AJL, Alves A, Pennycook SR, Johnston PR, Ramaley A, Akulov A, Crous PW.
(2008) Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. *Persoonia* 21: 29ï 55
Pinto CMF, Maffia L.A., Casal;I V.W.D., Cardoso A.A. (1998) *In vitro* effect of plant extracts on mycelia growth and sclerotial germination of *Sclerotium cepivorum. J. Phytopathol.* 146: 421-425.

Pirone P.P. (1978) Diseases and Pests of Ornamental Plants, Wiley Interscience. NewYork.

Prabakaran, M, Merinal, S. and Panneerselvam, A. (2011) Investigation of phylloplane mycoflora from some medicinal plants *Eur. J. Exp. Bio.,* 1 (2):219-225

Prenas M., Solanilla L.E., Monge S.R., Salcedo G., Palemzuela R.P. (1999) Antifugal activity of plant cystin . *Mol. Plant- Microbe Interact.* 12: 624-627.

Punithalingam E. (1980) Plant diseases attributed to *Botryodiplodia theobromae* Pat. Cramer, Vaduz.

Purohit D.K., Bilgrami K.S. (1969) Variations in the conidial morphology of genus *Pestalotiopsis. Indian Phytopathology* 22: 275 ï 279.

Qasem J.R., Abu-Blan H.A. (1996) Fungicidal activity of some common weed extracts against different plant pathogenic fungi. *J. Phytopathol.* 144: 157-161.

Rafiq M.M., Nasir A. and Bhatti M.A. (1984) Antifungal properties of certain common wild plants against different fungi. *Pak. J. Agri. Res*. 5: 236 ï 238.

Rahman A.U., Choudhary M.L., Farooq A., Ahmed A., Iqbal M., Z., Demirci, F. and Baser K.H. (1999) Antifungal activities and essential oil constituents of some spices from Pakistan. Proc. Int. Elctr. Confer. Synth. Org. Chem. (ECSOC-3)

Rai S.N and Chakrabarti S.K. (2001) Demand & Supply of Fuel wood & Timber in India, *Indian Forester* 127(3): 263 ï 279.

Ramdas K., Suresh G., Janardhan N., Masilamani S., (1998) *Pestic. Sci.* 52: 145.

Rangaswami G. (1975) In: *Diseases of crop plants in India,*

Ravindranath N.H & Sudha. P. (2004) Joint Forest Management in India. University Press, Hyderabad pp:1 ï 25

Ray R.C. (2004) Extracellular amylase production of *Botrydiplodia theobromae* and *Rhizopus oryzae* grown on cassava starch residue. *J. Envioren. Biol*. 25; 489 ï 494.

Reischer H. (1951) Growth of Saprolegniaceae in synthetic media. II. Nitrogen requirements and the role of Krebs cycle acids, *Mycologia* 43: 319-328.

Robbins W.J. and Kavanah F. (1938) Vitamin $B_1$ or its intermediates and growth of certain fungi. *Am. J. Botany* 25: 229 ï 263

Robbins W.J. and Ma R. (1945) Growth factors for Trichophyton mentagrophytes., *Amer. J. Bot.* 32: 509-523

Rosenberg H.R. (1942) Chemistry and Physiology of Vitamins, Interscience Publishers, Inc. New York .

Samuel G. (1927) On the short hole disease caused by *Cladosporium carpophium* and on the shoe hole effect, óAnn Botany, London, 41:375 ï 404

Sarbhoy A.K, (2005) Important diseases & medicinal plants in India. Second Global conference, Plant health-Global health, Nov -25-29, Udaipur ï 329-330

Sarbhoy A.K. Agarwal D.K. & Varshney J.L (1977- 81) *Fungi of India*

Schopfer W.H. (1934) Versuche iuber die Wirkung von reinen kristallisierten. Vitamin B1 auf Phycomyces., *Ber. Deut. Botan. Ges.* 52: 308-312.

Schopfer W.H. (1935) Etude sur les facteurs de croissance. Action de la vitamine cristalisee B1 et de l'extrait de germe de ble sur Rhizopus et d'autres Mucorinee., *Z. Vitaminforsch.* 4: 187-206.

Schopfer W.H. (1943) Plants and Vitamins. *Waltham, Mass., Chronica Botanica* Co., p.293.

Schopfer W.H. and Guilloud M. (1945) The nutrient requirements and synthetic activity of *Rhizopus suinus. Z. Vitaminforch*. 16: 181 ï 296.

Sharma S. and Goyal N. (2011) Biological studies of the plants from genus *Pluchea, Annals of Biological Research* 2(3): 25 ï 34.

Sharma S.K., Goyal. N. (2010) *Der Pharmacia Lettre*, 2(5), 308.

Sharma, P. D. (1974) Experimental studies of some micro-fungi from decaying shoots of *Setaria glauca. Trans. Brit. Mycol. Soc*. 63: 397-400.

Signh J., Tripathi N.N (1999) Inhibition of storage fungi of blackgram (*Vigna mungo* L.) by some essential oils. *Flavour Fragrance J.* 14: 1-4.

Singh N.I. (1981) Some new host records for India. *Indian Phytopathology* 34: 233 - 234

Singh R.P. (1993) Botanical Pesticides In: *Developing countries Current Status and Future Trends* (eds Dhaliwal G. S. and Singh) Common Wealth Publishers, New Delhi pp 236 ï 238

Singh R.S. (2005) History of Plant Pathology In: Plant Diseases, Oxford and IBH pub. New Delhi

Singh S., Pandey P.C. and Misra B.M. (1977) Twig blight and pod blackening of Cacao, *Indian Forester,* 103 ï 483 ï 485.

Singh U.P., Singh B., Singh R.B., (1980) The antifungal effect of Neem (*Azadirachta indica*) extracts on some soil borne pathogens of gram *Mycologia*. 72: 1077 ï 1093.

Soundharrajan R. Rajagopal V.R., Ramasamy R., Selladurai M., and Srinivasan N. (2003) Antifungal activity of some essential oils. *J. Agric. Food chem*. 51: pp. 7596 ï 7599.

Srinivasan, D., Nathan, S., Suresh, T. and Perumalsamy, O. (2001) Antimicrobial activity of certain Indian Medicinal Plants used in folkloric medicine. *J. Ethnopharmacol*., 4:217-220.

Steyaert R.L. (1949) Contributions aô lôetude monographic de *Pestalotia* de Not. Et Monochaetia Sacc. (Truncatella gen nov. Et *Pestalotiopsis* gen nov.) Bull jard. Bot. Bruxelles 19: 285 ï 354.

Steyaert R.L. (1953a) New and old species of *Pestalotiopsis. Trans Br Mycol. Soc.* 36: 235 ï 242.

Steyaert R.L. (1955) *Pestalotia Pestalotiopsis et Truncatella. Bull. Jard. Bot. Bruxelles* 25: 191 ï 199.

Steyaert R.L. (1961) type specimens of Spegazziniôs collections in the *Pestalotiopsis* and related genera (Fungi Imperfecti: Melanconiales) Darwinia (Buenos Aires) 12: 157 ï 190.

Strobel G.A. (2002) Rainforest endophytes and bioactive products. Critical Reviews in Biotechnology 22: 315 ï 333.

Sun H.J., Depriest P.T., Gargas A., Rossman A.Y., Friedmann E.I. (2002) *Pestalotiopsis maculans*: a dominant parasymbiont in North American lichens. *Symbiosis* 33: 215 ï 226.

Sun Og Lee, Gyung Ja Choi, Kyong Soo Jang, He Kyoung Lim, Kwang Yun Cho and Jin ï Cheol Kim, (2007) Antifungal activity of Five Plant Essential Oils as Fumigant Against Postharvest and Soil borne Plant Pathogenic fungi *J. Plant. Pathol,* Korea 23:*2*

Sutton B.C. (1980) The Coelomycetes: Fungi Imperfecti with Pycnidia Acervular and Stromata. Commonwealth Mycological Institute, Kew, Surrey, England.

Sutton BC. (1980) The Coelomycetes, Fungi Imperfecti with Pycnidia, Acervuli and stromata. Commonwealth Mycological Institute, Kew, Surrey, England.

Swada T., Yamahara S., Shimazu S., Ohta T. (1971) Evaluation of crude drugs by bioassay III. Comparison with local variation of the content and fungistatic action of the essential oil from the roots of *Curcuma longa* Shoyakugaku Zasshi, 22: 33 ï 517.

Swain, T. (1977) *Ann Rev. Plant Physiol.*:28 p 479 ï 501

Talukdar M.J. (1974) Plant disease in Bangladesh. *Bangla J. Agril Re*. 1: 61 ï 83

Tejesvi M.V., Mahesh B., Nalini M.S., Prakash H.S., Kini K.R., Subbiah V., Shetty H.S. (2005) Endophytic fungal assemblages from inner bark and twig of *Terminalia arjuna* W and A (Combretaceae) *World J. Microbiol. Biotechnol*. 21: 1535 ï 1540.

Tejesvi M.V., Mahesh B., Nalini M.S., Prakash H.S., Kini K.R., Subbiah V., Shetty H.S. (2007a) Genetic diversity and antifungal activity of species of *Pestalotiopsis* isolated endophytes from medicinal plants. *Fungal Divers*. 24: 37 ï 54.

The Wealth of India: (1998) A dictionary of Indian Raw materials and industrial products National Institute of Science communication, CSIR, New Delhi.

Tiwari D.P., Rajak R.C. and Nikhra M. (1981) A New species of *Phomopsis* causing leaf spot disease on *Tectona grandis* L. *Curr. Sci.* Nov.20, 50: (22) pp 1002.

Tomita F. (2003) Endophytes in Southeast Asia and Japan their taxonomic diversity and potential applications. *Fungal Diversity* 14: 187 ï 204.

Trapero M.A., Romero, Varo R., and Sanchez M.E. (2003) first report of *Pestalotiopsis maculans* in Nursery plants of *Arbutus unedo* and *Ceratonia siliqua* in Spain, *Plant disease*. pp 1263.

Trivedi P.C. and Sharma N. (2002) Screening of Leaf extracts of some plants for their nematicidal and fusarial properties against *Meloidgyne incognita* and *Fusarium oxysporum, Asian J. Exp. Sci.* Vol. 16 No. 1and 2, pp. 21 ï 28.

Tuset JJ, Hinarejos C, Mira JL (1999) First report of leaf blight on sweet persimmon tree by *Pestalotiopsis theae* in Spain. *Plant Dis* 83:1070

Ushiki J., Hayakawa Y., Tadano T. (1996) Medicinal plants for suppressing soil borne plant diseases. I. Screening for medicinal plants with antimicrobial activity in roots. *Soil. Sci. Plant Nutr*. 42:423-426.

Varma Jaya and Dubey N.K. (1999) Prospective of botanical and microbial products as pesticides of tomorrow, *Current Science*, Vol 76:2 pp. 172 ï 179.

Venkatasubbaiah P, Grand LF, Dyke CGV (1991) A new species of *Pestalotiopsis* on Oenothera. *Mycologia* 83(4):511ï 513

Vir, D. and Sharma R.K. (1985) Studies on the fungicidal properties of neem oil. *Indian J Plant Pathol.* 3: 241 ï 24

Vitale A, Polizzi G (2005) Occurrence of *Pestalotiopsis uvicola* causing leaf spots and stem blight on bay laurel (Laurus nobilis) in Sicily. *Plant Dis* 89(12):1362

Wijesekara ROB, Ratnatunga C.M., Durbeck K. (1997) *The Dillistation of Essential Oils. Manufacturing and Plant Contructionn Handbook* Eschborn, Federal Republic of Germany: Protrade, Department of Foodstuffs and Agricultural Products.

Wirth J. C., Nord F.F. (1942) Essential steps in the enzymatic breakdown of hexoses and pentoses. Interaction between dehydrogenation and fermentation. *Arch. Biocehm*. 1:143 ï 163.

Xu L, Kusakari S, Hosomi A, Toyoda H, Ouchi A (1999) Postharvest disease of grape caused by *Pestalotiopsis* species. *Ann Phytopathol Soc Japan* 65:305ï 311

Yasuda F, Kobayashi T, Watanabe H, Izawa H (2003) Addition of *Pestalotiopsis* spp. to leaf spot pathogens of Japanese persimmon. *J Gen Plant Pathol* 69:29ï 32

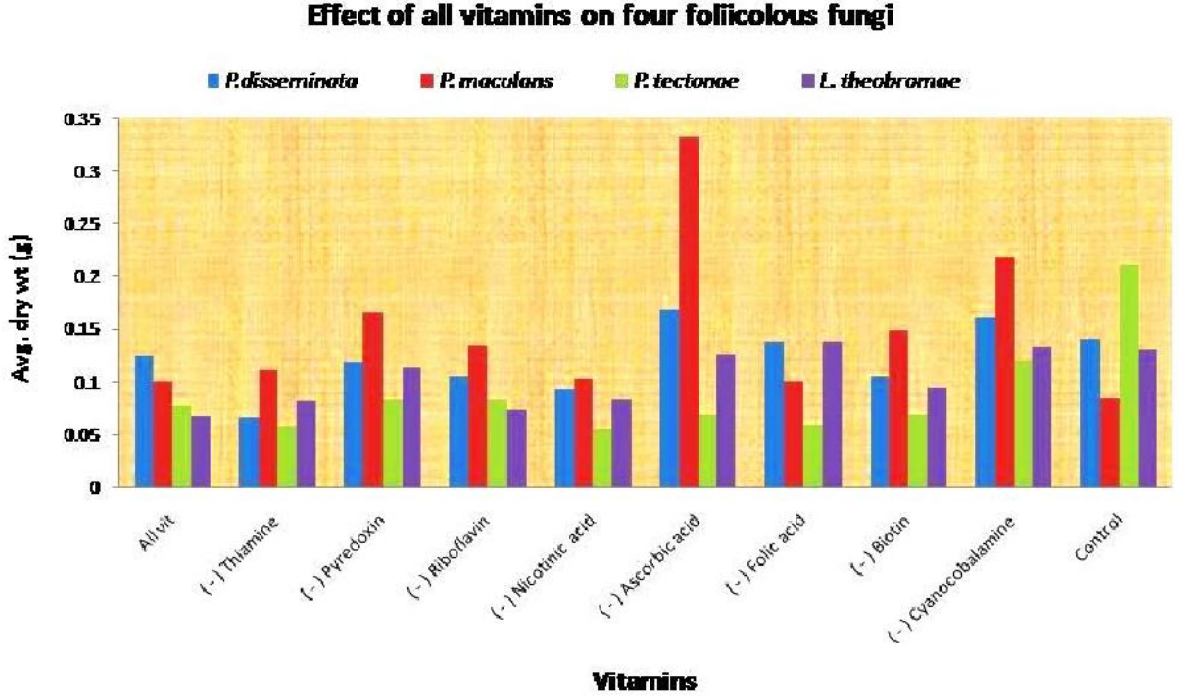
Younis M., Mehmood K., Rashid A. And Ashiq A. (2004) Effect of carbon, nitrogen sources and ascorbic acid on the colony growth and acervulus production of *Pestalotia psydii, Int. J. Agri. Biol*. Vol.6 No. pp.1110-1112.

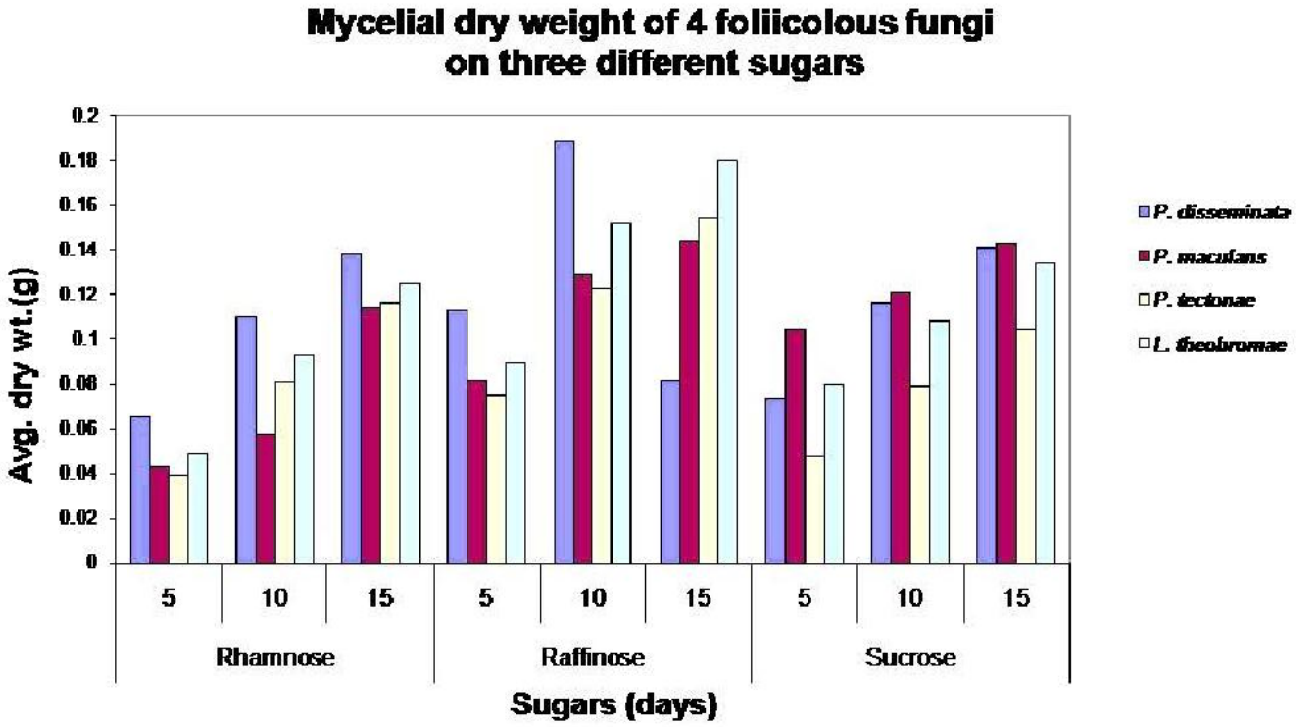
Zafar I., Mussarat S., Farrakh and Sheraz B. (2002) Antifungal properties of some indigenous plants from Peshwar valley. *Asian J. Plant Sci*. 1(6): 708 ï 709

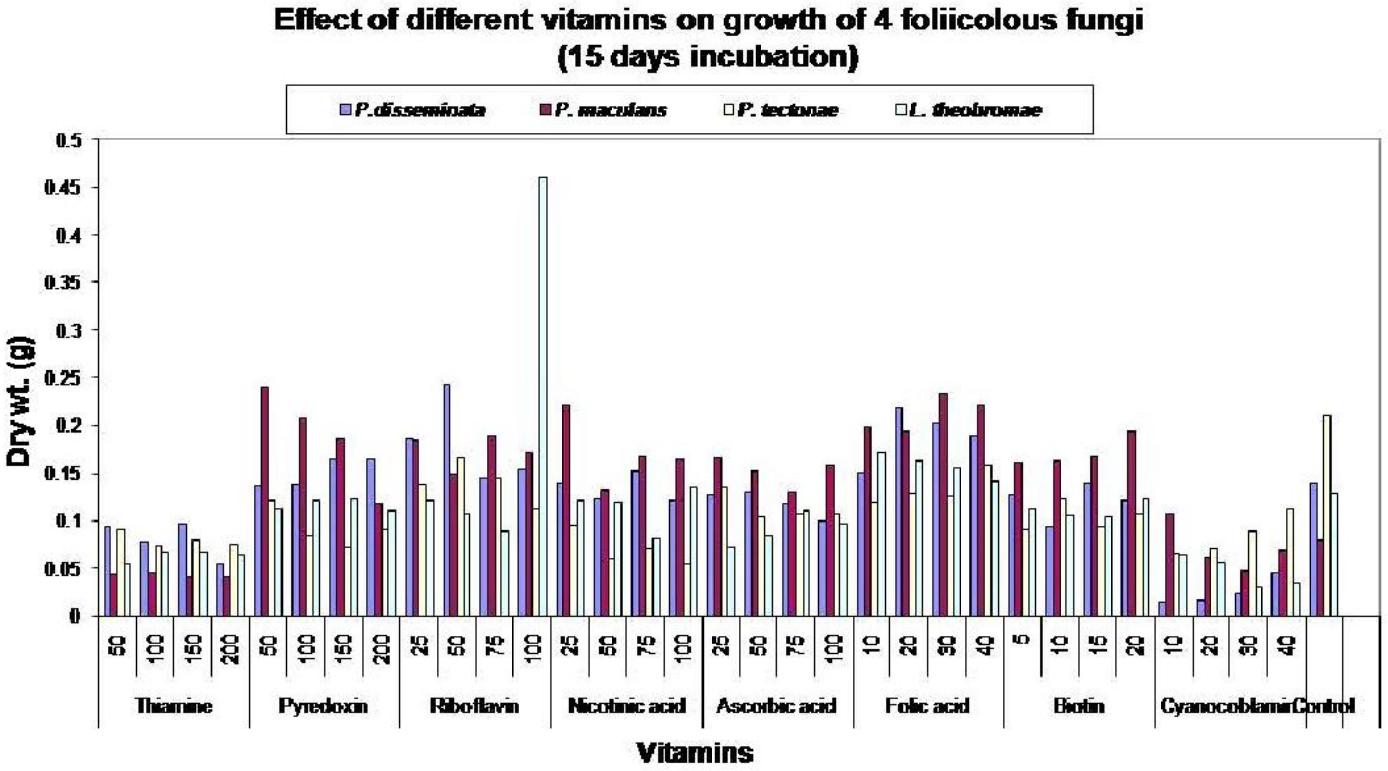
Zalokar M. (1954) Studies on biosynthesis of carotenoids in *Neurospora crassa*. *Arch. Biochem. Biophys.* 50: 71-80.

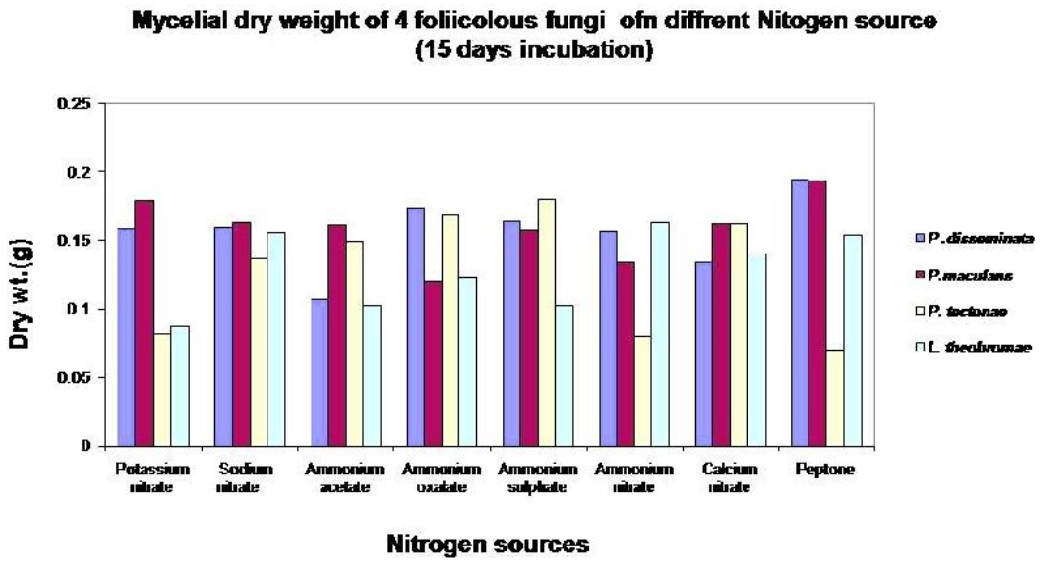
Zambonelli A, Dô Aulerio A.Z., Binachi A, Albasini A. (1996) Effects of essential oils on phytopathogenic fungi *in vitro. J. Phytopathol.* 144:491-494.

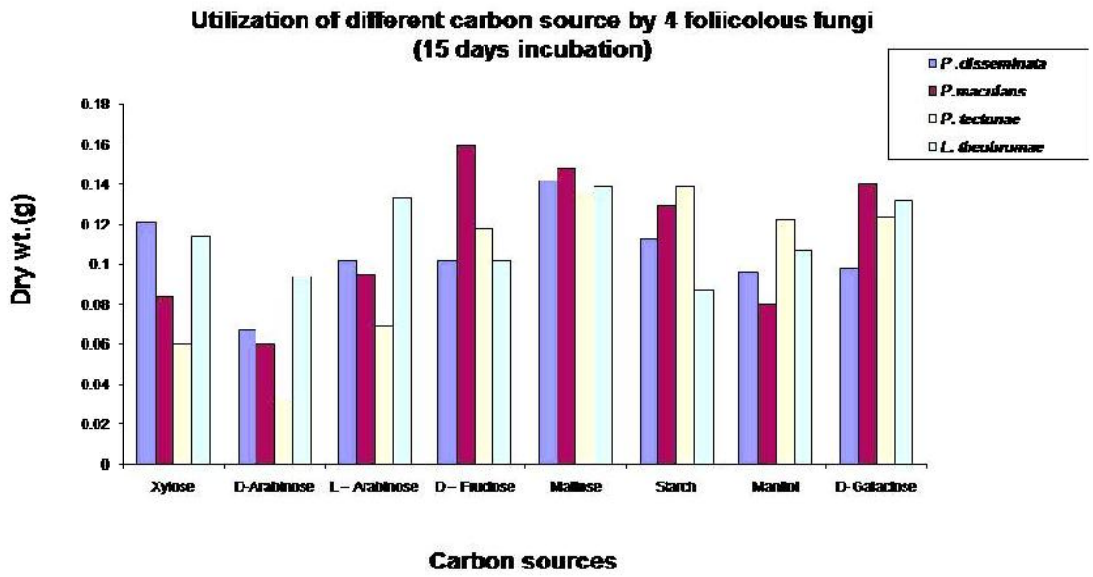
Zhu P. L. (1989) Study on identification and taxonomy of *Pestalotiopsis* spp. from common ornamental plants. Ms. Thesis. Zhejiang Agricultural University, China


Effect of all vitamins on four follicolous fungi
P.disseminata
P. maculans
P. tectonae
L. theobromae
Avg. dry wt (g)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
All vit
(-) Thiamine
(-) Pyredoxin
(-) Riboflavin
(-) Nicotinic acid
(-) Ascorbic acid
(-) Folic acid
(-) Biotin
(-) Cyanocobalamine
Control
Vitamins

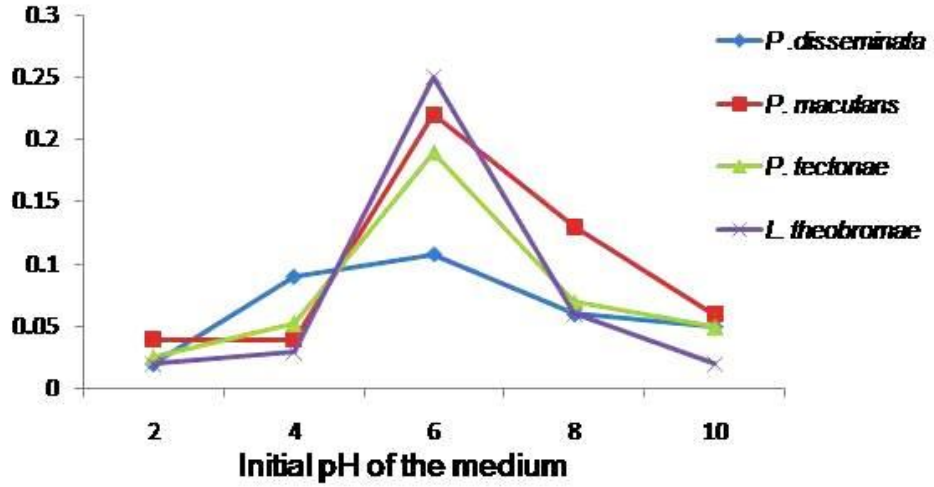

Mycelial dry weight of 4 foliicolous fungi
on three different sugars
Avg. dry wt.(g)
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
5
10
15
5
10
15
5
10
15
Rhamnose
Raffinose
Sucrose
Sugars (days)
P. disseminata
P. maculans
P. tectonae
L. theobromae


Effect of different vitamins on growth of 4 foliicolous fungi
(15 days incubation)
P.disseminata
P. maculans
P. tectonae
L. theobromae
Dry wt. (g)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
50
100
150
200
50
100
150
200
25
50
75
100
25
50
75
100
25
50
75
100
10
20
30
40
5
10
15
20
10
20
30
40
Thiamine
Pyredoxin
Riboflavin
Nicotinic acid
Ascorbic acid
Folic acid
Biotin
Cyanocoblamin
Control
Vitamins

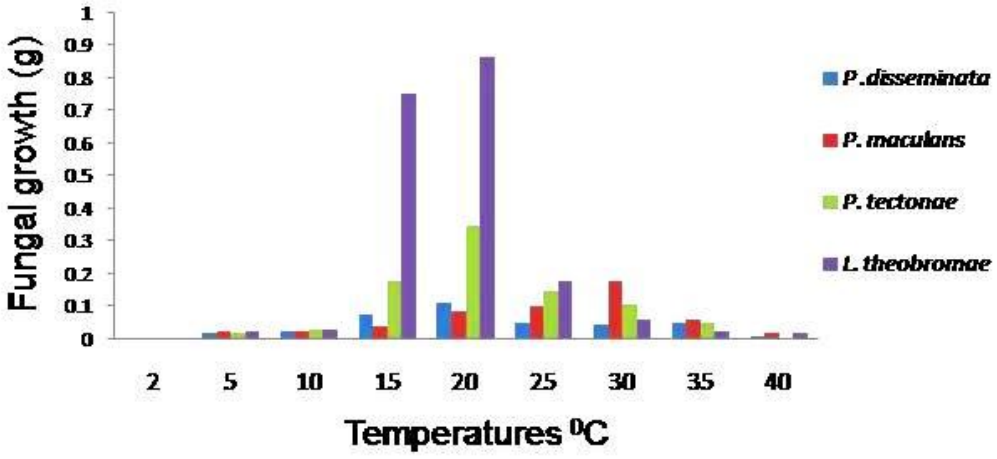

Mycelial dry weight of 4 foliicolous fungi ofn diffrent Nitogen source
(15 days incubation)
Dry wt.(g)
0.25
0.2
0.15
0.1
0.05
0
Potassium nitrate
Sodium nitrate
Ammonium acetate
Ammonium oxalate
Ammonium sulphate
Ammonium nitrate
Calcium nitrate
Peptone
Nitrogen sources
P.disseminata
P.maculans
P. tectonae
L. theobromae

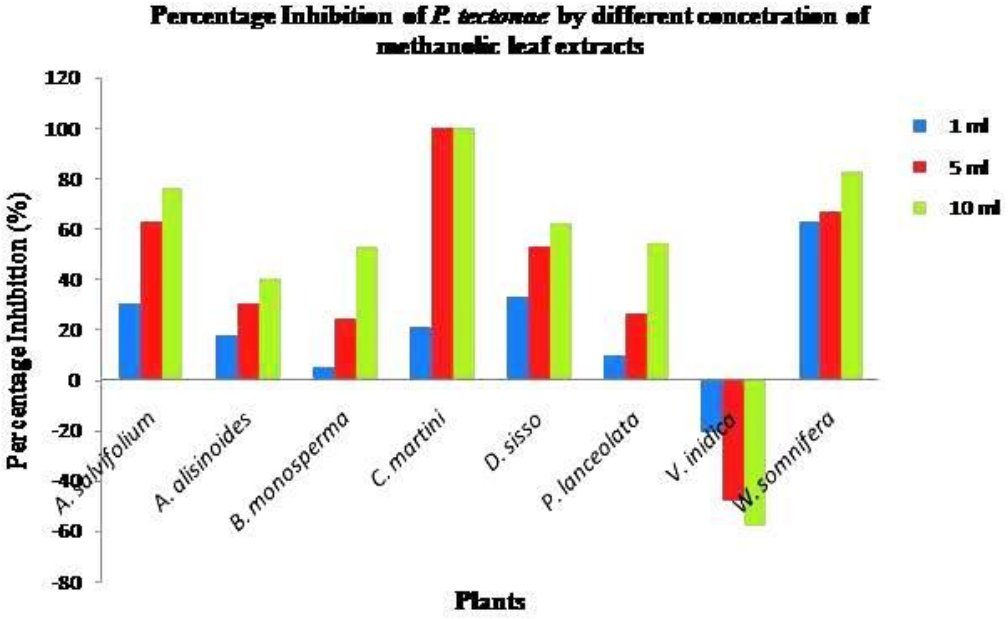

Utilization of different carbon source by 4 follicolous fungi
(15 days incubation)
P.disseminata
P.macrdans
P. tectunae
L. theobromae
Dry wt.(g)
0.18
0.16
0.14
0 12
0.1
0.08
0.06
0.04
0.02
0
Xylose
D-Arabinose
L – Arabinose
D – Fructose
Maltose
Starch
Manitol
D-Galactose
Carbon sources

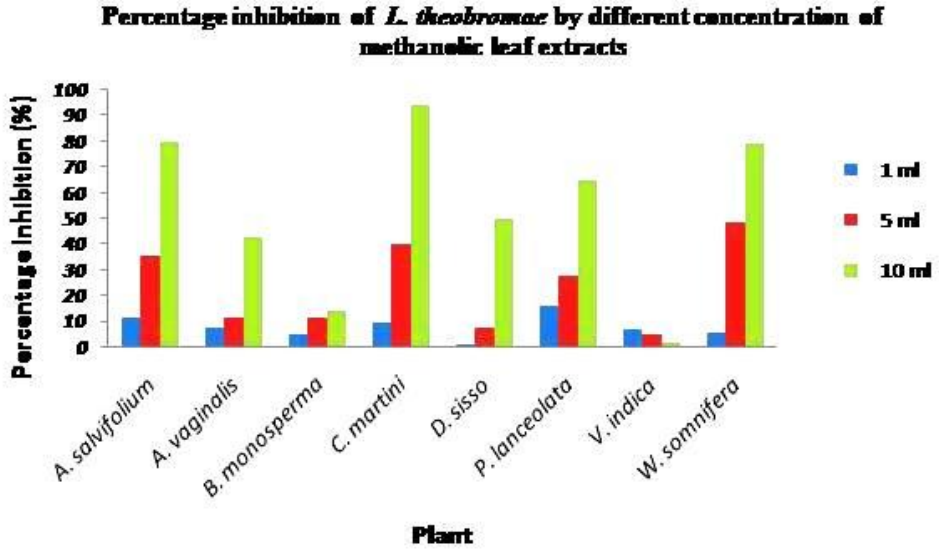
Graph showing growth at various potentials of Hydrogen



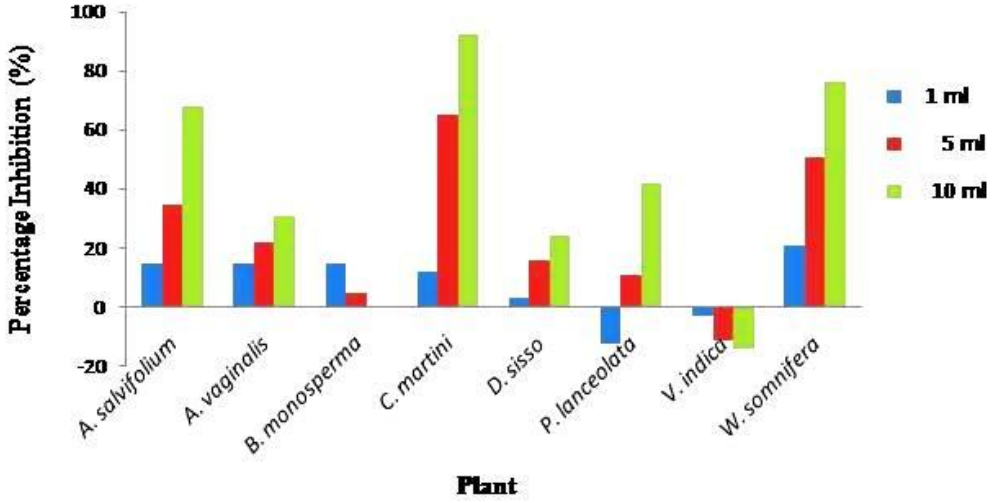

Graph showing growth at various temperatures ($^0$C)




Percentage Inhibition of P. tectonae by different concetration of methanolic leaf extracts
Percentage Inhibition (%)
120
100
80
60
40
20
0
-20
-40
-60
-80
A. salvifolium
A. alisinoides
B. monosperma
C. martini
D. sisso
P. lanceolata
V. inidica
W. somnifera
1 ml
5 ml
10 ml
Plants


Percentage inhibition of L. theobromae by different concentration of methanolic leaf extracts
Percentage Inhibition (%)
100
90
80
70
60
50
40
30
20
10
0
A. salvifolium
A. vaginalis
B. monosperma
C. martini
D. sisso
P. lanceolata
V. indica
W. somnifera
1 ml
5 ml
10 ml
Plant

**Percentage inhibition of *P. disseminata* by different concentration of methanolic leaf extracts**




**Percentage Inhibition of *P. maculans* by different concentration of methanolic leaf extracts**

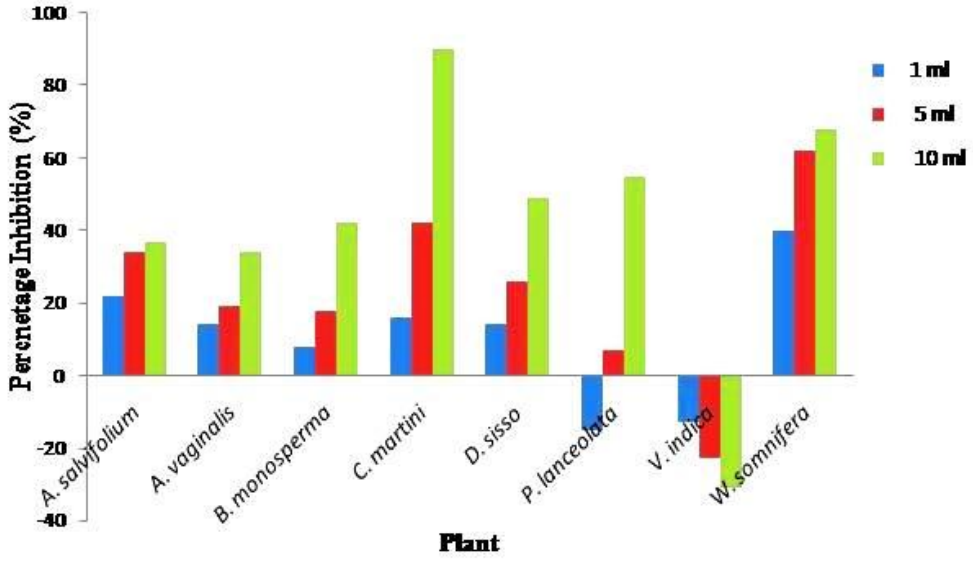


**Fig. 1 Percentage inhibition of *F. pallidoroseum* in metahnolic and aqueous extracts of certain plants.**

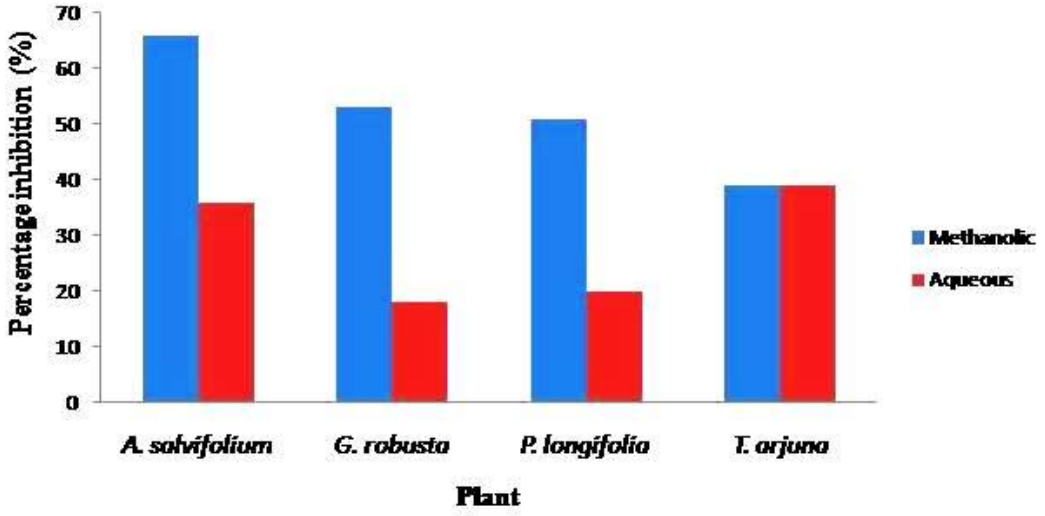



**Fig. 2 Percentage inhibition of *T. subthermophila* in methanolic and aqueous extracts of certain plants**

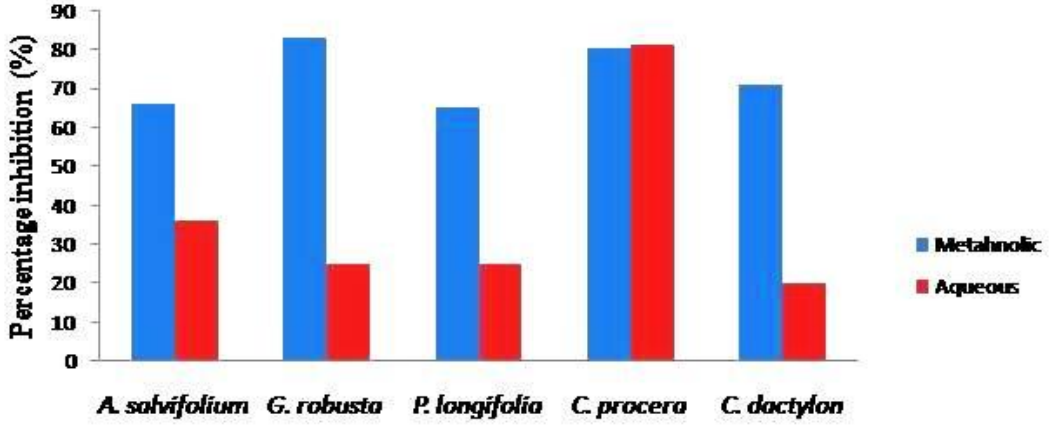
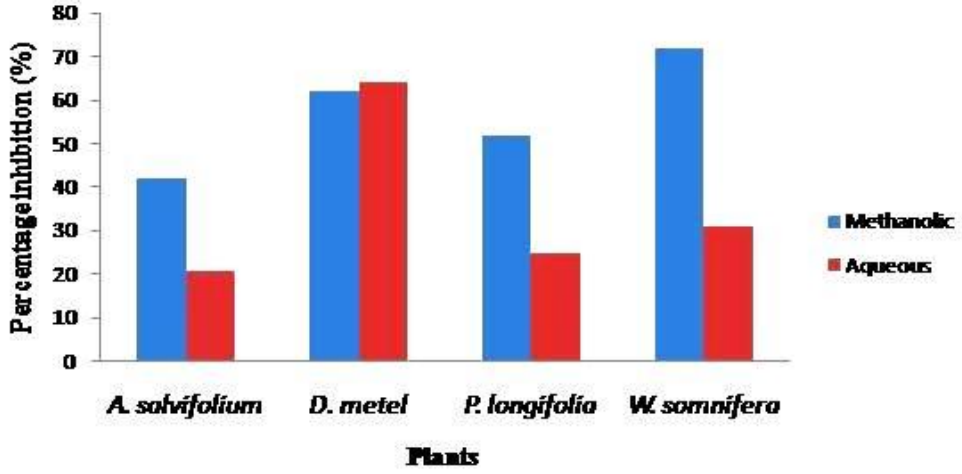


**Fig. 3 Percentage inhibiton of *D. rostrata* in aqueous and methanolic extract of certain plants**



## Illustration of photographs

**Plate – I**

Fig. A,B ï Ratanmahal Wild Life sanctuary

Fig. C, D ï Shoolpaneshwar WLS

Fig. E ï Jambughoda WLS

Fig. F ï Pavagadh forest area

**Plate – II**

Fig. A, C, E ï Diseased leaf of *Tectona grandis.*

Fig. B ï Fungal spores of *Lasiodiplodia theobromae*

Fig. D ï Spores *of Phomopsis tectonae*

Fig. F ï *Fusarium pallidoroseum* conidia.

Fig. G ï Ascospores of *Thelevia subthermophila*

**Plate – III**

Fig. A, C, E, - Leaf spot disease symptom of *Bambusa arundinacea*

Fig. B. ï Fungal spores of *Drechslera rostrata*

Fig. D ï Fungal spores of *Melanconiopsis microspora*

Fig. F ï Conidia of *Pestalotiopsis maculans*

**Plate – IV**

Fig. A ï Pathogenecity test on *T. grandis* leaf

Fig. B,C,D ï Reappearance after infection in T. grandis leaf.

Fig. E ï Condia of *Pestalotiopsis disseminata* isolated from *Terminalia arjuna* leaf.

Fig. F ï Condia of *Gleoesporium gleoesporoides* isolated from *T. arjuna* leaf.

**Plate – V**

Fig. A ï Paper chromatogram showing utilization of sucrose by *Pestalotiopsis disseminata*.

Fig. B ï Paper chromatogram showing utilization of sucrose by *P. maculans.*

Fig. C ï Paper chromatogram utilizing sucrose by *Phomopsis tectonae.*

Fig. D ï Paper chromatogram utilizing *Lasiodiplodia theobromae*

**Plate – VI**

Fig. 1 ï Growth of *Pestalotiopsis disseminata* in methanolic leaf extract of *Alangium salviifolium.*

Fig. 2 ï Growth of *P. disseminata* in methanolic extract of *Alysicarpus vaginalis.*

Fig.3 ï Growth of *P. disseminata* in methanolic leaf extract of *Cymbopogon martini*.

Fig. 4 ï Growth of *P. disseminata* in methanolic extract of *Pluchea lanceolata*.

**Plate – VII**

Fig. 1 - Growth of *Pestalotiopsis maculans* in methanolic leaf extract of *Alangium salviifolium.*

Fig. 2 ï Growth of *P. maculans* in methanolic extract of *Alysicarpus vaginalis.*

Fig.3 ï Growth of *P. maculans* in methanolic leaf extract of *Cymbopogon martini*.

Fig. 4 ï Growth of *P. maculans* in methanolic extract of *Pluchea lanceolata*.

**Plate VIII**

Fig.1 ï Growth of *P. maculans* in methanolic leaf extract of *Butea monsoperma*

Fig. 2 ï Growth of *P.maculans* in methanolic extract of *Pluchea lanceolata*.

Fig. 3 ï Growth of *P.maculans* in methanolic extract of *Withania somnifera*.

**Plate IX**

Fig. 1 - Growth of *Phomopsis tectonae* in methanolic leaf extract of *Alangium salviifolium.*

Fig. 2 ï Growth of *P. tectonae* in methanolic extract of *Butea monosperma*

Fig.3 ï Growth of *P. tectonae* in methanolic leaf extract of *Cymbopogon martini.*

Fig. 4 ï Growth of *P. tectonae* in methanolic extract of *Withania somnifera*.

**Plate X**

Fig. 1 - Growth of *Lasiodiplodia theobromae* in methanolic leaf extract of *Alangium salvifolium.*

Fig.3 ï Growth of *L. theobromae* in methanolic leaf extract of *Cymbopogon martini*.

Fig.3 ï Growth of *L. theobromae* in methanolic leaf extract of *Dalbergia sisso.*

Fig. 4 ï Growth of *L. theobromae* in methanolic extract of *Withania somnifera*.

**Plate XI**

Plants selected.

**Plate XII**

Fig. 1 - Growth of *Drechslera rostrata* in methanolic leaf extract of *Datura stramonium.*

Fig.3 ï Growth of *Thelevia subthermophila* in methanolic leaf extract of *Datura stramonium*.

Fig.3 ï Growth of *Fusarium pallidoroseum* in methanolic leaf extract of *Terminalia arjuna*.

**PLATE XI**


*1 - Drechslera rostrata on methanolic leaf extract of Datura stramonium*

*2 - Thelevia subthermophila on D. stramonium*

*3 - Fusarium pallidoroseum on methanolic leaf extract of Terminalia arjuna*

**PLATE - X**


1
Alangium salvifolium leaf extract
Control
A
Lasiodiplodia
B
C
2
Cymbopogon martini leaf extract
Control
A
Lasiodiplodia
B
C
3
Control
A
Lasiodiplodia
B
C
4
Withania somnifera leaf extract
Control
A
Lasiodiplodia
B
C

**PLATE - IX**

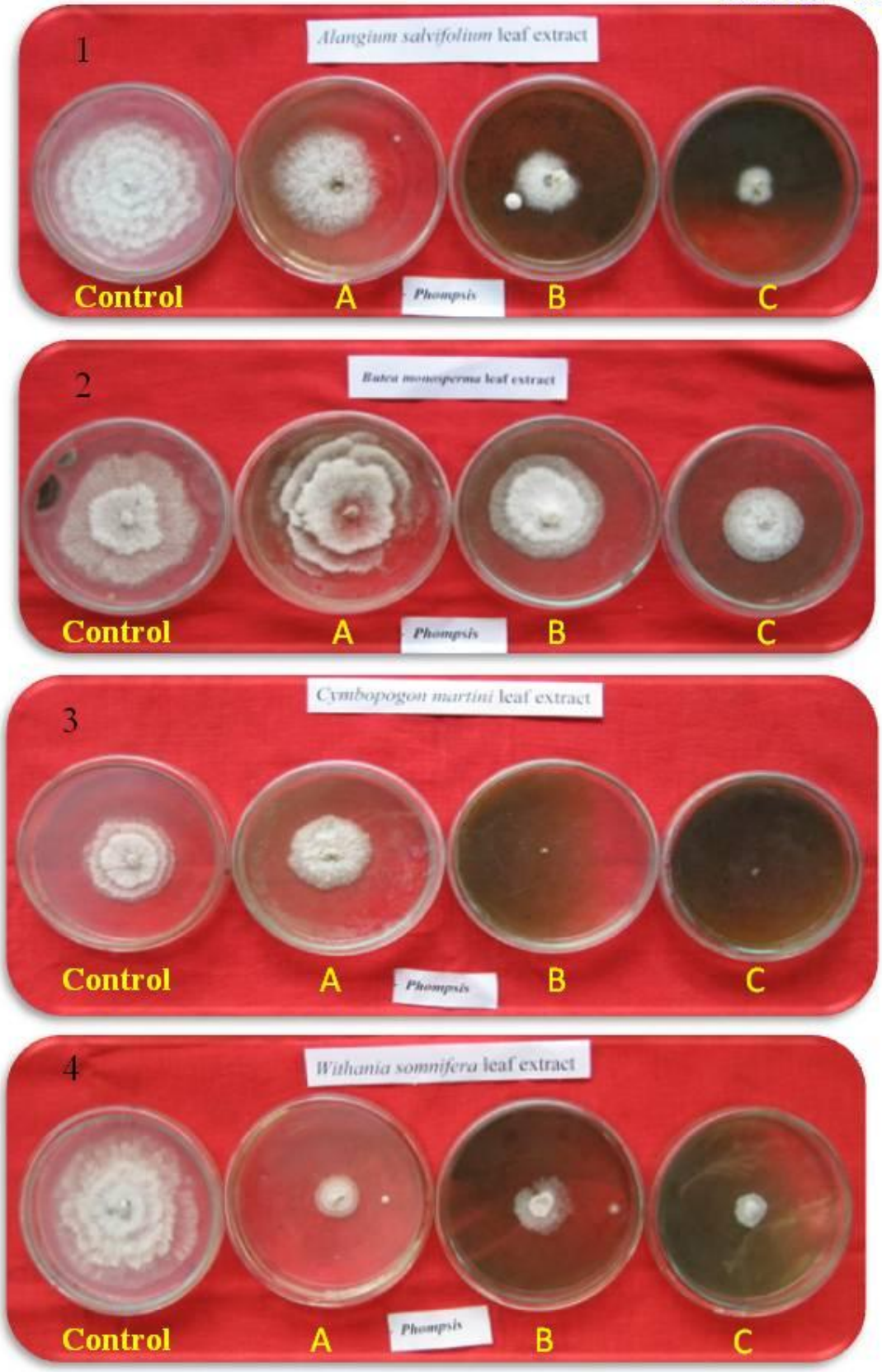
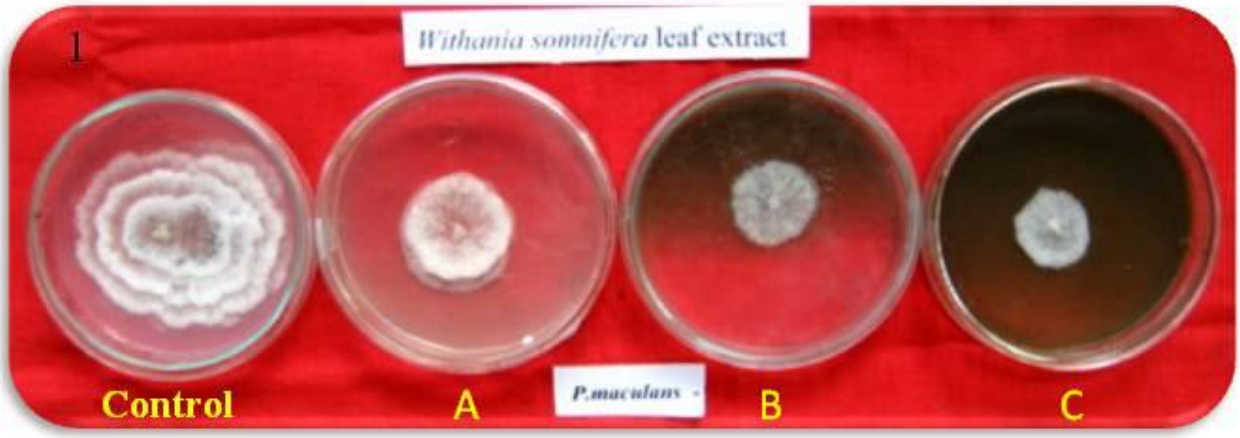

1
Alangium salvifolium leaf extract
Control
A
Phomopsis
B
C
2
Butea monosperma leaf extract
Control
A
Phomopsis
B
C
3
Cymbopogon martini leaf extract
Control
A
Phomopsis
B
C
4
Withania somnifera leaf extract
Control
A
Phomopsis
B
C

**PLATE - VIII**


1
Withania somnifera leaf extract
Control
A
P.maculans
B
C

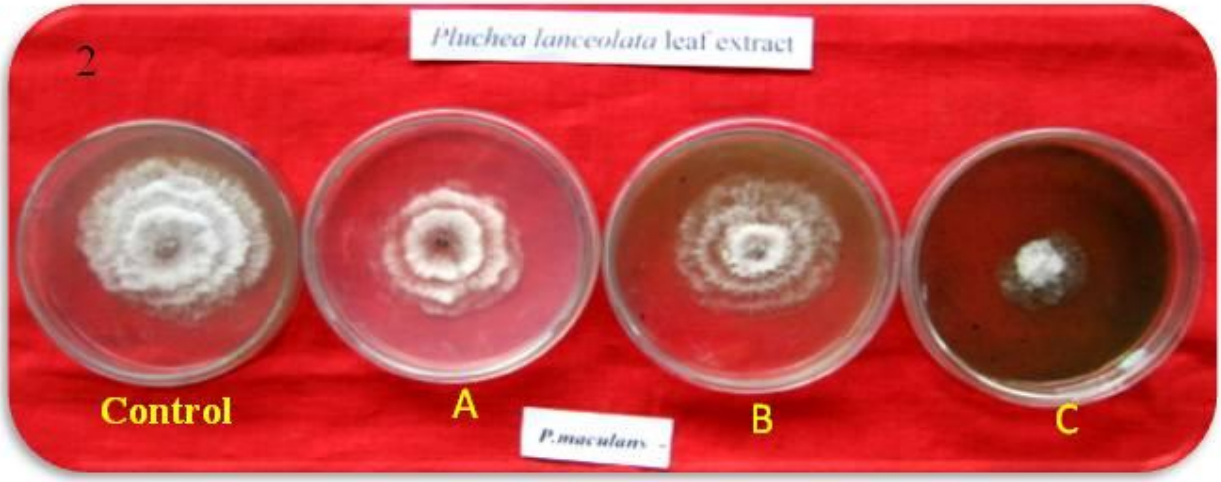

2
Pluchea lanceolata leaf extract
Control
A
P.maculans
B
C

**PLATE - VII**

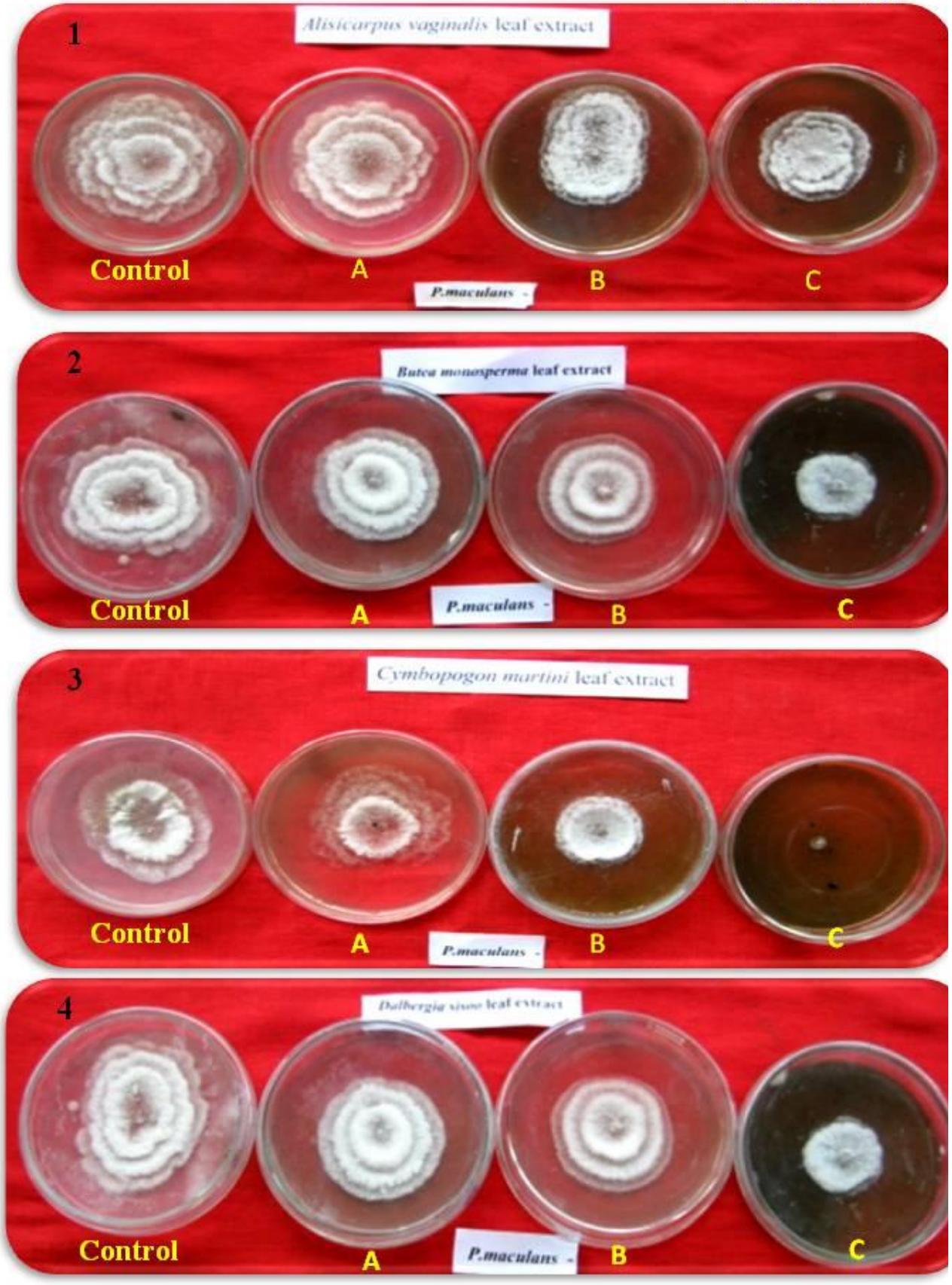

1
Alisicarpus vaginalis leaf extract
Control
A
P.maculans -
B
C
2
Butea monosperma leaf extract
Control
A
P.maculans -
B
C
3
Cymbopogon martini leaf extract
Control
A
P.maculans -
B
C
4
Dalbergia sissoo leaf extract
Control
A
P.maculans -
B
C

**PLATE - VI**


1
Alangium salvifolium leaf extract
Control
A
P.disseminata
B
C
2
Alisicarpus vaginalis leaf extract
Control
A
P.maculans
B
C
3
Cymbopogon martini leaf extract
Control
A
P.disseminata
B
C
4
Pluchea lanceolata leaf extract
Control
A
P.disseminata
B
C

**PLATE - V**

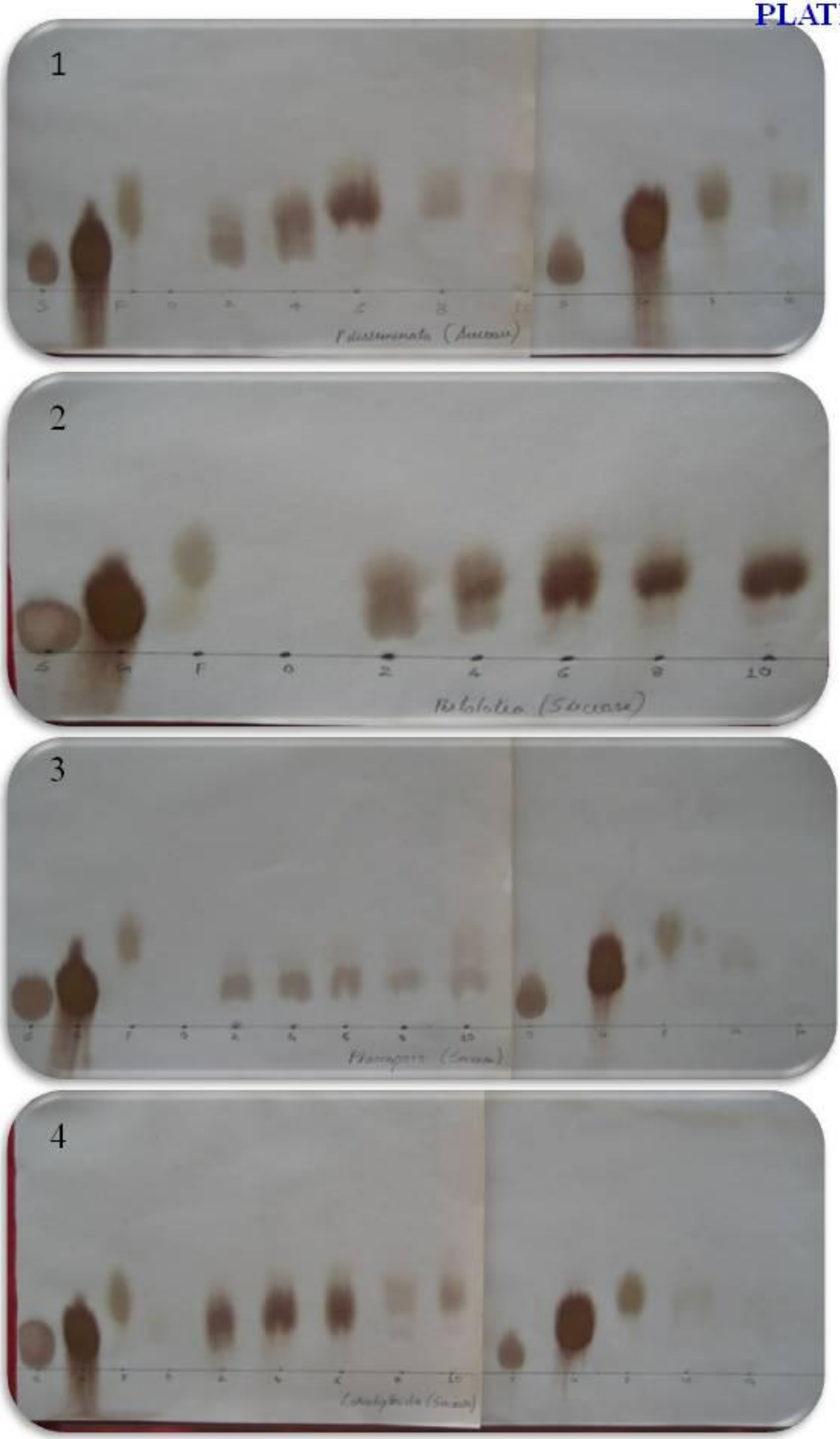


**PLATE - IV**





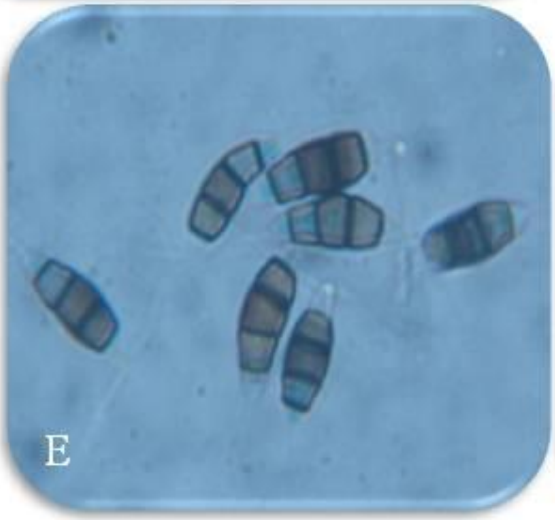
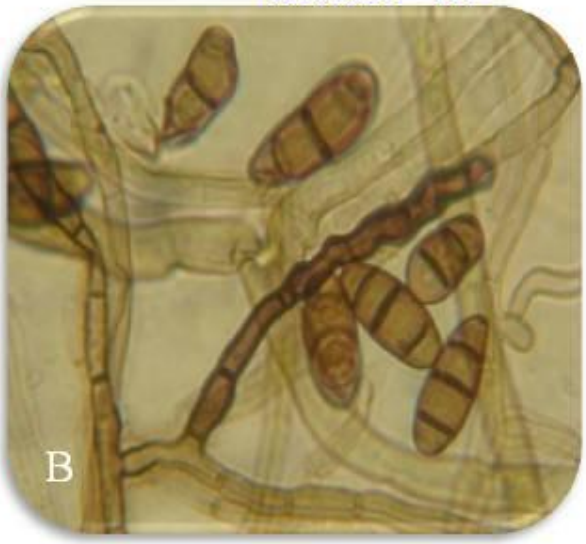



**PLATE - III**





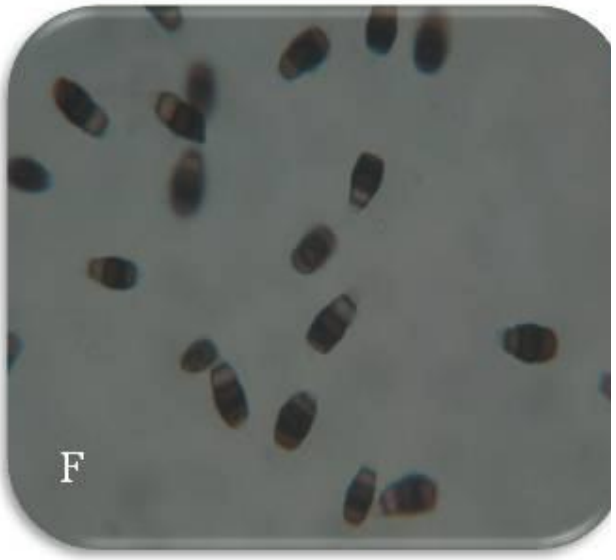
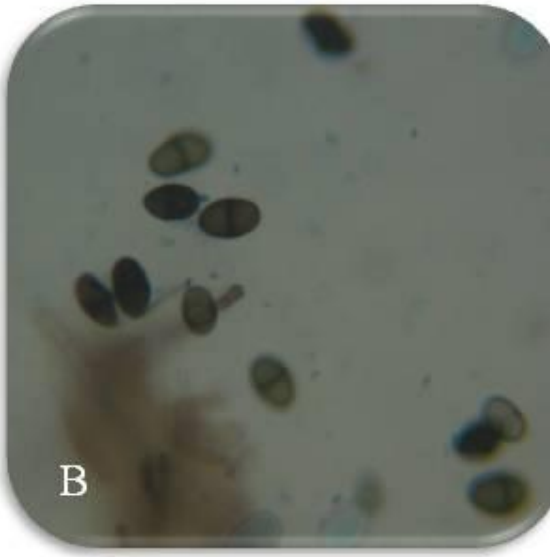
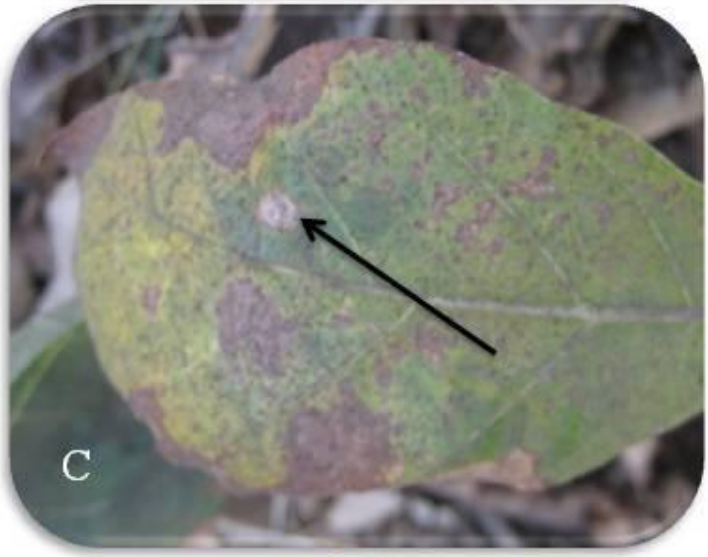
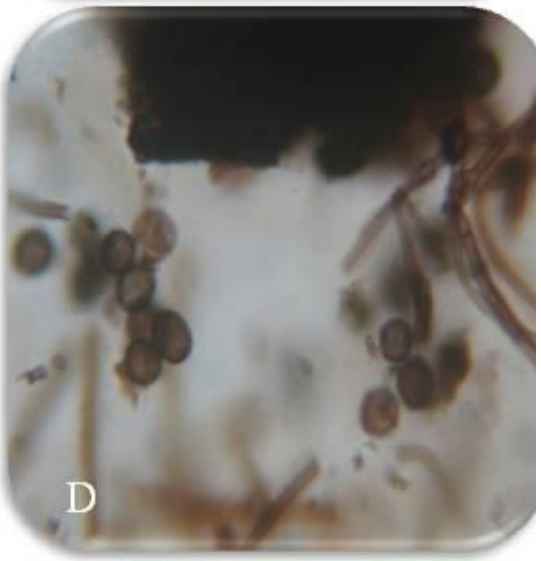


**PLATE - II**



F

G

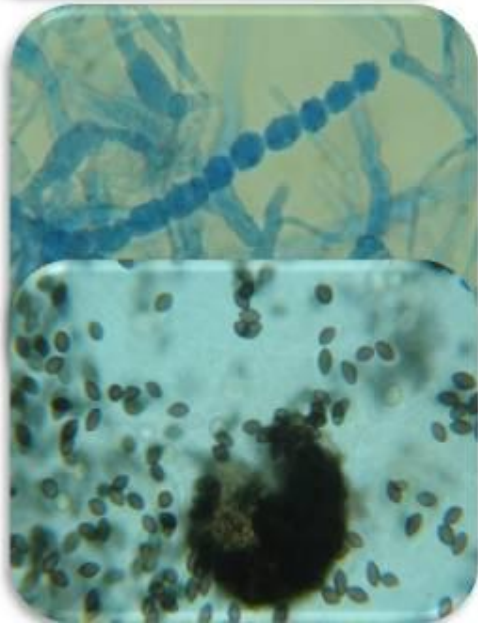


**PLATE - I**


A

B


C


D


E


F

# APPENDICES

## 16

### Antifungal property of certain leaf extracts against two phytopathogens

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**Abstract**

*Tectona grandis* L.f. is economically important forest tree. It is found prominently in dry deciduous forests and produce high quality of wood. Two fungi *i.e., Fusarium pallidoroseum* (Cooke) Sacc. and *Thielavia subthermophila* Mouch were isolated from the infected leaves of teak. Pathogenecity test for both these fungi resulted in appearance of disease symptoms within 5 ï 7days after inoculation. Reisolations from different inoculated leaves confirmed that both fungi were pathogenic. Considering the non pollutive and biodegradable nature of ecofriendly plant based alternatives, various leaf extracts have been tried as botanical pesticides.

The inhibitory effect of methanolic and aqueous extracts of plant was assessed against both the pathogens by poisoned food technique (Nene and Thapliyal, 1979). Observations were recorded after 7 days. Out of 5 plants tested against fungus *T. subthermophila*, *Grevillea robusta* A. Cunn. (83%) recorded highest mean inhibition of mycelial growth followed by *Alangium salviifolium* L.f. (66%). Extract of *Polyalthia longifolia* (Sonn.) Thw. (65%) *Heterophragma adenophyllum* Seem. (53%) *Terminalia arjuna* Roxb. (48%) were also

effective. For *F. pallidoroseum* maximum inhibition was obtained in leaf extract of *A. salviifolium* (66%) followed by *G. robusta* (53%), *P. longifolia* (51%), *T. arjuna* (39%) *H. adenophyllum* (39%). Leaf extracts of *Grevillea* and *Alangium* can be used at commercial scale to control the two leaf infecting pathogens.

**Key words:** *Tectona grandis, Fusarium pallidoroseum, Thielavia subthermophila,* extract, *Grevillea robusta*

## Introduction

Teak, (*Tectona grandis* L.,) indigenous to India, Myanmar and a few South East Asian countries, is one of the most versatile hardwood species. In India, teak grows naturally and is raised extensively in plantations. Sagwan or Teak is a tall and handsome deciduous tree belonging to family Verbenaceae. It is called sagon, saigon, saj, taku, kayum, etc. in local languages. It grows well in warm climate and well-drained soil. Teak wood is considered to be one of the best timbers available anywhere. Leaves are simple, opposite, large, round, broad, pointed and thick in structure. New leaves appear in May-June. Teak is affected by a few serious diseases both in nurseries and plantations. A survey was conducted in Pavagadh and Jambughoda forest to find out leaf spot pathogens.

## Materials and Methodology

Isolations of fungal species that were found associated with disease symptoms were made separately on PDA medium contained Petri dishes. Fungal cultures were inoculated on healthy leaves to test the pathogenicity. Fungi *F. pallidoroseum* and *T. subthermophila* belonging to Fungi imperfecti and Ascomycotina were isolated from Teak leaves. Identification of cultures was done by Plant Pathology Division of IARI, New Delhi. To test the antifungal property leaves of various tree species were oven dried at 70 $^{0}$C, 20g powdered

material was used for Soxhlet extraction using 200ml of methanol solution. Three concentrations of 1ml, 5ml and 10 ml each from extract were used to observe growth of fungi by Poisoned food technique

**Results and Discussion**

Isolation from various Teak leaves revealed the presence of fungi like *Alternaria sp., Curvularia* sp*., Phomopsis tectonae*. *Fusarium pallidoroseum* and *Thielavia subthermophila*. During Pathogenicity test wavy brown ring appeared on the leaf. When pathogen *T. subthermophila* was infected on fresh leaves of Teak, black spot encircling yellowish ring appeared. Re-isolation from inoculated diseases leaves confirmed that both fungi are pathogenic. Antifungal activity of dried leaves of *Grevillea robusta, Terminalia arjuna, Polyalthia longifolia, Heterophragma adenophyllum* was tested against both the pathogenic fungi. Percentage inhibition at 10 % concentration was higher as compared to 1 ml and 5 ml. Except for *T. subthermophila* leaf extract of *Grevillea robusta* recorded highest inhibition (83%) followed by *Alangium salviifolium* (66%). Extract of *Polyalthia longifolia* (65%), *Heterophragma adenophyllum* (53%), *Terminalia arjuna* (56%) were also effective. For *F. pallidoroseum* maximum inhibition was obtained in leaf extracts of *A. salviifolium (*66%) followed by *G. robusta* (53%), *P. longifolia* (51%), *T. arjuna* (39%) *H. adenophyllum* (39%).

**References**


1. Arya, C. and Arya A. (2002) New leaf spot diseases of Social Forestry trees ï I *J. Mycol. Pl. Pathol*, 32(2): 260-262.
2. Dadwal, V.S. and Jamalludin (2001) A note on basal canker of Teak in plantations. *Indian Forester* 127(3): 365-366.
3. Hooda K.S., Bhatt J.C., Joshi D, Sushil S.N. and Gupta H.S., (2008) Biocontrol agents *vis – a vis* fungicides in managing various diseases of tomato (*Lycopersicon esculentum* Mill.) in hills of Uttarakhand *Ind. Phytopath*. 61 (3): 331 ï 336.

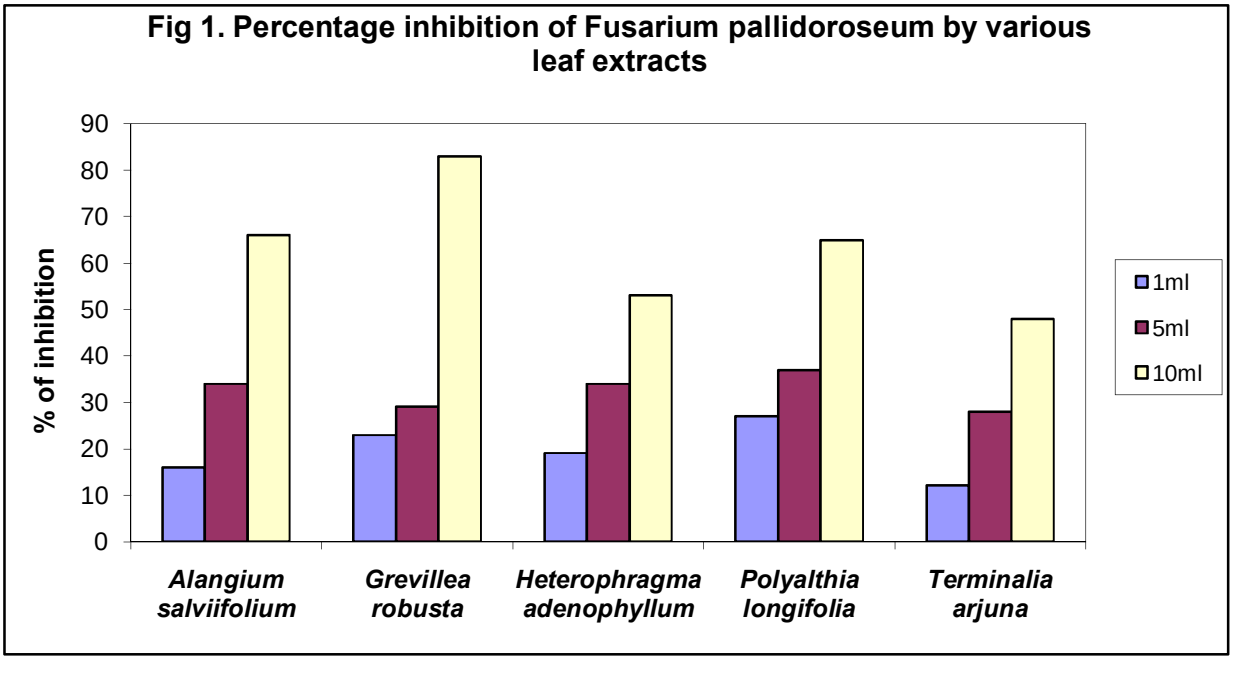
4. Utpal K., Deka P.K. Dutta, Gogoi R. and Borah P.K., (2008) Management of leaf spot disease complex of betelvine by bioagents and plant extracts *Ind. Phytopath* 61(3): 337 - 342.

**Percentage inhibition of *Fusarium pallidoroseum at* different concentration of plant extracts**

| Sr. no. | Plant Selected | Methanolic extract | | | Aqueous extract | | |
|---|---|---|---|---|---|---|---|
| | | 1 ml | 5 ml | 10 ml | 1 ml | 5 ml | 10 ml |
| 1. | *Alangium salviifolium* | 16 | 34 | 66 | 36 | 25 | 30 |
| 2. | *Grevillea robusta* | 23 | 29 | 53 | 17 | 15 | 13 |
| 3. | *Heterophragma adenophyllum* | 19 | 34 | 39 | 11 | 24 | 25 |
| 4. | *Polyalthia longifolia* | 27 | 37 | 51 | 9 | 16 | 20 |
| 5. | *Terminalia arjuna* | 12 | 28 | 39 | 50 | 29 | 39 |

**Percentage inhibition of *Thielavia subthermophila* at different concentration of plant extracts**

| Sr. no | Plant Selected | Methanolic extract | | | Aqueous extract | | |
|---|---|---|---|---|---|---|---|
| | | 1 ml | 5 ml | 10 ml | 1 ml | 5 ml | 10 ml |
| 1. | *Alangium salviifolium* | 16 | 34 | 66 | 23 | 25 | 36 |
| 2. | *Grevillea robusta* | 31 | 53 | 83 | 18 | 20 | 25 |
| 3. | *Heterophragma adenophyllum* | 15 | 37 | 53 | 0 | 9 | 20 |
| 4. | *Polyalthia longifolia* | 13 | 52 | 65 | 10 | 15 | 25 |
| 5. | *Terminalia arjuna* | 28 | 56 | 48 | 20 | 17 | 50 |



# Antifungal potential of certain leaf extracts against three phytopathogens

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Plants diseases play a major role in the destruction of natural resources in agriculture and forestry. Among the Foliicolous fungi members of fungi imperfecti appear to be most aggressive. Usually chemical compounds are used to control leaf spot diseases, but they in fact help pathogens in developing resistance against fungicides. Unfortunately more the specific effect of a chemical on an organism, greater the probability of decreasing the effect through genetic shifts in population, whereas broad spectrum fungicides produce undesirable consequences on non ï target organisms (Tjamos *et al.,* 1992) to reduce the synthetic fungicides, a few botanicals *viz*. neem oil, need seed extract, mahua and pomgam oil have been tested and found t o possess fungicidal properties against certain pathogens (Rajappan *et al.,* 1997) Arya (1988) found aqueous shoot extract of *Ephedra foliata* and leaf extract of *Eucalyptus occidentalis* effective against *Phomopsis viticola* at 25%. At higher concentration (75%) leaf extract of neem was most effective causing 82.3 % spore inhibition. Tripathi (2005) tested 24 angiospermic plants. Leaf extract in ethyl acetate) of *Citrus aurantifolia*, *Murraya koenigii, Nerium indicum, Prunus persica, Ocimum gratissimum* and (ethyl alcohol) extract of *Acacia nilotica* showed good activity. Benzene extracted leaf extract of *Ipomoea fistulosa* ï a profusely growing weed was found inhibitory to *Aspergillus niger, Colletotrichum gloeosporiodes* and two species of *Fusarium* (Mittal *et al*., 2004)

**Table no:1 Percentage inhibition of *Fusarium pallidoroseum* at different concentration of leaf extracts**

| Sr. no. | Plant Selected | Methanolic extract | | | Aqueous extract | | |
|---|---|---|---|---|---|---|---|
| | | 1 ml | 5 ml | 10 ml | 1 ml | 5 ml | 10 ml |
| 1. | *Alangium salviifolium* | 16 | 34 | 66 | 36 | 25 | 30 |
| 2. | *Grevillea robusta* | 23 | 29 | 53 | 17 | 15 | 18 |
| 3. | *Heterophragma adenophyllum* | 19 | 34 | 39 | 11 | 24 | 25 |
| 4. | *Polyalthia longifolia* | 27 | 37 | 51 | 9 | 16 | 20 |
| 5. | *Terminalia arjuna* | 12 | 28 | 39 | 50 | 29 | 39 |
| 6. | *Dalbergia sisso* | - | 12 | 22 | - | **-3** | 12 |
| 7. | *Eclipta alba* | - | 15 | 24 | - | 3 | 17 |

**Table no: 2 Percentage inhibition of *Thelevia subthermophila* at different concentration of leaf extracts**

| Sr. no | Plant Selected | Methanolic extract | | | Aqueous extract | | |
|---|---|---|---|---|---|---|---|
| | | 1 ml | 5 ml | 10 ml | 1 ml | 5 ml | 10 ml |
| 1. | *Alangium salviifolium* | 16 | 34 | 66 | 23 | 25 | 36 |
| 2. | *Grevillea robusta* | 31 | 53 | 83 | 18 | 20 | 25 |
| 3. | *H. adenophyllum* | 15 | 37 | 53 | 0 | 9 | 20 |
| 4. | *Polyalthia longifolia* | 13 | 52 | 65 | 10 | 15 | 25 |
| 5. | *Terminalia arjuna* | 28 | 56 | 48 | 20 | 17 | 50 |
| 6. | *Calotropis procera* | 60 | 75 | 80 | 50 | 53 | 51 |
| 7. | *Datura metel* | 12 | 48 | 51 | 73 | 33 | 29 |
| 8. | *Cynadon dactylon* | 65 | 12 | 71 | 57 | 63 | 20 |
| 9. | *Withania somnifera* | 13 | 36 | 56 | 18 | 35 | 31 |

**Table no: 3 Percentage inhibition of *Drechslera rostrata* at different concentration of leaf extracts**

| Sr. no. | Plant Selected | Methanolic extract (Conc) | | | Aqueous extract (Conc) | | |
|---|---|---|---|---|---|---|---|
| | | 1 ml | 5 ml | 10 ml | 1 ml | 5 ml | 10 ml |
| 1. | *Calotropis procera* | 52 | 31 | 42 | 48 | 37 | 21 |
| 2. | *Datura metel* | 10 | 68 | 62 | 59 | 38 | 64 |
| 3. | *Polyalthia longofolia* | 13 | 31 | 52 | 13 | 20 | 25 |
| 4. | *Withania somnifera* | 28 | 62 | 72 | 13 | 43 | 31 |

Zafar *et al.,* (2002) reported that chloroform extracts of leaves of *M. azedarach* was active against *Fusarium chlamdosporum* while hexane, ethanol and water extracts were not. In a similar kind of work Bajwa et al., (2006) reported the antimycotic activity of aqueous and dichloromethane fractions of *Cicer arietinum* against *Drechslera tetramera* and *D. hawaiiensis.*

Leaf decoction of *Calotropis procera, Datura stramonium* were found to be effective in suppressing uredospore germination on detached leaves of wheat. (Bhatti, 1988). Hasa *et al.,* 91992) that leaf extracts of *Datura stramonium* reduced the development of rust pustules on the leaves of wheat. Mughal *et al.,* (1996) observed that aqueous extracts of *Datura alba* and *Withania somnifera* inhibited the growth of *Alternaria alternata, A. brassicola* and *Myrothecium roridum.*

According to Karim *et al.,* (2008) *Calotropis procera* leaf extract showed maximum fungal growth inhibition of *Candida albicans and Microsporum boulardi.* The bacteriocidal activity of *Calotropis procera* could be due to presence of calactin, mudarin and protein called calotropin which are active constituents of *C. Procera* (Parotta, 2001).

Differences in growth rate were exhibited with respect to the concentration employed. The periodic assays revealed significant reduction in fungal growth rate in all higher concentrations. The comparison between different aqueous and methanolic leaf extract concentrations showed that the percentage colony growth inhibition was significantly greater in methanolic extract in contrast to aqueous extract.

# Two new Leaf spot disease of *Bambusa arundinacea* (Retz.) Roxb. from Gujarat, India

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**Keywords** *:Bambusa arundinacea, leaf spot, Pestalotiopsis maculans*, *Drechslera rostrata.*

**Abstract**

Survey was conducted in Pavagadh forest region of Panchmahal District of Gujarat. An area with dense Bamboo cover was located at a distance of 50 km from Vadodara and in the Arboretum of M.S. University campus during August ï September 2009. Leaves with spots at the tip and leaves with lesions in the centre of lamina of *Bambusa arundinacea* were collected. After isolation on PDA medium, two new fungal pathogens *Pestalotiopsis maculans* (Corda) Nagraj and *Drechslera rostrata* (Drech) Rich & Fras were recorded. These are new host record for the country.

## Introduction

Pavagadh is a Hill Station, situated about 46 km away from Vadodara in the municipality of Panchmahal district in Gujarat state in western India. It is known for a famous Mahakali temple which draws thousands of pilgrims everyday. It is situated at an elevation of 823 m above sea level at 22 º 28ô00 N 73 º 30ô02ôE. Area of this locality Chapaner ï Pavagadh Archaelogical Park has been in scripted by UNESCO as World Heritage site in 2004.

Besides *Tectona grandis*, bamboos are planted by forest department in Pavagadh forest area. Bamboos are tall perennial, arborescent grasses belonging to Bambucaceae, a tribe under Poaceae comprising of four sub ï tribes: Arundinaceae, Eubambuseae, Dendrocalamceae and Melocannceae, generally inhabiting humid tropical, sub tropical regions of the world. They form an important constituent of many deciduous and evergreen forests. Areas under *Bambusa arundinacea* with 75 clumps/ha are classified as dense forest. (Varma and Bahadur, 1980).

In India one third of the entire population of bamboos is being utilized for constructional purposes. Single mat and veneer boards have been made from the green culms of *B. arundinacea*. It is well suited for making bamboo - ply. It is used for supporting crops such as sugarcane, betel vines and other climbing crops. Since the culms and rhizomes contain nutritive elements N,P,K, and Ca, they are suitable for preparing compost or manure. Trays made from this species are used for rearing silkworms in sericulture industry. Charcoal from bamboo is reported to have more calorific value than wood ï charcoal, it is used by goldsmiths. *B. arundinacea* yield a wax with low melting point which is used as a base for shoe polishes in place. Leaves of *B. arundinacea* used as are much valued as fodder, particularly during scarcity. In India seeds of *B. arundinacea* are extensively eaten by the poor during famines. Seeds resemble paddy but are bigger in size. Seeds are pickled, used for making beer (Anonymous, 1965).

**Materials and Methods**

Diseases leaves were collected from Pavagadh forest area, an World Heritage site located near Vadodara and from Arboretum, M.S. University of Baroda campus. Infected leaves

were washed in tap water then surface sterilized with freshly prepared 0.1 % $HgCl_2$ solution for 1 min. Both fungal pathogens *D. rostrata and P. maculanas* were isolated and maintained on Potato Dextrose Medium.

**Result and Discussion**

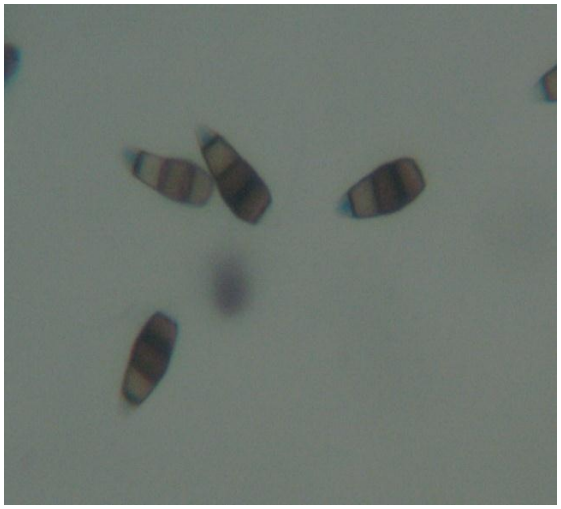
Hino (1938) first use the term ñ*Fungorum bambusicolorum*ò (bambusicolous fungi), but did not give any fungi growing on any bamboo substrates, which include leaves, culms, branches, rhizomes and roots. The genera of bamboo with the highest numbers of fungi recorded globally are *Arundinacea, Bambusa, Phyllostachys* and *Sasa*. Species of *Bambusa* in particular have been found to support a high fungal diversity. This is probably due to a larger number of collections, as it is one of the most widespread genera in tropical and subtropical Asia (Dransfield and Widija, 1995).

**Disease Symptoms:** Leaves with yellowish brown tips appeared in case of infection with *Drechslera rostrata* (Drech) Rich & Fras. and in case of infection caused by *Pestalotiopsis maculans* (Corda) Nagraj symptoms appeared as brown oval lesions in the mid rib region of the older leaves.

Identification of both pathogens is done based on morphological characters ( Fig.a, b).
Cultures were identified and confirmed from Agharkar Institute, Pune and IARI respectively.
The fungi were inoculated on healthy leaves and Kochôs postulates were confirmed. Since the two pathogens are not cited in literature (Jamalludin *et al.,*1989*,* Bilgrami *et al*., 1981).
Hence the two pathogens constitute new host record on the leaves of bamboo.

**Fig. Showing conidia of**

***Pestalotiopsis maculans*** **(**Corda) Nagraj



***Drechslera rostrata*** (Drech) Rich & Fras

**References**


Anonymous (1965) Wealth of India, CSIR, New Delhi

Bilgrami K.S., Jamulludin, Rizvi M.A. (1981) Part I Fungi of India, Today and Tomorrow pub., New Delhi

Bilgrami K.S., Jamulludin, Rizvi M.A. (1981) Part II Fungi of India Today and Tomorrow pub., New Delhi.

Dransfield, S. and Widija, E.A. (1995) Plant Resources of South- East Asia, PROSERA Foundation, Backhuye Puhu, Leiden, Netherlands.

Jamalludin, Goswami, M.G. Ojha B.M. (1989) Fungi of India, Scientific pub, Jodhpur

Harsh, N.S.K. Nath, V. and Jamalludin (1990) New forest disease record for India, *Journal of Tropical Forestry* Vol. 6(2) 175 ï 176

Hino, I. (1938) Illustrations Fungorum bambusicolorum. Bulletin of the Miyakazi College of Agricultural Forestry 10: 55 ï 64

Sarbhoy A.K., Agarwal, Varshney Fungi of India (1977-81)

Varma D.M. and Bahadur 1980 *Ind. Forester Rec*, N S, Bot 6(1) pp 9

# Diversity of Arbuscular Mycorrhizal Fungal Spores present in the Rhizospheric soil of four different Grasses and strategies to promote Plantation in degraded Forest area in Gujarat, India

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**SUMMARY**

Arbuscular Mycorrhizal fungi (AMF) are the most important members of soil microbial community in ecosystem. These useful organisms form an indespensible component of any fertile soil. It is said that a good quality of soil is almost equal to 1kg of gold. Symbiotic relationships of AMF provide an alternative for survival of plants in highly stressed areas, this association helps in uptake of P as well as other minerals, maintain better water balance, increase plant biomass and produce growth promoting substances. By application of such organisms use of synnthetic fertilizers can be reduced. Stratagies to promote plantation in poor or degraded soil includes introduction of native AMF in soil. Pelleting of different seeds will be tried with small root pieces to incorporate AMF. During present study a survey was undertaken in degraded forest areas with certain grasses in (Godhara and Baria divisions) 115 kms from Vadodara city in India. Rhizospheric soil samples from 25 different places were collected. Soil samples of *Heteropogon contortus* showed more number of AM spores (220) as compard to *Themeda triandra* (165). In Kalitalai *Chloris barbata* showed more number of spores (150), while it was only 110 in soil sample analyzed from Rampara. The percentage occurence of AM spores was more in Bandheli. Analysis of AM spores resulted into identification of different species

belonging to 3 genera i.e. *Glomus*, *Gigaspora*, and *Acaulospora*. Since *Glomus* was present predominantly in most of the cases it was selected for further study.

Key words: AM Fungi, *Heteropogon contortus*, *Themada triandra*, *Chloris barbata*, *Glomus, Gigaspora*

## Introduction:

Mycorrhizas are symbiotic association between plant roots and certain fungi. These associations have evolved with plants since the colonization of dry land by plants began as a survival mechanism for fungi and higher plants. Both endure the existing environments of low soil fertility, periodic drought, diseases, extreme temperature and other natural stresses. AM fungi cannot complete their life cycle without host plants. Benefits derived by plants from this relationship includes increase nutrient uptake, more water absorption, increased nutrient mobilization, production of feeder roots, longevity as well as stress tolerance etc. (Manoharachary *et al.*,2008). AM fungi are naturally occurring fungal component of soil microbiota in most of terrestrial ecosystems as well as in some aquatic plants (Stenland and Charvat 1994, Manoharachary *et. al.,* 2008). However, it is important to distinguish between specificity (the ability to colonize), effectiveness (plant response to colonization), and ineffectiveness (the amount of colonization) because AM are widely different in these abilities depending on the environment. They do have wide host ranges, however and are capable of long term relationship with many different plants. In order for this partnership to work at atleast four elements must be in place: a) Appropriate root morphology, b) Fungal structures able to penetrate the plant cell, c) Extra radical mycelia which are root like vegetative fungal structure growing in the soil and d) Soil condition.

AM fungi are the dominant component of the rhizosphere soil and transfer many assimilates to the roots. This alters the root exudation pattern and hence changes the microbial population dynamics of the rhizosphere and rhizoplane regions (Brundrett 2004). The fungi responsible are classified in the phylum Glomeromycota, of order Glomales. They are assumed to be unculturable and, except for germination, wholly dependent on photosynthetic plants. AM fungi are used to be classified as Vesicular Arbuscular Mycorrhizae (VAM) but research uncovered that a major suborder did not form thin walled, lipid filled vesicles, so they are referred to as AM associations today. There is no evidence for specificity between plants and AM fungi (Smith and Read 1997). Mycorrizal fungi act as providers and protectors for plants. For example N,P,K are deficient in certain soils and can be increased in plant intake by mycorrhizae ( Norland, 1993) . Other essential nutrients such as Ca, Mg, S ,Fe, Zn, Al & Na have been shown to increase in plants with AM fungi ( Daft & Hacskaylo, 1976) .

Baria taluka of Dahod district in Gujarat has 29353.39 ha. as reserved forest area. Possibility of raising trees and grass is very higher here and in certain other areas of the state. Survey was conducted in different areas of Rampara, Kali Talai areas of Baria division Forest in Central Gujarat region, India.The study area falls between in 22° 41' 60 N latitude and 73° 54' 0 E Longitude.

## Materials and Methods

**Common Grasses selected for the Study include:**

**1. *Dicanthium annulatum* (Forsk.) Stapf. (Zinzvo)**

Stems of this perennial grass usually woody at the base with strong wiry roots and tufted leaves then geniculately ascending. Nodes may be beared or not. Leaves rigid, glaucous, glabrous or hairy above with tubercle- based hairs, sheath beared at tip, ligule oblong, obtuse. Spiklets variable in size 2.5 to 6.2 cm, pinkish or nearly white.

**2. *Heteropogon contortus* L. (Nani-Shukali)**

Plants are perennial, stems erect from a decumbent rooting base, 50-70 cm tall, branched, compressed, glabrous, seriate and leafy. Leaves are flat, glabrous, acuminate, sheaths shorter than internodes, ligule short, membranous, ciliate. Racemes 8-15 cm long, few to many, axillary and terminal. Sessile spiklets 0.4 -0.5 cm long, small, naked.

**3. *Themeda triandra* Forsk. (Butani, Marar)**

It is perennial, densely tufted herb. Stem is stout or slender, erect or geniculate and ascending, subsimple or branched, nodes glabrous. Leaves are linear, flat. Sheaths compressed, keeled, smooth. Panicle narrow, reaciform, branched. Involucral spikelets are whorled, sessile, persistent, lanceolate, acute, oblong, glabrous, male, glumes-3. Pedicellate spiklets are linear-lanceolate.

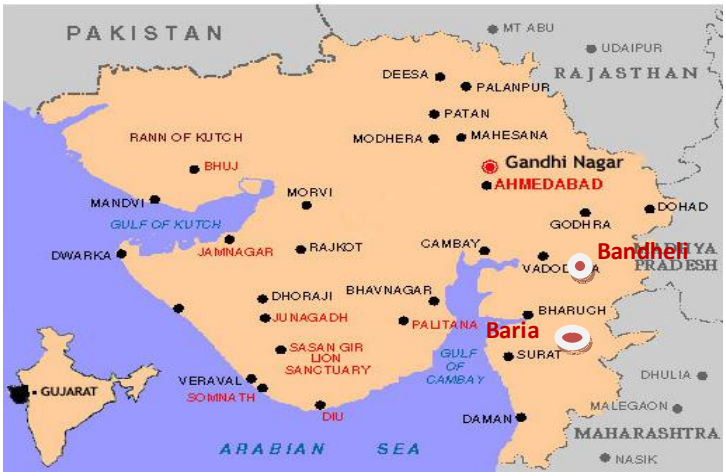
**4. *Chloris barbata* L. (Mindadiu, Kaliu)**

A member of family Poaceae this grass is perennial. Leaf-sheaths glabrous on surface. Ligule is a ciliolate membrane, leaf-blades involute. Inflorescence is composed of racemes, racemes 2ï 3; paired or digitate, spreading; unilateral. Spikelets pendulous, solitary, sessile. Spikelets comprising 1 fertile floret; with diminished florets at the apex. Spikelets cuneate, laterally compressed. Glumes persistent; similar; shorter than spikelet; thinner than fertile lemma gaping. Lower glume lanceolate, upper glume has primary vein smooth or scaberulous, in upper glume lateral veins are absent.

## Isolation of AM fungi by Wet Sieving and Decanting Method:

Wet Sieving and Decanting method as suggested by Gerdemann and Nicolson (1963) was used to obtain AM spores from rhizospheric soil. The method includes mixing of 100g soil with 1litre of tap water and then decanting it in a stack of sieves. In the present case 5 sieves ranging from 63

to 500 µm were used. The AM spores were separated on different sieves, based on their size. They were then picked up by hypodermic syringe using a stereo microscope. Permanent slides were prepared in polyvinyl alcohol glycerol. Similar types of 5 spores were placed in a vial containing 0.05% streptomycin sulphate for further use. VAM spores were collected, photographed and on the basis of their colour, spore size, identification has been done.



## RESULTS:

**Table 1: Number of AM spores per 100g isolated from different soil samples of Baria division and economic importance of these grasses.**

| Sr. No. | Plants | Location | Sample No. | No. of spores/100g soil | Economic Importance |
|---|---|---|---|---|---|
| 1. | *Heteropogon contortus* | Bandheli, Godhra | A | 220 | Used as fodder. Roots are diuretic and sometimes used in rheumatism. |
| | | ,, | B | 137 | |
| | | ,, | C | 205 | |
| | | ,, | D | 150 | |
| | | ,, | E | 110 | |

| | | | | | |
|---|---|---|---|---|---|
| | | Kalitalai, Baria | F | 130 | |
| | | ,, | G | 98 | |
| | | Rampara, Baria | H | 105 | |
| 2. | *Chloris barbata* | Kalitalai, Baria | A | 150 | Used as fodder in young stage. |
| | | ,, | B | 97 | |
| | | Rampara, Baria | C | 85 | |
| | | ,, | D | 110 | |
| 3. | *Themada triandra* | Bandheli, Godhra | A | 157 | Palatable when young and unpalatable when mature. Shows some amount of tolerance to drought. |
| | | ,, | B | 165 | |
| | | Rampara, Baria | C | 95 | |
| | | ,, | D | 115 | |
| | | Kalitali, Baria | E | 97 | |
| | | ,, | F | 120 | |
| 4. | *Dicanthium annulatum* | Rampara, Baria | A | 85 | Highly esteemed fodder grass. Palatable both when young and mature. Controls soil erosion because of its elaborate root system. |
| | | ,, | B | 125 | |
| | | Kalitalai, Baria | C | 140 | |
| | | ,, | D | 100 | |

**Table 2: Identification of AM spores based on different morphological characters of 4 different grasses**

| Sr. No. | Name of AM Fungi | Colour of the Spore | Size (µm) | Wall layers | Thickness of wall (µm) | Thickness of Hyphae (µm) |
|---|---|---|---|---|---|---|
| 1 | *Glomus glomerulatum* Sieverding | Yellow-Brown | 64 | 2 | 6.4 | --- |
| 2 | *G. claroides* Schenck& Smith | Yellow-Brown | 76.8 | 2 | 12.8 | --- |
| 3 | *G. glomerulatum* Sieverding | Yellow-Brown | 64 | 2 | 6.4 | --- |
| 4 | *G. clarum* Nicolson & Shank. | Yellow-Brown | 92.8 | 2 | 9.6 | --- |
| 5 | *G. macrocarpum* (Tul.&Tul.) Berch& Fortin | Yellow-Brown | 86.4 | 2 | 6.4 | --- |
| 6 | ,, | Yellow-Brown | 88.2 | 2 | 19.6 | --- |
| 7 | *G. claroides* Schenck& Smith | Yellow-Brown | 70.4 | 2 | 6.4 | --- |
| 8 | *G. clarum* Nicolson & Shank. | Hyaline | 85.7 | 2 | 6.4 | --- |
| 9 | *G. etnicatum* Becker & Gerd. | Yellow | 83.2 | 2 | 6.4 | --- |
| 10 | ,, | Yellow | 73.6 | 2 | 6.4 | --- |
| 11 | *G. citricola* Tang. & Zang. | Hyaline-Yellow | 57.6 | 2 | 6.4 | --- |
| 12 | *G. geosporum* (Nicol. & Gerd.)Walker | Yellow-Brown | 294.4 | 2 | 9.6 | --- |

| | | | | | | |
|---|---|---|---|---|---|---|
| 13 | *G. fasciculatum* (Thaxter) Gerde.& Trappe emend. Walker & Koske | Hyaline-Yellow | 124.8 | 3 | 9.6 | --- |
| 14 | ,, | Yellow-Brown | 112 | 2 | 9.6 | --- |
| 15 | *G. agrregatum* (Schenck Smith) emend. Koske | Yellow-Brown | 131.2 | 2 | 12.8 | --- |
| 16 | *G. fasciculatum* (Thaxter) Gerde.& Trappe emend. Walker & Koske | Yellow-Brown | 92.8 | 2 | 9.6 | --- |
| 17 | *Gigaspora albida* Scenck& Smith | Brown | 86.4 | 2 | 6.4 | 6.4 |
| 18 | *Glomus mosseae* Nicol.& Gerd. | Hyaline<br>Yellow | 70.4 | 2 | 6.4 | --- |
| 19 | *Gigaspora ramisporophora* Spain, Siverding & Schenck | Brown | 284.8 | 2 | 12.8 | 12.8 |
| 20 | *Gigaspora albida* Scenck& Smith | Yellow-Brown | 220.8 | 2 | 16 | 12.8 |
| 21 | *Glomus fuegianum* (Spegazzii) | Yellow-Brown | 67.2 | 2 | 6.4 | --- |
| 22 | *G. hoi* Berch. & Trappe | Yellow-Brown | 73.6 | 3 | 9.6 | --- |
| 23 | ,, | Yellow-Brown | 92.8 | 3 | 12.8 | --- |
| 24 | *G. macrocarpum* (Tul. &<br>Tul.) Berch & Fortin | Hyaline-Brown | 121.6 | 2 | 9.6 | --- |
| 25 | *Gigaspora decipiens* Hall & Abbot. | Brown | 259.2 | 2 | 9.6 | 9.6 |
| 26 | *G. fasciculatum* (Thaxter) Gerde. & Trappe emend. Walker & Koske | Hyaline-Yellow | 124.8 | 3 | 9.6 | --- |

| | | | | | | |
|---|---|---|---|---|---|---|
| 27 | *Gigaspora candida* Bhattacharjee,Mukherji, Tiwari & Skoropad | Brown | 204.8 | 2 | 16 | 16 |
| 28. | *Acaulospora laevis* Gerde.& Trappe | Yellow-Brown | 183.15 | 3 | 6.66 | 6.66 |

Note: - size of hyphae not recorded

--- = Absent

**Table 3: Identification of different spores of *Glomus* based on different morphological characters**

| Slide No | Name of AM Fungi | Colour of spore | Size (µm) | Wall layers |
|---|---|---|---|---|
| 1 | *Glomus fasciculatum* (Thaxter) | Yellow to brown | 108.8 | 3 |
| 2 | *G. aggregatum* (Shenck. & Smith) emend. Koske | Brown | 105.6 | 2 |
| 3 | *Glomus etunicatum* Becker & Gerdemann | Yellow to brown | 128 | 2 |
| 4 | *Glomus monosporum* Gerdemann & Trappe | Yellow to brown | 169.7 | 2 |
| 5 | *Glomus convolutum* Gerde.& Trappe. | Yellow to brown | 83.2 | 2 |
| 6 | *G. macrocarpum* (Tul. & Tul) Berch & Fortin | Yellow to brown | 108.8 | 2 |
| 7 | *G. maculosm* Schenck & Smith | Hyaline to yellow | 121.2 | 2 |
| 8 | *G .claroides* Schenck & Smith | Yellow to Brown | 92.8 | 2 |
| 9 | *G. melanosporum* Gerdemann & Trappe | Yellow to | 157.6 | 2 |

| | | | | |
|---|---|---|---|---|
| | | brown | | |
| 10 | *G. caledonium* (Nicol. & Gerd) Trappe & Gerdemann | brown | 139.3 | 2 |
| 11 | *G. intraradices* Schenek & Smith | Yellow to Brown | 109.1 | 2 |
| 12 | *G. tenerum* (Tandy) Mc Gee | Orange | 150.1 | 2 |
| 13 | *Glomus geosporum* (Nicol. And Gerd.) Walker | Yellow to Brown | 196.9 | 2 |
| 14 | *G. hoi* Berch. & Trappe. | Yellow to Brown | 112 | 2 |
| 15 | *G.mossae* (Nicol & Gerd) Gerdemann & Trappe | Yellow to brown | 127.3 | 2 |

## Conclusions:

It is evident from Tables above that rhizospheric soil of different grasses harbored –

- 15 species of *Glo*mus
- 4 species of *Gigaspora*
- Only one species of *Acaulospora*

**ACKNOWLEDGEMENTS**

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**REFERENCES:**


- Adholeya A and Gaur A. 1994 Estimation of VAM fungal spores in soil *Mycorrhiza News* 6(1): 10 – 11

- Brundrett M. 2004 Diversity and classification of mycorrhizal associations *Biol Rev* 79: 473-495.

- Daft M. and E. Hacskaylo. 1976. Arbuscular mycorrhizas in the anthracite and bituminous coal wastes of Pennsylvania. *Journal of Applied Ecology.* 13: 523 – 531.

- Gerdemann J W and Nicolson Y. H. 1963 Spores of mycorrhizae Endogone species extracted from soils by wet sieving and decanting *Transactions of British Mycological Society* 46: 235 – 244

- Manoharachary C., Swarupa Rani S. and Kunwar I.K. 2008 Arbuscular Mycorrhizal Fungi Associated With Some Plants of Apocynaceae *J Mycol Pl Pathol* 38(1): 91-92

- Manoharachary C.,Ameetha P. Kunwar I.K. and Reddy V.S. 2008 Arbuscular Mycorrhizal Fungi in Relation to Plant Growth in *Dalbergia sissoo J Mycol Pl Pathol* 38(1): 97-101

- Mehrotra V. S. and Baijal U. 1994 Advances in the taxonomy of Vesiular Arbuscular fungi Biotechnology in India pp. 227 – 286.

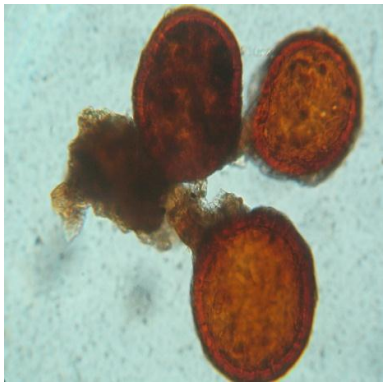
- Norland M.R. 1993. Soil facotrs affecting mycorrhizal use in surface mine reclamation. US Department of Interior, Bureau of Mines, Information Circular/9345 pp.21.

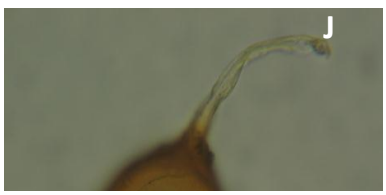
- Schenck N. C. and Perez Y. 1990 *Manual for the identification of VA Mycorrhiza (VAM) fungi* Florida: Synergistic Publisher 241 pp.

- Smith S.E and Read D.J. 1997. *Mycorrhizal Symbiosis*. 2nd ed. Academic Press, London. pp 1-589

- Stenland D.L. and Charvat I.D. 1994 Vesicular arbuscular mycorrhizae in floating wetland mat communities dominated by *Typha*. *Mycorrhiza* 4: 131-137


**Plate- I**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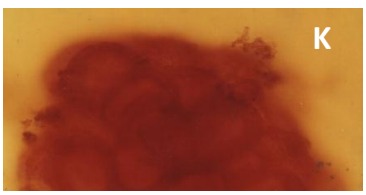
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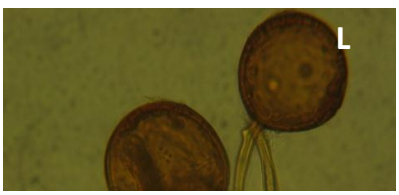
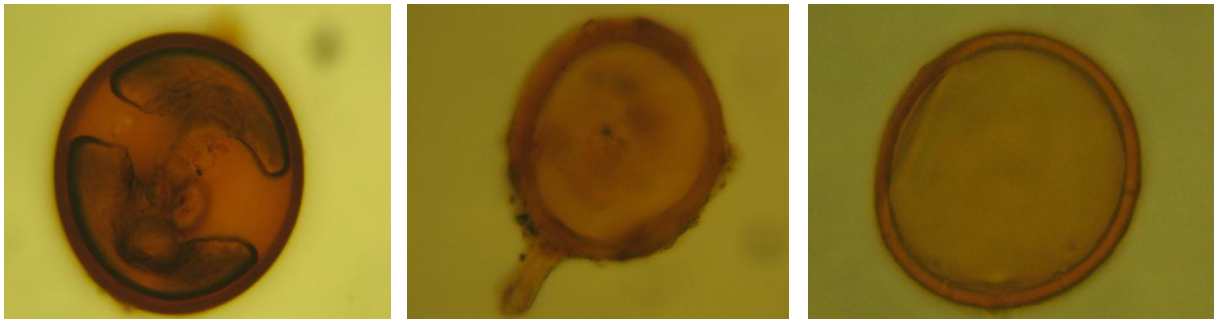
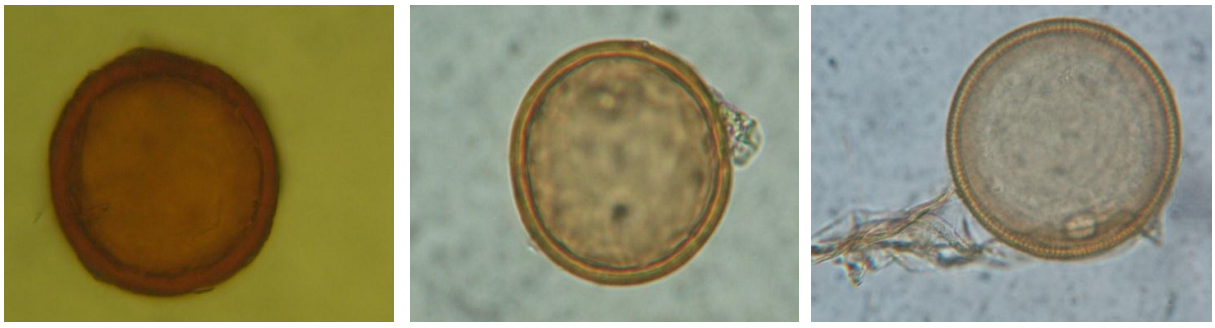
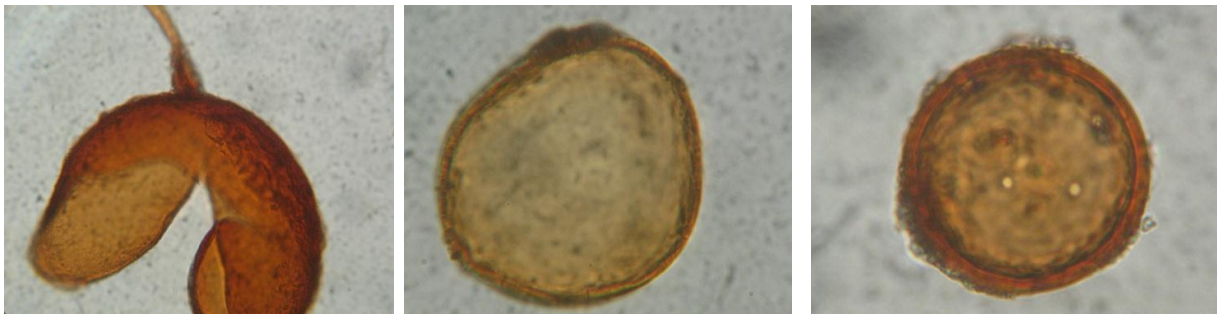
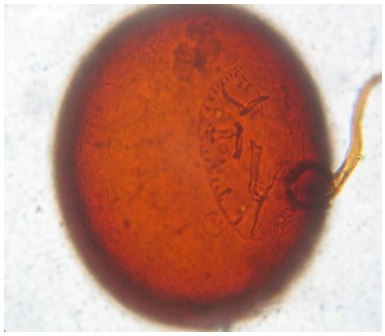
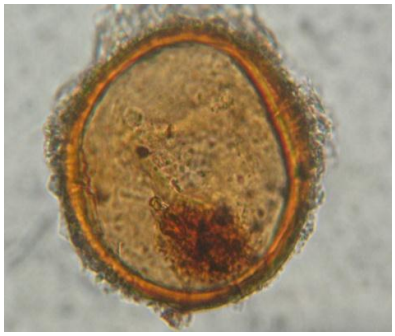

L

**Plate-II**








**J**

**Plate- I**

A) & B) - Grasslands of Baria Division

C) *Heteropogon contortus*

D) *Themada triandra*

*E*) *Dicanthium annulatum*

F) *Chloris barbata*

G) *Glomus aggregatum*

H) *Glomus clarum*

I) *Gigaspora albida,*

J) *Glomus fasciculatum*,

K) *Sclerocystis microcarpa*

L) *Glomus aggregatum*

**Plate-II**

A) *Glomus melanosporum*

B) *Glomus mosseae*

C) *G.glomerulatum*

D) *G.claroides*

E) *G.citricola*

F) *G. macrocarpum*

G) *Gigaspora candida*

H) *G. etunicatum*

I) *G.geosporum*

J) *Gigaspora ramisporophora*

K) *G.hoi*

L) *Gigaspora decipiens*

# Phylloplane mycoflora of *Madhuca indica* L. and *Diopsyros melanoxylon* Roxb.

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**Abstract**

Indian region is one of the most diverse biogeographic regions of the world, embracing a wide range of topography from perpetually snow covered high Himalayas to plains at sea level. A great variety of climatic and altitidinal variations coupled with varied ecological habitats has contributed immensely to the rich diversity. National Forest Policy 1988 advocates for increasing forest productivity and meet the demands of timber, fuel, fiber, paper etc.

Phylloplane fungi have been poorly studied as compared to endophytes, saprobes and pathogenic fungi. Nutrients stimulatory for microorganism on phyllosphere and phylloplane regions consist primarily of plant materials *(e.g.* pollen grains, old petals, etc.) or insect excreta. Stimulation of necrotrophic (destructive) fungi by pollen grains (Fokkema, 1981) and aphids honey dews (Fokkema *et al,* 1983) is well documented. Fungi may be blown from near or for off places and settle on leaf lamina. These fungi may grow when conditions are favorable and can cause diseases, the fungi may be necrotrophic and obligate parasites. Such a study is required to find out ecological niches in order to control them. A study was under taken on leaves of *Madhuca* and *Diospyros* to find out phylloplane fungi. Results indicated that more number of viable fungi were recorded by serial dilution technique.

## Introduction

Numerous investigations have been carried out on the fungal flora of leaf surfaces of several plants growing or cultivated in many parts of the world by several researchers (Abdel-Fattah *et al.*, 1977; Abdel-Hafez, 1981, 1984, 1985; Abdel-Hafez *et al*., 1995; Eicker, 1976; Khallil and Abdel-Sater, 1993; Mazen *et al*., 1985; Nagaraja, 1991; Perez and Mauri, 1989; Sharma, 1974).

Tropical forests in Jambughoda have large number of *Madhuca* and *Diospyros* trees. *M. indica* suffers from**,** *Cylindroncladium scorpariu, Pestalotia dichaeta, Pestalotia* sp*., Phyllachora madhuca, Polystictus steinbelianus, Scopela echinulata, Sarcinella* sp*. Sphaceloma madhucae*

***Diospyros melanoxylon*** Roxb. is attacked by *Aecidium rhytismodieum, A. miliar, Cercospora kaki, Pseudocercospora kelleri, Sarcinella gorakhpurensis.* It is essential that diseases must not occur in *D. melanoxylon* tree as its leaves are used for making local cigarette (Bidi) and Madhuca flowers and oil obtained from seeds is used for different purposes.

Phyllosphere is the immediate vicinity of leaf surface, where microbial communities are constantly in dynamic state due to exo-and endogenous sources of nutrients. It is a complex terrestrial habitat that is characterized by presence of a variety of microorganisms including bacteria, filamentous fungi and yeasts. Phylloplane fungi are the mycota growing or the surface of leaves. There are two groups of phylloplane 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.

Most of the methods used for the study of phylloplane fungi have advantages and disadvantages. Direct observation for example, enables one to determine the distribution of the phylloplane population and provides useful information such as the growth forms and spatial distributions of a variety of microorganisms. Direct microscopy gives a more precise estimate of population size than plate count methods (Baker, 1981). Direct count methods give higher densities than impressions because some organisms may remain on the plant when the adhesive tape is removed. It is not however, easy to determine the viability and identity of propagules unless the groups are separated by their morphological differences using microscopy (Last and Warren, 1972; Baker 1981; Parbey *et al.,* 1981). The irregular nature of the plant surface, moreover, makes the examination difficult at high magnification (Paton, 1982).

Leaf washing facilitates the óidentification of colonizersô from chance initiators (Last and Warren, 1972). Culture condition may also affect the subsequent growth of the propagules (Parberry *et al.,* 1981) and some organisms may not grow on the medium provided (Baker, 1981).

In the leaf impression method, fungal spores on the leaf surface stick onto the agar. This may give rise to very dense fungal and bacterial growth on the agar plate (Lee *et al*., 2002). When the spore technique is used only fungal colonies with mature fruiting bodies will be isolated and immature fungal colonies, or species that need water for spore release, will be neglected.

Rai and Singh (1981) have investigated the antagonistic activities of some phylloplane fungi (of mustard and barley) against *Alternaria brassicae and Drechslera graminea.* The antagonists are *Aureobasidium pullulans, Epicoccum purpurascens, Cladosporium cladosporioides* and *Alternaria alternata.* The most significant effects were

observed when the spores of leaf surface fungi or their metabolites were sprayed on leaves prior to inoculation of the pathogens.

**Materials and Methods**

There are several methods for investigating phylloplane fungi such as tape impressions followed by culturing (Langvad, 1980) and spore ï fall techniques (Lamb and Brown 1970, Dickinson 1973, Langvad 1980, Vardavakis 1988). With tape impressions a thin agar film is pressed on to the leaf surface that can be incubated in Petri dishes containing filter paper moistened with glycerol. The spore fall technique involves attaching the leaf onto the inside of the Petri dish lid above agar, using the premise that fungal spores can fall or are short onto the agar surface.

Three methods: direct observations, leaf washing method, cellotape method were followed to study phylloplane fungi on *Diospyros melanoxylon, Madhuca indica* leaves.

The above two plants were chosen to study because they come in abundant source, easily available *D. melanoxylon* is one of the Minor Forest Produce and has high economic value.

Leaves of *D. melanoxylon* and *M. indica* were sampled and brought to laboratory in polythene bags. The leaves were washed in sterile distilled water and collected in conical flask. 1 ml of water after washing the leaves was placed on PDA containing plates and were incubated at $25^0$C and examined until fungal colonies appeared. Sterile mycelia were subcultured to PDA medium.

The fungi were identified on basis of taxonomic keys when fruiting occurred.

**Results and Discussion**

The fungi present in infection court may be influnced by other microbes and environmental conditions. A survey conducted on phylloplane microflora by cellotape and serial dilution plate method revealed presence of 13 different fungi in the *D. melanoxylon* and eight in case

of *M. indica* it is intersting to note that leaves when infected by these fungi may produce toxic compounds. The fungi like *Aspergillus, Candidus, Alternaria alternata, Nigrospora sphaeraca* and *Phyllactinia* sp. Were recovered by dilution plate technique only. *A niger* and *Alternaria alternata* are most common airbone fungi these are common aero allergens.

**Table 1: List of fungi isolated from the phylloplane of *Diospyros melanoxylon* and *Madhuca indica***

| **Sr. No.** | **Fungi** | ***Diospyros melanoxylon*** | ***Madhuca indica*** |
|---|---|---|---|
| 1. | *Alternaria* sp. | √ | √ |
| 2. | *Aspergillus awamori* | √ | √ |
| 3. | *Aspergillus fumigatus* | √ | √ |
| 4. | *Aspergillus niger* | √ | √ |
| 5. | *Cladosporium cladosporoides* | √ | - |
| 6. | *Colletotrichum capsici* | √ | √ |
| 7. | *Curvularia lunata* | √ | - |
| 8. | *Fusarium oxysporum* | √ | - |
| 9. | *Penicillium citrinum* | - | √ |
| 10. | *Pestalotiopsis* sp. | √ | - |
| 11. | *Pestalotiopsis versicolor* | √ | - |
| 12. | *Phyllactinia* sp. | √ | - |
| 13. | *Rhizopus stolonifer* | √ | √ |
| 14. | *Trichoderma* sp. | √ | √ |

**Note -** Presence of fungi based on 5 inoculms placed on PDA plates.

**Table 2: List of fungi recorded and their percentage frequency of occurence by two different methods.**

| Sr. No. | Species | *Madhuca indica* | | *Diospyros melanoylon* | |
|---|---|---|---|---|---|
| | | Cellotape method | Dilution | Cellotape | Dilution |
| | **Zygomycetes** | | | | |
| 1. | *Mucor heimalis* | 2 | 5 | 2 | 2 |
| 2. | *Rhizopus stolonifer* | 2 | 5 | 5 | 5 |
| | **Ascomycetes** | | | | |
| 3. | *Chaetomium globosum* | 1 | 5 | 2 | 5 |
| 4. | *Phyllactinia* | - | - | - | 2 |
| | **Anamorphic fungi** | | | | |
| | ***Hyphomycetes*** | | | | |
| 5. | *Aspergillus candidus* | - | 2 | - | 3 |
| 6. | *A. niger* | 2 | 10 | 2 | 10 |
| 7. | *A. awamori* | - | - | 2 | 2 |
| 8. | *Alternaria alternata* | - | 5 | - | 10 |
| 9. | *Cladosporium cladosporiodes* | 1 | 2 | - | 2 |
| 10. | *Curvularia lunata* | 2 | 8 | 4 | 10 |
| 11. | *Fusarium oxysporum* | - | 5 | 2 | 6 |
| 12. | *Nigrospora sphaerica* | - | 2 | - | 2 |
| 13. | *Penicillium citinum* | 2 | 10 | 2 | 4 |
| 14. | *Trichoderma viride* | 4 | 10 | 2 | 10 |
| | **Coelomycetes** | | | | |
| 15. | *Pestalotiopsis* sp. | 2 | 2 | 2 | 6 |
| 16. | *Colletotrichum capsici* | 1 | 2 | - | 2 |

NOTE: Presence ( - ) absence of colonies based on average of 3 replicates. Numericals show percentage frequency.

## References


Abdel-Fattah, H. M., Moubasher, A. H. and Abdel-Hafez, S. I. (1977) Fungus flora of root and leaf surface of broad bean cultivated in Oases, Egypt. *Naturalia Monspeliensia Ser. Bot.* 27: 167-177.

Abdel-Hafez, S. I. (1981) Phyllosphere and phylloplane fungi of wheat cultivated in Saudi Arabia. *Mycopathologia* 75: 33-38.

Abdel-Hafez, S. I. (1984) Rhizosphere and phyllosphere fungi of four fern plants growing in Saudi Arabia. *Mycopathologia* 85: 45-52.

Abdel-Hafez, S. I. (1985) Leaf surface fungi of *Argemone mexicana* growing in Saudi Arabia. *Cryptogamie*, *Mycol*. 6: 69-78.

Abdel-Hafez, S. I., El-Said, A. and Gherabawy, Y. A. (1995) Mycoflora of leaf surface stem, bagasse and juice of adult sugarcane (*Saccharum officinarum* L.) plant and cellulolytic ability in Egypt. Bull. Fac. Sci., Assiut Univ. 24: 113- 130.

Baker J. H. 1981. Direct observation and enumeration of microbes on plant surfaces by light microscopy. In: Blak-emanJP, ed. Microbial ecology of the phylloplane. Lon-don: Academic Press. p 3-14.

Baker JH. 1981. Direct observation and enumeration of mi-crobes on plant surfaces by light microscopy. In: Blak-emanJP, ed. Microbial ecology of the phylloplane. Lon-don: Academic Press. p 3-14.

Dickinson C.H. (1973) Effects of ethirimol and zineb on phylloplane microflora of barley. *Trans Br Mycol Soc* 60:423-431.

Fokkema N.J. (1973) The role of saprophytic fungi in antagonism against *Drechslera orokiniana (Helminthosporium sativum*) on agar plates and on rye laves with pollen. *Physiol. Plant. Pathol.* 3: 195- 205.

Lamb RJ, Brown JF. 1970. Non-parasitic microflora on leaf surfaces of *Paspalum dilatatum, Salix babylonica* and *Eucalyptus stellulata. Trans. Br. Mycol. Soc.* 55:383-390.

Langvad F. 1980. A simple and rapid method for qualitative and quantitative study of the fungal flora of leaves. *Can J Bot* 26:666-670.

Last F. T. and Warren RC. 1972. Non-parasitic microbes colonizing green leaves: their form and functions. Endeavour 31:143-150.

Lee O.H.K and Hyde K.D. (2002) Phylloplane fungi in Hongkong mangroves: evaluation of study methods. *Mycologia*, 94(4), 596-606.

Nagaraja,T.G.(1991) rhizosphere and phyllosphere studies in *strychnous nux*-vomica linn. Ad. Plant sci. 4:171-173.

Parbery J. H. Brown JF, Bofinger VJ 1981. Stastical methods in the analysis of phylloplane population In Blakeman JP, ed microbial ecology of phylloplane. London Academic Press P 47-65.

ParberyJH, Brown JF, Bofinger VJ. 1981. Statistical methods in the analysis of phylloplane populations. In: Blake-man JP, ed. Microbial ecology of the phylloplane. Lon-don: Academic Press. p 47-65.

Paton A. M. (1982) Light microscope techniques for the mi-crobiological examination of plant materials. In: Rhodes-Roberts ME, Skinner FA, eds. Bacteria and plants. London: Academic Press. p 235-243.

Rai B. and Singh D. B. (1981) Effect of leaf extracts of mustard and barley on growth behavior of some phylloplane microfungi. Bulletin of the Torrey botanical club. 108(4) pp. 419-421.

Vardavakis E. (1988) Seasonal fluctuation of non-parasitic mycoflora associated with leaves of *Cistus incanus*, *Arbutus unedo* and *Quercus coccifera. Mycologia* 80:200- 210.

**Fig: Graph showing occurence of phylloplane fungi by Cellotape and dilution plate methods**