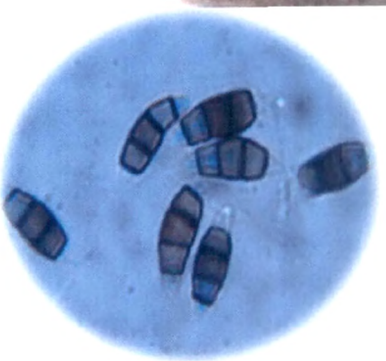
RESULTS AND DISCUSSION



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 – 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 74° 37' to 74° 11' E Longitude and

between 22° 32' to 22° 35'N 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. `Pani'.

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 – 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 – 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 – 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 Amphispharíaceae (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 – 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 Koch's 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 – 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 – 0.3 % azadirachtin and 30 – 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 – 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 – hydroxyvindole) isolated from the roots of *Allium fistulosum*, inhibits the protein synthesis of *Fusarium oxysporum* (Phay *et al.,* 1999). Eugenol (4 – allyl – 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 – 3 – O-b-D- apiofuranosyl -12) –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,β – 1,4 – 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 $P \leq 0.05$ 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

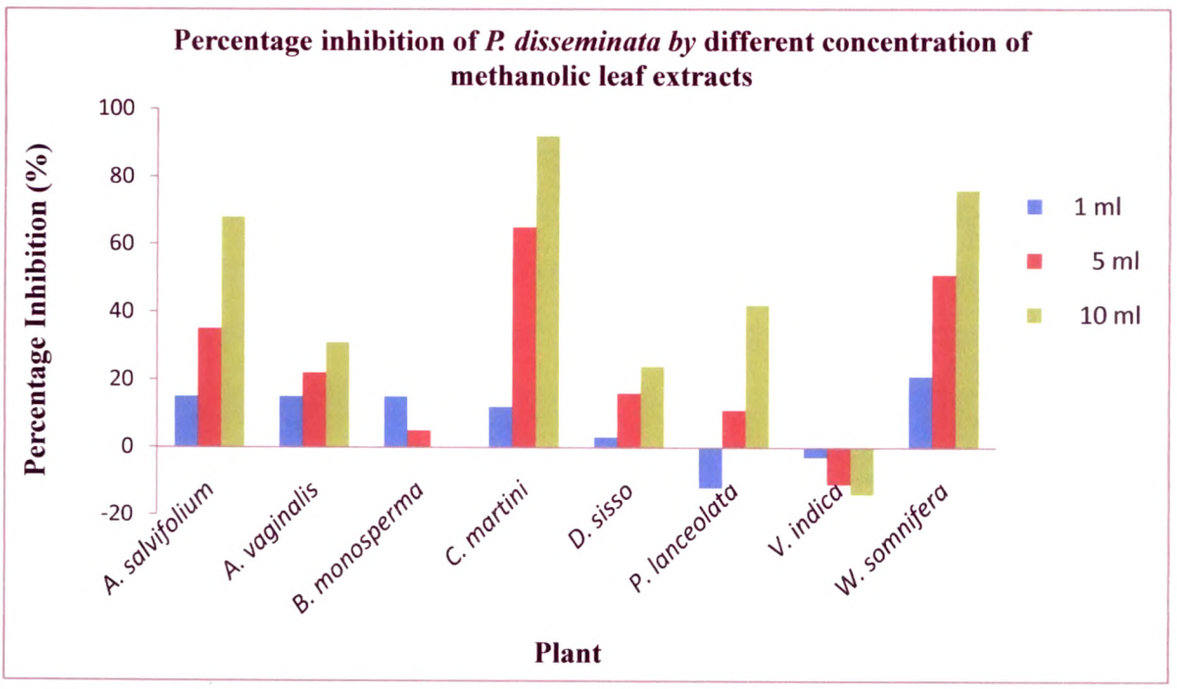
Results were significant at $P \leq 0.05$ level by one way ANOVA

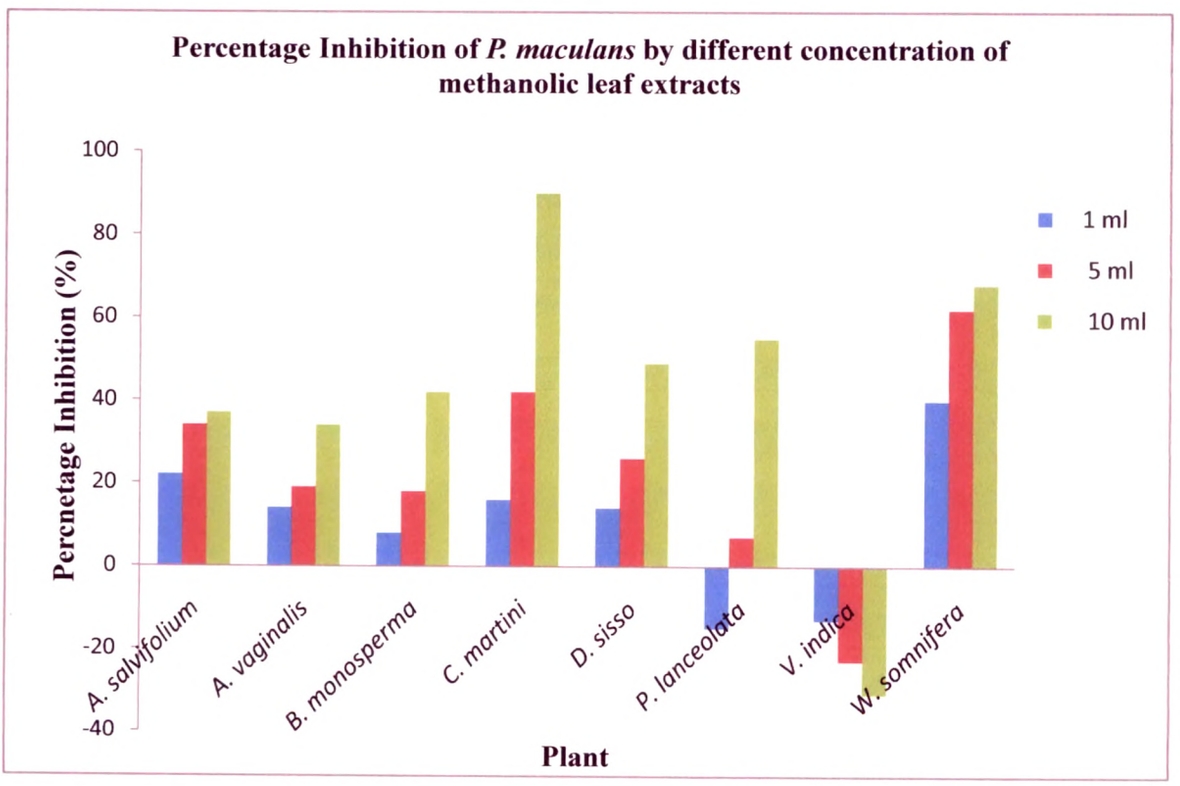
Table: 3.4 Percentage inhibition of *Phomopsis tectonae* at different concentration of leaf Extracts

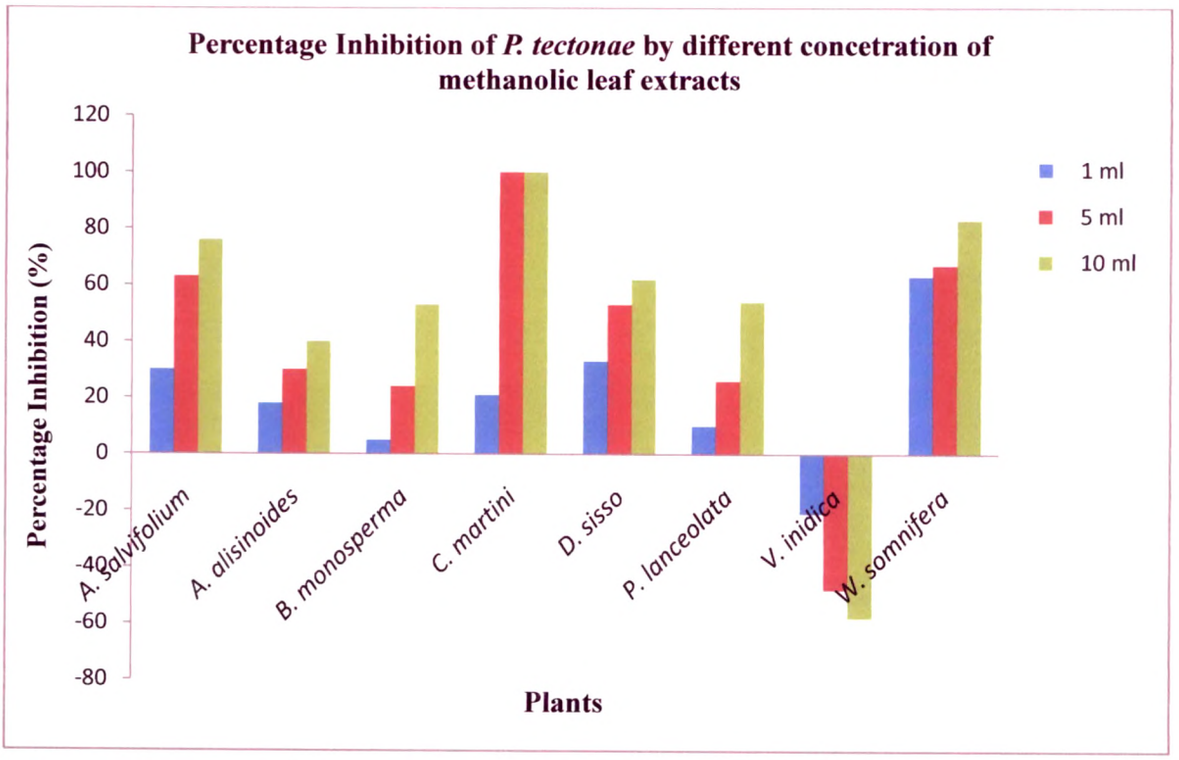
Sr. no	Plant Selected	Methanolic extract		
		1% *	5% *	10% *
1.	*Alangium salviifolium*	30 ±0.57	63 ±2.0	76 ±2.51
2.	*Alysicarpus vaginalis*	18 ±1.52	30 ±1.0	40 ±3.51
3.	*Butea monosperma*	5 ±2.30	24 ±2.0	53 ±2.0
4.	*Cymbopogon martini*	21 ±4.0	100 ±0.0	100 ±0.0
5.	*Dalbergia sisso*	33 ±2.0	53 ±1.52	62 ±1.15
6.	*Pluchea lanceolata*	10 ±3.05	26 ±2.08	54 ±1
7.	*Vogelia indica*	-21 ±2.0	-48 ±1.0	-58 ±3.05
8.	*Withania somnifera*	63 ±2.0	67 ±0.57	83 ±1.0

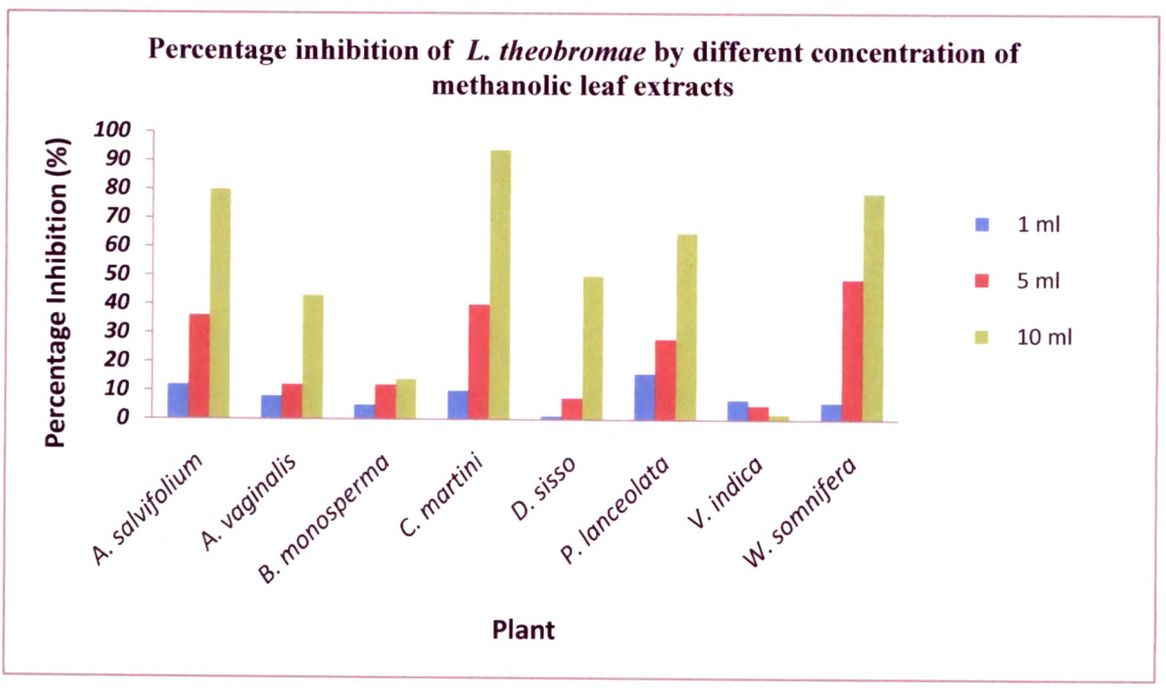
* indicates each compound values are based on three replicates
Results were significant at $P \leq 0.05$ level by one way ANOVA

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.*


Percentage inhibition of P. disseminata by different concentration of methanolic leaf extracts
Percentage Inhibition (%)
100
80
60
40
20
0
-20
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. maculans by different concentration of methanolic leaf extracts
Percnetage Inhibition (%)
100
80
60
40
20
0
-20
-40
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. 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

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 Micheli's 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 Pasteur's 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.	Coon's	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 & Hawker's	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 & Hawker's	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.	Elliot's	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.	Richard's	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.	Czapek's	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 ±0.02	7.00	0.39 ±0.03	7.05	0.22 ±0.005	7.00	0.18 ±0.005	7.00
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 $P \leq 0.05$ level by one way ANOVA

Observation from Table no:1 revealed that best growth of *P. disseminata* was obtained on Host decoction followed by Richard's, Czapek's, PDA, DOX's, Modified Asthana & Hawker's, Asthana & Hawker's, Elliot's and Coon's medium. Statistical analysis revealed best growth on *T. arjuna* leaf decoction, Richard's, Czapeck's while it was poor on all other mediums.

The growth of *P. maculans* was maximum on Richard's followed by DOX, Czapek's and was poor on all the others. Richard's medium supported maximum growth of *P. tectonae*

followed by PDA, *T. grandis* decoction, DOX, Czapek's medium while poor growth was accomplished on rest all mediums.

Lasiodiplodia theobromae achieved maximum yield on Czapek's followed by Richard's after 15 days incubation, moderate growth was observed on PDA, *T. grandis* decoction followed by modified Asthana & Hawker's medium and was poor on rest others. The excellent sporulation was observed on Modified Asthana & Hawker's 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 constituent's constant throughout the studies. (Arya, 1985)

Though modified Asthana & Hawker's 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 Hawker's 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 wt.(g)*	Final pH*	Dry wt.(g)*	Final pH*	Dry wt.*	Final pH*	Dry wt.(g)*	Final 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

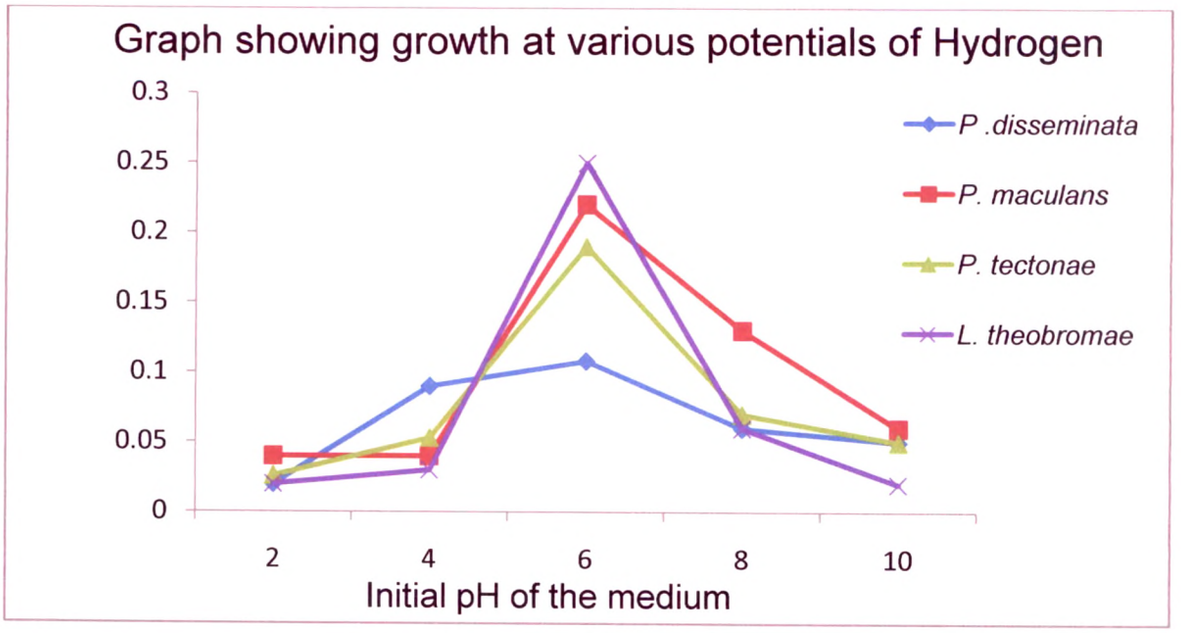
* indicates each compound values are based on three replicates
Results were significant at $P \leq 0.05$ level by one way ANOVA

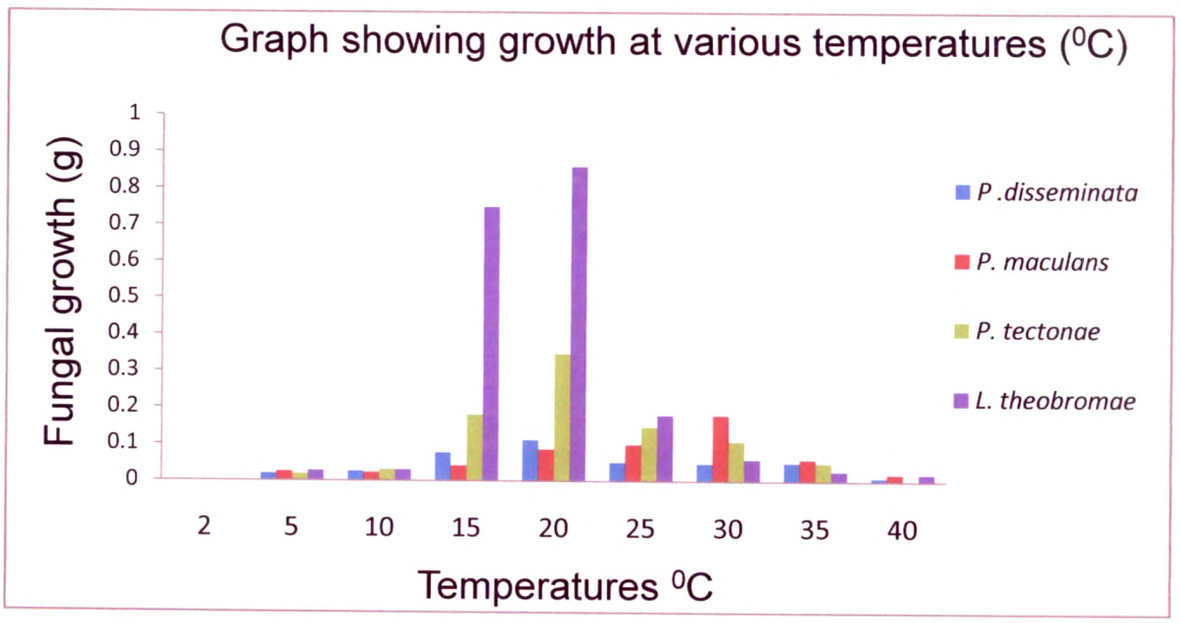
Table 3.7 Mycelial dry wt. and final pH of four different foliicolous fungi at different temperatures

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 $P \leq 0.05$ 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 theobromae* 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 – 36 ^{0}C. Best growth was observed


Graph showing growth at various potentials of Hydrogen
0.3
0.25
0.2
0.15
0.1
0.05
0
2
4
6
8
10
Initial pH of the medium
P .disseminata
P. maculans
P. tectonae
L. theobromae


Graph showing growth at various temperatures (0C)
Fungal growth (g)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
5
10
15
20
25
30
35
40
Temperatures 0C
P .disseminata
P. maculans
P. tectonae
L. theobromae

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 – 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 – wall, like cellulose, chitin, and pectin substances contain carbon in varying form and concentration, and thus provide the structural frame – work of the fungal cell. In their functional role, carbon compounds are still more significant, because fungi, being chemotrophs, obtain all their energy – 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 – 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

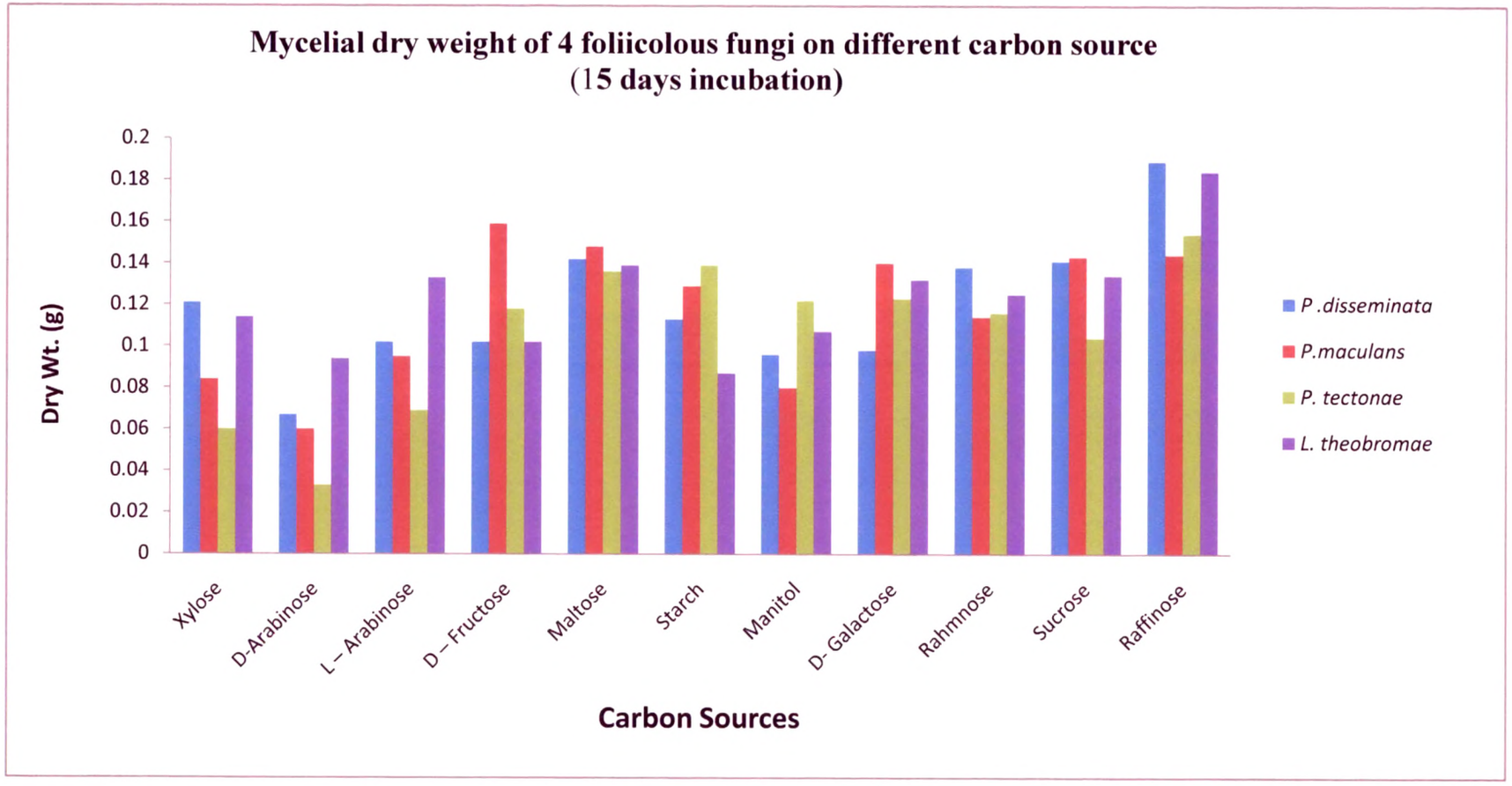
set up. The mere fact that there is a proof of their efficiency to derive food from different natural substrates.

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 – 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 – 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 $P \leq 0.05$ 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


Mycelial dry weight of 4 foliicolous fungi on different carbon source
(15 days incubation)
Dry Wt. (g)
0.2
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
Rahmnose
Sucrose
Raffinose
Carbon Sources
P .disseminata
P.maculans
P. tectonae
L. theobromae

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 – 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 *Lasiodiplodia 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 – 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 – 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 – 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 α – 1 – 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.* α – β – 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: α – D – Glucopyranosyl – 1 – 2 – β – D – 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 Benedict's solution.

Oligosaccharides

It consists of 2 – 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 – 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 – 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

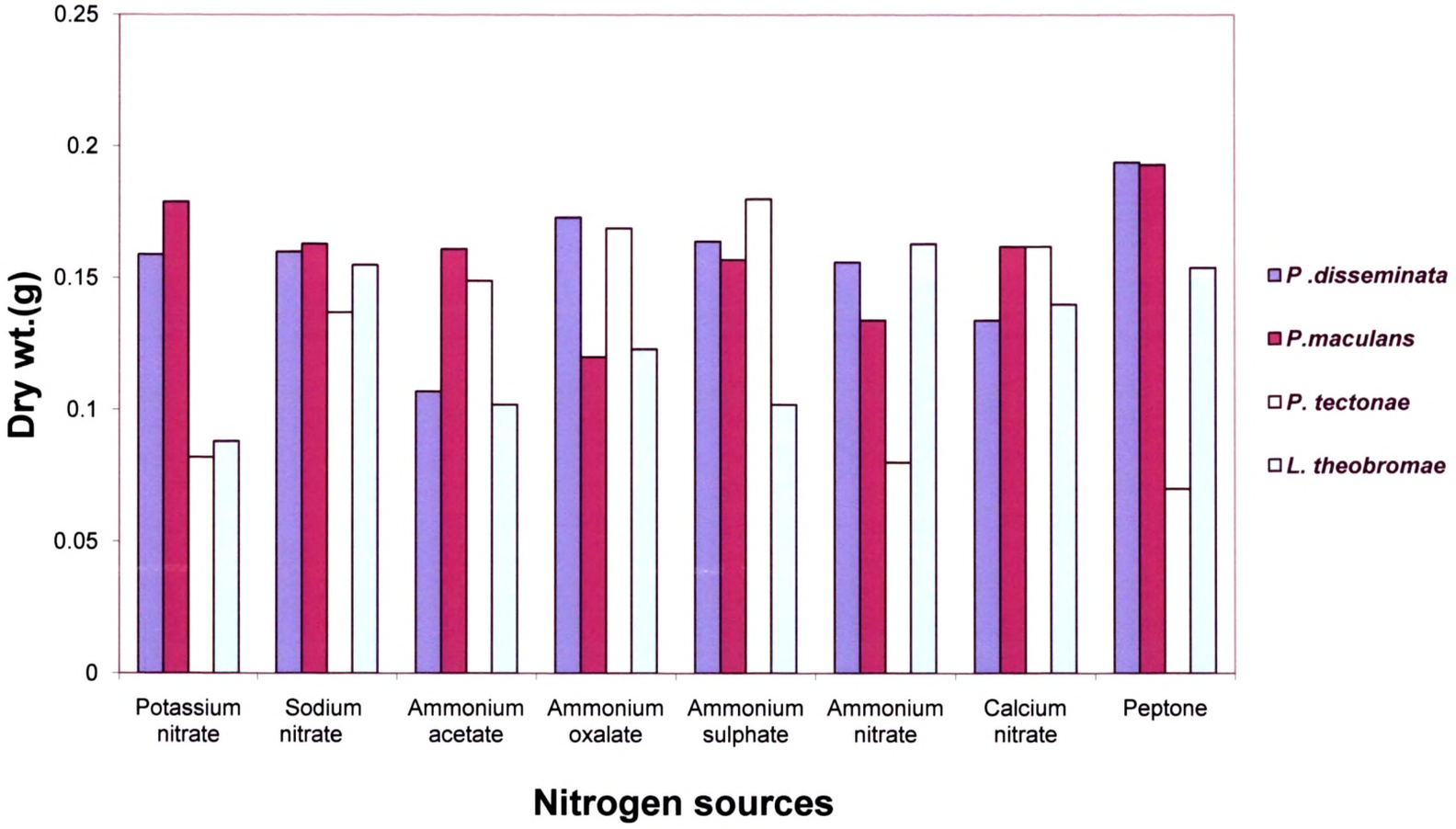
Sr. No.	Nitrogen Source	*Pestalotiopsis disseminata*		*Pestalotiopsis maculans*		*Phomopsis tectonae*		*Lasiodiplodia theobromae*	
		Dry wt.(g)*	Final pH*	Dry wt.(g)*	Final pH*	Dry Wt.(g)*	Final pH*	Dry Wt. (g)*	Final pH*
1.	Potassium nitrate	0.159 ± 0.09	6.36	0.179 ± 0.27	6.00	0.082 ± 0.10	5.03	0.088 ± 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 $P \leq 0.05$ 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.*

Mycelial dry weight of 4 foliicolous fungi ofn diffrent Nitogen source (15 days incubation)



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 – 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 α-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 5'-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 –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 – 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 – 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 (Є-N-Biotnyl – 1 – 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 "Folic acid" (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 – 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 – carbon fragment which constituents a pool of reactive C -1 intermediate. This 1 – carbon units are found in the form of formyl derivates of folic acid which acts as co –enzymes in reactions involving transfer of 1 carbon compound during the synthesis of various cell constituents. For example N^5, N^{10} – methelene tetra hydo folic acid is known to act as the co – enzyme in glycin and serine inter conversions. Under the catalytic influence of various derivatives of tetra hydro folic acid. 1 – 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 – 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 – 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 $P \leq 0.05$ level by one way ANOVA

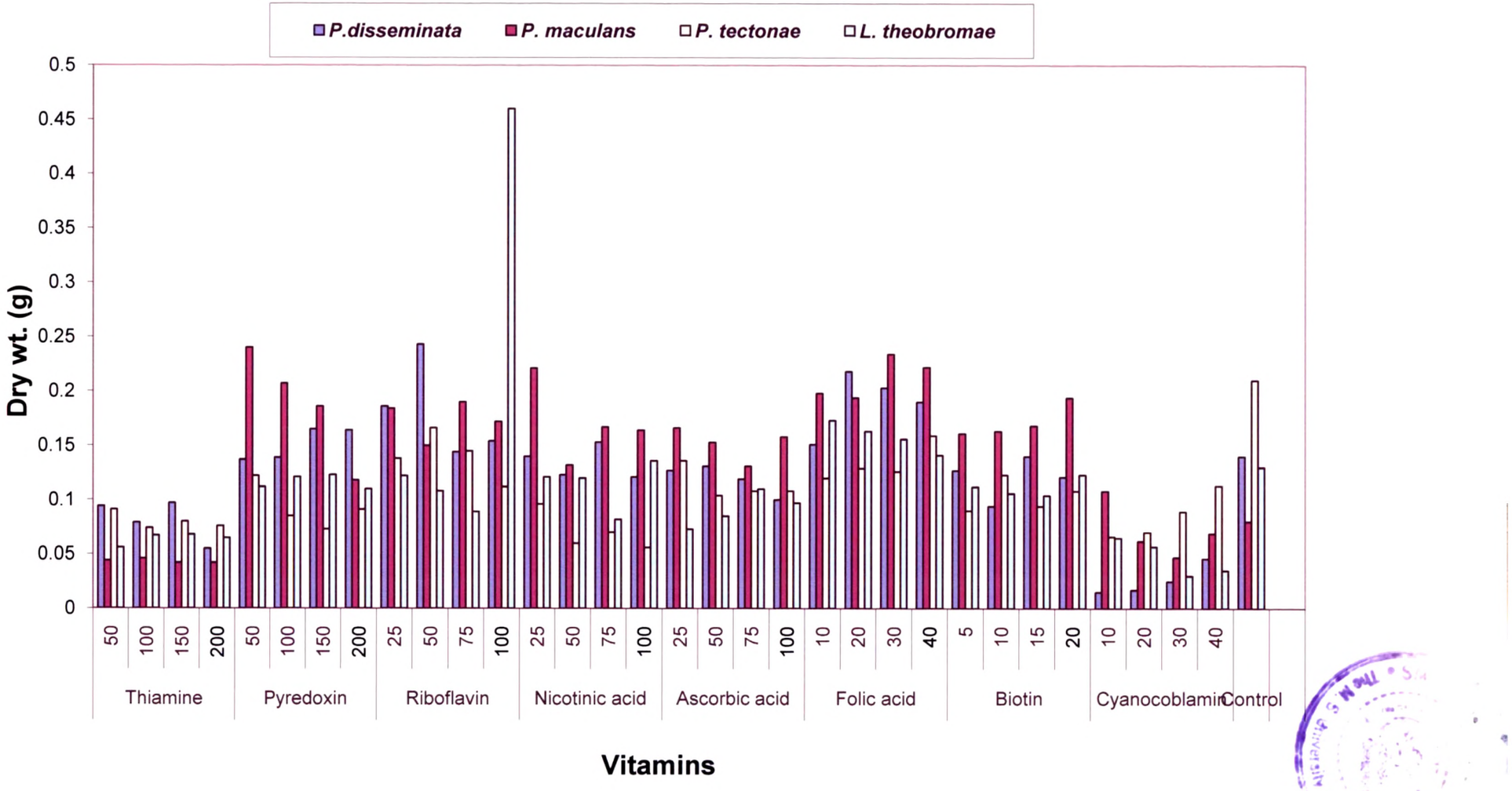
Table: 3.10 (b) Effect of different vitamins on growth of 4 foliicolous fungi after 15 days incubation

	* 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 $P \leq 0.05$ level by one way ANOVA

Effect of different vitamins on growth of 4 foliicolous fungi (15 days incubation)



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 $P \leq 0.05$ 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 'A' 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.*

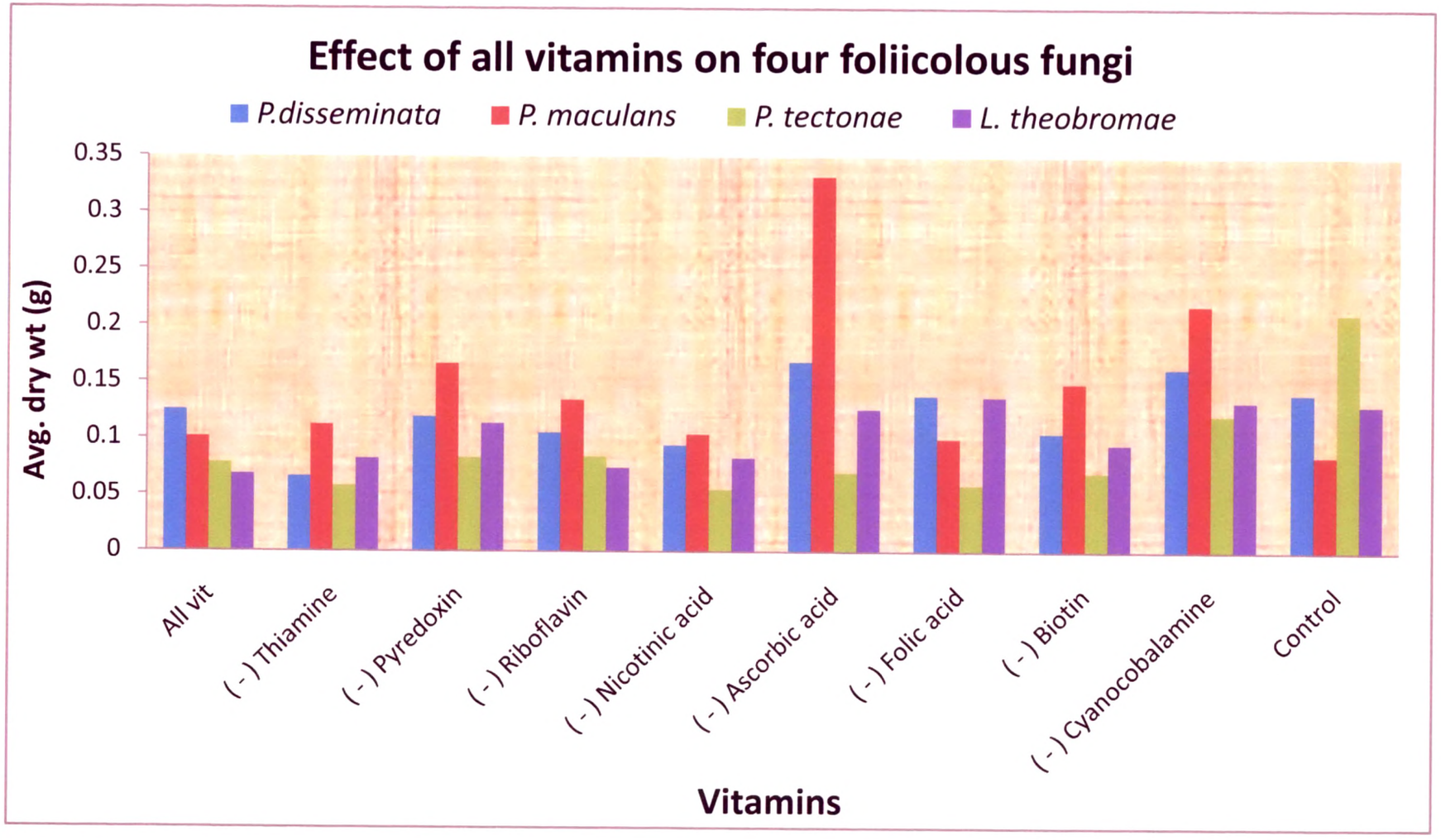
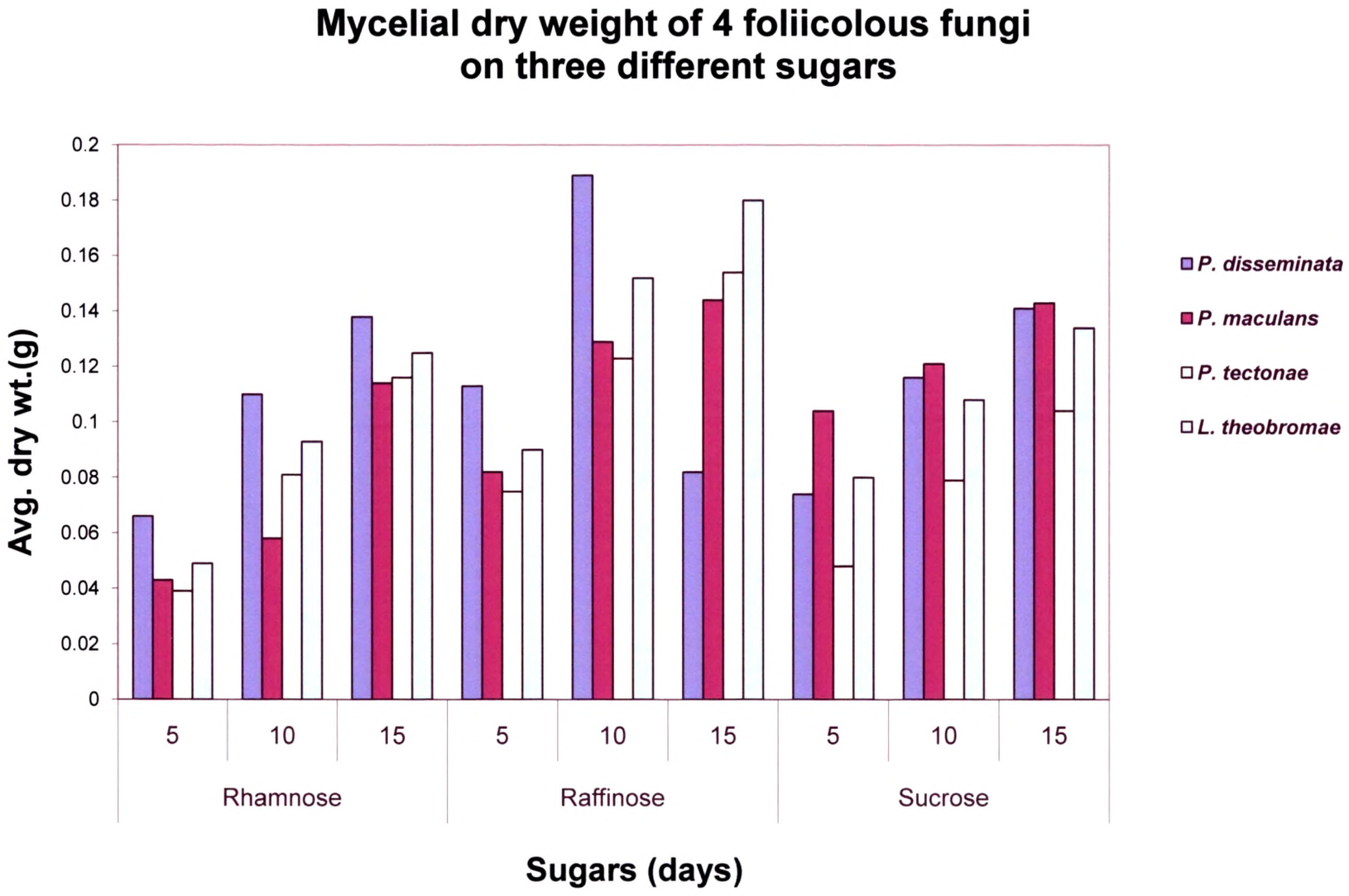

Effect of all vitamins on four foliicolous fungi
P.disseminata
P. maculans
P. tectonae
L. theobromae
Avg. dry wt (g)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
All vit
(-) Thiamine
(-) Pyredoxin
(-) Riboflavin
(-) Nicotinic acid
(-) Ascorbic acid
(-) Folic acid
(-) Biotin
(-) Cyanocobalamine
Control
Vitamins

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		-	2	12
	Glucose		2	4	-
	Fructose		4	8	-
	Galactose		-	8	
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			12	
	Glucose		6	4	
	Fructose		8	4	
	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		-	12	
	Glucose		8	8	
	Fructose		8	10	
	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		-	4	-
	Fructose		-	8	
	Galactose		-	8	

* indicates each compound values are based on three replicates

Results were significant at $P \leq 0.05$ level by one way ANOVA


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

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 – 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* (Mendel's, 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.

Survey was conducted in Ratan Mahal WLS, Jambughoda WLS, Baria Division, Shoolpaneshwar WLS, Pavagadh forest area, for collection of diseases leaves of certain forest tree species. Isolation was done to find out associated leaf infecting fungi. Sixteen different types of leaf spot fungi were isolated from certain tree species.

From the leaves of *Tectona grandis* fungi like *Alternaria alternate, Fusarium pallidoroseum, Lasiodiplodia* theobromae, *Phomopsis tectonae, Thielavia subthermophila,* were isolated. From the leaves of *Terminalia arjuna Gloeosporium gloeosporoides, Pestalotiopsis disseminata* were recovered. From *Bambusa arundinaceae* leaves *Colletotrichium capsici, Curvularia prasadi, Drechslera rostrata, Melanconiopsis microspora, Pestalotiopsis maculans,* were isolated. *Fusarium roseum* was isolated from leaves of *Madhuca indica.*

Isolated different foliicolous fungi were maintained on PDA slants and identification was done on the basis of their morphological and cultural characteristics. Their pathogenicity trials of isolated fungi were performed to confirm the Koch's postulates

Physiological studies including effect of different culture media, pH, Temperature and utilization of sugars by certain foliicolous fungi were done.

Selection of Suitable Media

To record the variations in cultural characters (growth and sporulation), four selected fungi *i.e. L. theobromae Phomopsis tectonae, P. disseminata and P. maculans* were grown in 8 different culture media. All the four fungi showed maximum mycelial dry weight in Richard's medium followed by Czapek's media but sporulation was absent in two cases.

Sporulation of 4 fungi was better on Modified Asthana & Hawker's media 'A'. The growth was poor on host decoction and Coon's medium.

Effect of Temperature

Effect of different temperatures on growth and sporulation of 4 foliicolous fungi was observed. The fungi were grown at 9 different temperatures between 2°C – 40°C. Best growth and sporulation of *P. disseminata, L. theobromae* and *P. tectonae* were obtained at 20^0C and of *and P.* maculans at 30°C.

Effect of pH

Effect of different pH was observed on growth and sporulation of selected four fungi. Five different initial hydrogen ion concentrations were maintained. Modified Asthana and Hawker's medium 'A' was used as a basal medium. *P. tectonae, L. theobromae, P. disseminata* and *P. maculans.* showed maximum mycelial growth and very good sporulation at pH 6.

Utilization of Sugars

Chromatographic studies were undertaken to detect the utilization of mono, di and poly saccharides.

In *L. theobromae* sucrose, D-glucose, fructose was present upto 4 days and 10^{th} day Glucose & fructose was present in *Phomopsis tectonae* and in *P. disseminata.* On 14^{th} day sucrose was utilized completely in all the four fungi.

Rhamnose was utilized in 14 days by all four fungi but the spots became lighter in 12^{th} and 14^{th} day showing traces of sugars. In Raffinose the break down product was present upto 8 days. After 8 days only one sugar was prominent upto 15 days but in less concentration. In *P. tectonae* breakdown of sugar was at 8 days. After 14 days only one sugar was present while no growth was found in *P. maculans*

Effect of different Vitamins

Utilization of 8 different vitamins *i.e.* Thiamine, Pyridoxine, Riboflavin, Nicotinic acid, Folic acid, Ascorbic acid, Biotin, Cyanocobalamine was observed on 4 selected fungi.

The growth of *P. disseminata* and *P maculans*: was better in Folic acid, followed by Riboflavin and Nicotonic acid at all concentration.

In *P.* tectonae and *L. theobromae* growth was better in Biotin and Folic acid at all concentration followed by ascorbic acid and riboflavin. The inhibitory effect of Cyanocobalamine was observed on all four fungi at different concentrations. In vitamin thiamine there was decrease in dry weight of all four organisms at different concentration in compare to control sets.

Effect of different Nitrogen Sources

Utilization of Nitrogen compounds on all four fungus was observed. Peptone was a better source of nutrition for *P. disseminata* and *P. maculans*

Better growth of *P. tectonae* was recorded on Ammonium sulphate source. In *L. theobromae* Ammonium nitrate was a better source.

Phylloplane study of *Disopsyros melanoxylon* and *Madhuca indica*

Phylloplane studies were performed by three methods: direct observations, leaf washing method, cellotape method. Results indicated more number of viable fungi by serial dilution method. Fungi reported from *D. melanoxylon* and *M. indica* were *Aspergillus awamori, A. fumigatus A. niger, Cladosporium cladosporoides, Colletotrichum capsici, Curvularia lunata, Pestalotiopsis versicolor; Phyllactinia* sp., *Fusarium oxysporum, Rhizopus stolonifer.*

Biocontrol of foliicolous fungi by Botanical pesticides

Botanical pesticides were prepared by Soxhlet extraction method and tested by Poisoned Food technique. Best results were obtained in methanolic extract of *Cymbopogon martini* followed by *Withania somnifera* and *Alangium salvifolium, while Butea monsperma* and *Dalbergia sisso* leaf extract inhibited growth of fungi at higher concentration.

Percentage frequency occurrence showed *A. niger, P. citrinum , T. viride, Mucor hemalis, Rhizopus stolonifer* by dilution method in *M. indica*, while in *D. melanoxylon T. viride, Curvularia lunata, A. alternata, Pestalotiopsis* occurrence was more on phylloplane.