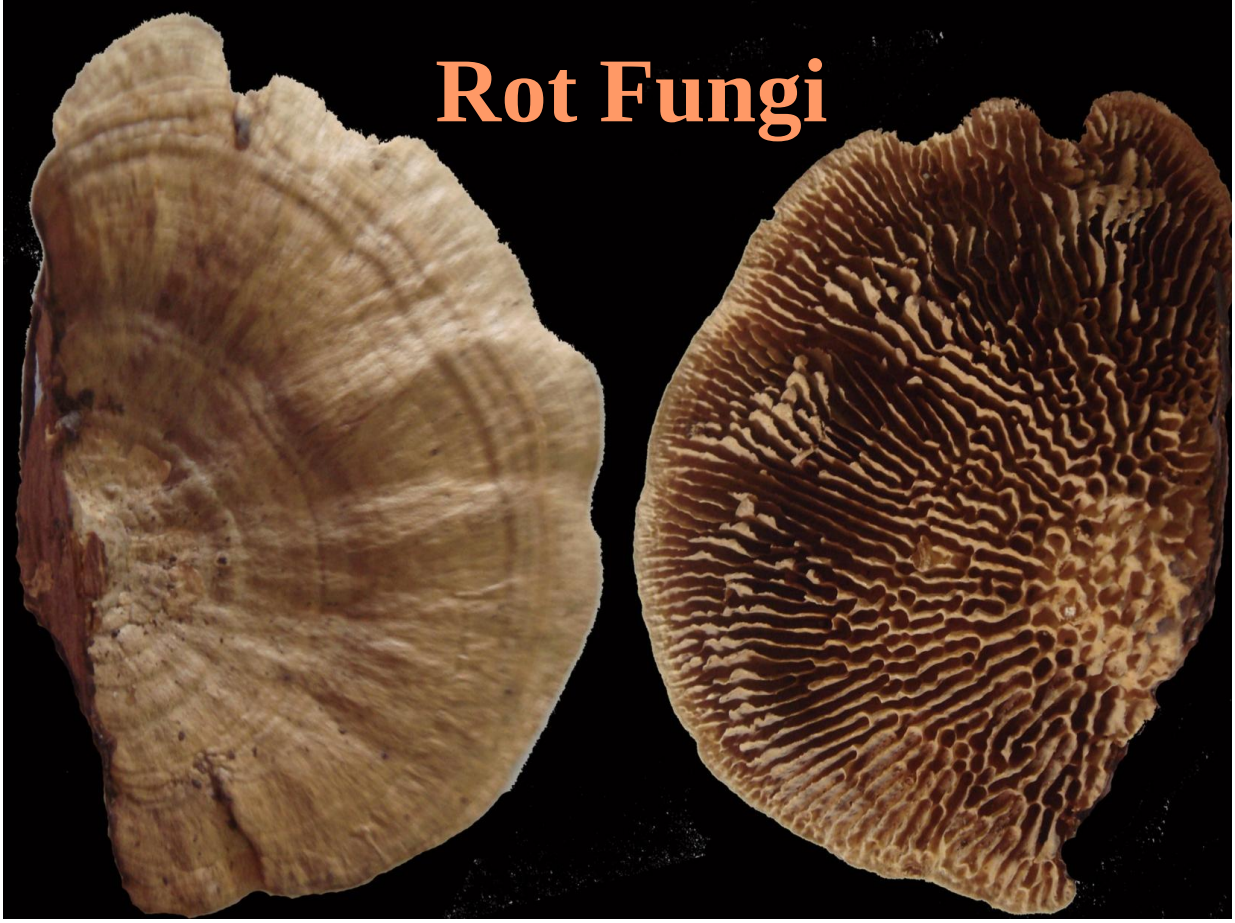
Study of the Decay Pattern & Enhancement of Enzyme Production For Better Biopulping By Certain White Rot Fungi



Study of the Decay Pattern & Enhancement of Enzyme Production For Better Biopulping By Certain White Rot Fungi

Thesis Submitted to
The Maharaja Sayajirao University of Baroda Vadodara

For the degree of
Doctor of Philosophy in Botany

By
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Under the guidance of
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Date: 20-12-2013

CERTIFICATE

The thesis entitled "Study of the Decay Pattern & Enhancement of Enzyme Production For Better Biopulping By Certain White Rot Fungi" submitted by Ms. Amee N. Padhiar contains the original research work carried out by her in the Seed Anatomy Laboratory of the Department of Botany, The Maharaja Sayajirao University of Baroda. It has been prepared in accordance with the University norms under my direct supervisions. It is further certified that this work has not been submitted earlier to any other University/ Institute for any degree.

Dr. (Mrs.) Susy Albert
Associate Professor,
Guide
Department of Botany,
Faculty of Science
The M. S. University of Baroda

Dedicated to God

&

My Parents

ACKNOLEDGEMENT

Looking back, I am surprised and at the same time very grateful for all I have received through these years. It has certainly shaped me as a person and has led me where I am now. My Ph. D. would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of the study.

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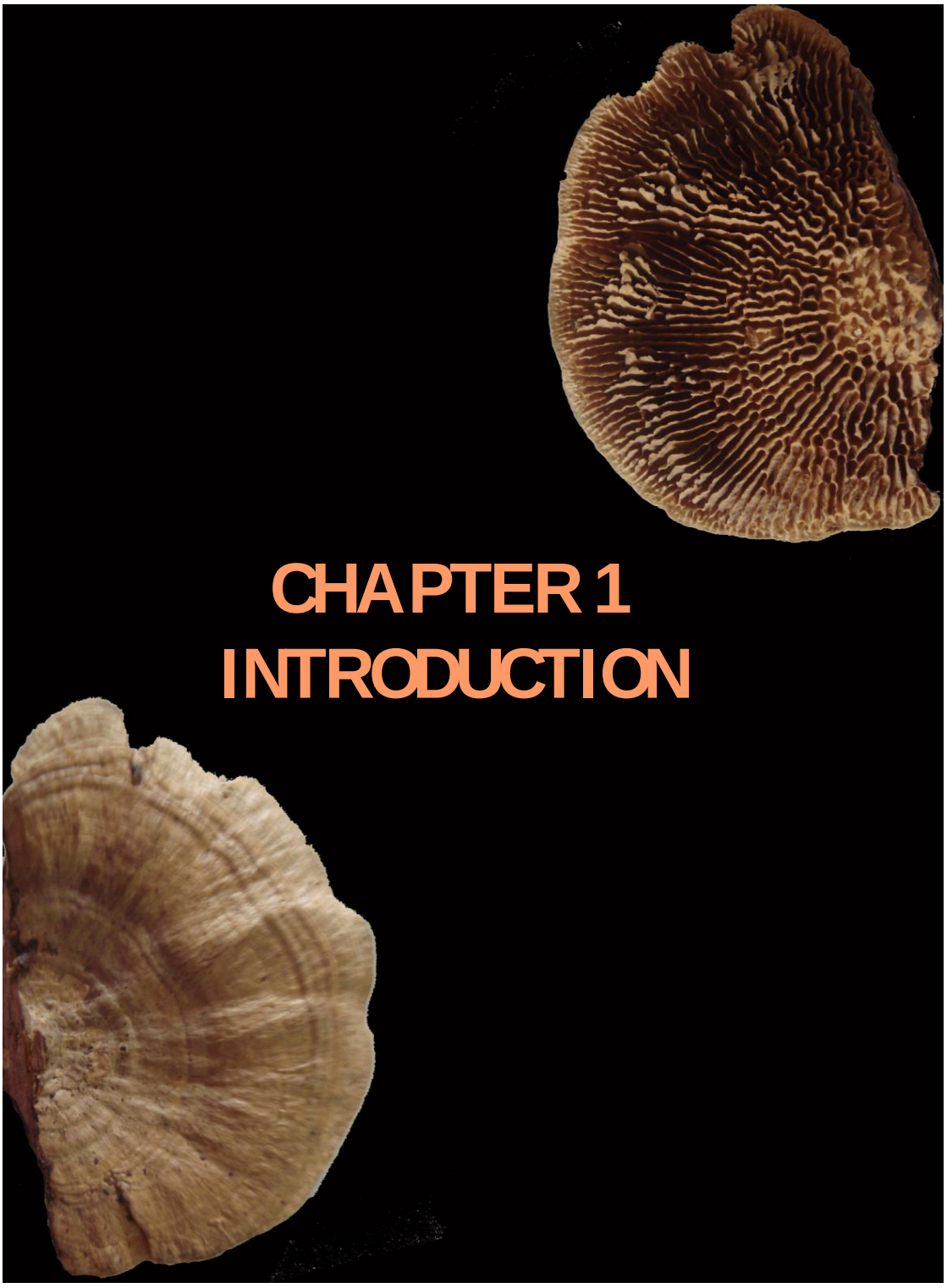
Amee Padhiar

INDEX



CHAPTER 1
INTRODUCTION

Lignocellulose:

Lignocellulosic materials are most abundant and renewable sources of carbon on the earth. Lignocellulose is the major component of woody plants and non woody plants such as grasses and it is a major source of renewable organic matter. It has 3 components lignin, hemicellulose and cellulose (Higuchi 1997). Lignocellulosic biomass includes materials such as agricultural residues (Corn straw and Wheat straw), forestry residues, Saw dust, wood, portions of municipal solid waste (waste paper) and various industrial wastes. Traditionally lignocellulosic material has been used in paper making, biomass fuels, composting, animal feed etc. Large amount of lignocellulosic waste are generated through forestry and agricultural practices, and this waste is often disposed off by biomass burning. However the huge amounts of residual plant biomass considered as waste can be potentially be converted into various different value added products including bio-fuels, bio-fertilizers, chemicals, cheap energy sources for fermentation, improved animal feeds and human nutrients (Howard *et al*. 2003).

Lignocellulose composition of plants is influenced by genetic and environmental factors and differs considerably. Following table shows the chemical composition of various lignocellulosic materials (Betts *et al*. 1991).

Raw materials	**Lignin (%)**	**Cellulose (%)**	**Hemicellulose (%)**
Hard wood	18-25	40-55	24-40
Soft wood	25-35	45-50	25-35
Grasses	10-30	25-40	25-50

Lignin in the grasses as shown in above table is very less compared to hard and soft wood. So the grasses can be a suitable raw material for paper and pulp industry. In Gujarat there are about 300 different species of grasses. Out of this there are many grasses which are consumed by the cattles as they are palatable, but some are unpalatable which are not consumed by cattle. These are grasses like *Arundo donax*, *Imperata cylindrica* etc. which can be utilized for paper pulp industry. The grasses reported to yield excellent material for paper pulp are *Saccharum spontaneum, S. bengalense, Arundo donax, Phragmites karka, Themeda arundinacea and T. villosa.* All these grasses can be used in admixture with bamboo pulp (Bor 1960).

According to Malherbe and Cloete (2003) the chemical properties of the components of lignocellulose make them a substrate of enormous biotechnological value. Lignin is one of the complex natural polymer, of the wood - together with the cellulose and hemicelluloses. It is second to cellulose in abundance on earth.

It has been observed that in the paper industry bamboo and wood are used in the ratio of 70:30. Looking at the scarcity of grasses and wood in the forest an effort has been made here to use another unpalatable grasses for making paper.

Theoretically all plants can be used as a source of cellulosic fibers for paper industry (Rousu *et al.* 2002). However for a plant material to be considered suitable for production of cellulosic fibers it must accomplish several requirements of a raw material and technical requirements for processing into adaptable to practical agricultural methods and produce dry matter and good quality fiber yield at economically attractive level (Tofanica *et al.* 2011).

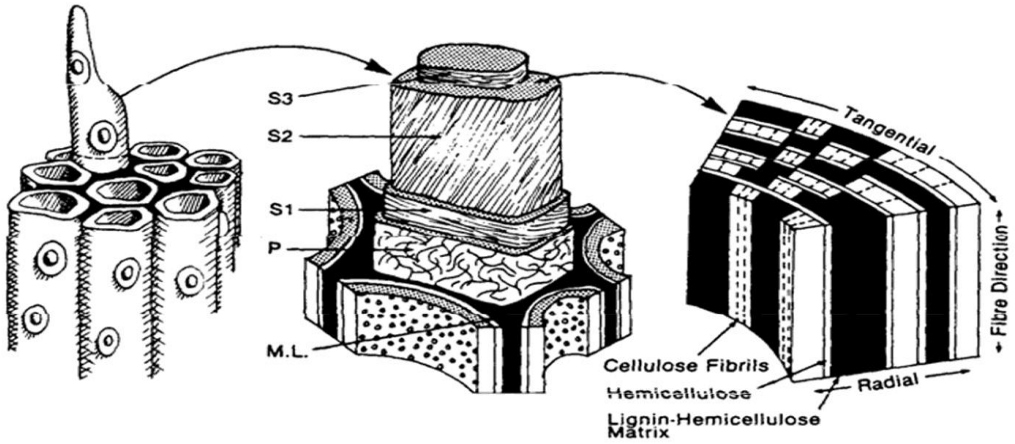
The importance of plant materials fiber dimensions and their derived values (slenderness ratio, flexibility coefficient and runkel ratio) on pulp and mechanical strength is well documented. In an extensive review of literature, Dinwoodie (1965) stressed the importance of the three derived values on pulp strength. Saika *et al.* (1997), Ogbonnaya *et al.* (1997) and Ververis et al. (2004) have successfully used those derived values to assess the suitability of various woody and non woody fiber raw materials for pulp and paper manufacture. In order to maximize the exploitation of non-woody plant fibers for paper pulp production, a more complete understanding of the chemical composition is also required, evaluation of which will help improving the industrial processes in which they are used as raw materials.

One of the objectives in the present study was to examine the fiber dimensions of some woody and non woody plants (grasses), to estimate their suitability for paper production. This was conducted by calculating various indices for paper production and comparing their examined properties with those of soft woods and hardwoods, which are taken as reference materials. These soft and hard woods are traditionally used for paper production. Also the chemical composition of the different non woody fibers have been evaluated.

Structure of wood:

Wood is a porous plant material consisting only xylem tissue. In hardwoods (angiosperm plants and deciduous trees) xylem cells consists of fibers, vessels, and ray parenchyma cells. These cells are responsible for support, nutrient storage, water transport and nutrient transport between plant roots and the leaves (Eriksson *et al.* 1990). Wood cell walls, tracheids and fibers, consist of several layers which differ in their

structure and chemical composition (Fig. 1). The major components of the wood cell walls are three biopolymers, cellulose, hemicellulose, and lignin (Harris and Stone 2008). Lignin is mainly present in the middle lamella region of wood, but most of the lignin is present within the secondary wall. Figure 1 shows that in the secondary wall lignin is mixed with and covalently bonded to the hemicelluloses and the cellulose fibrils are embedded in the lignin-hemicellulose matrix.



(a) (b) (C)

Figure1: Molecular architecture of woody tissues: (a) bundle of contiguous wood cells, (b) wall layers in cut-away view of single cell, and (c) section of secondary wall illustrating the relationship of hemicellulose and lignin to the cellulose fibrils. Cell wall layers are P (primary); and S_1, S_2 and S_3 (secondary). The middle lamella (M.L.) separates the cells. (Kirk and Cullen, 1998)

2-5% of the wood dry weight is made up of extractives (Sjöström and Westermark 1998), which are non-structural constituents of wood broadly divided into terpenes, resins, and phenols (Kuhad *et al.* 1997). These different organic compounds have different roles like, acting as a nutrition reserve for the living wood cells, and giving protection against microbial degradation (Sjöström 1993).

Wood components:

Cellulose:

Cellulose is the most abundant and significant polysaccharide on the earth. Wood species contain 40-45% (as dry weight) cellulose (Eriksson *et al.* 1990). Cellulose is linear polymer of glucose units, linked by β-1,4-glucosidic bonds (Fig. 2) and the degree of polymerization is up to about 15000 glucose units in one polymeric chain (Kuhad *et al.* 1997). In wood cell wall the long, cellulose chains are stabilized by hydrogen bonds to form microfibrils and further they form cellulose fibers. Cellulose is present in a major amount in thick S_2 layer of xylem element secondary wall (Fig. 1) where the fibrillous structure gives mechanical strength (Argyropoulos and Menachem 1997). The highly organized crystalline cellulose is not easy to degrade whereas amorphous non-organized cellulose is more susceptible to enzymatic degradation (Kuhad *et al.* 1997).

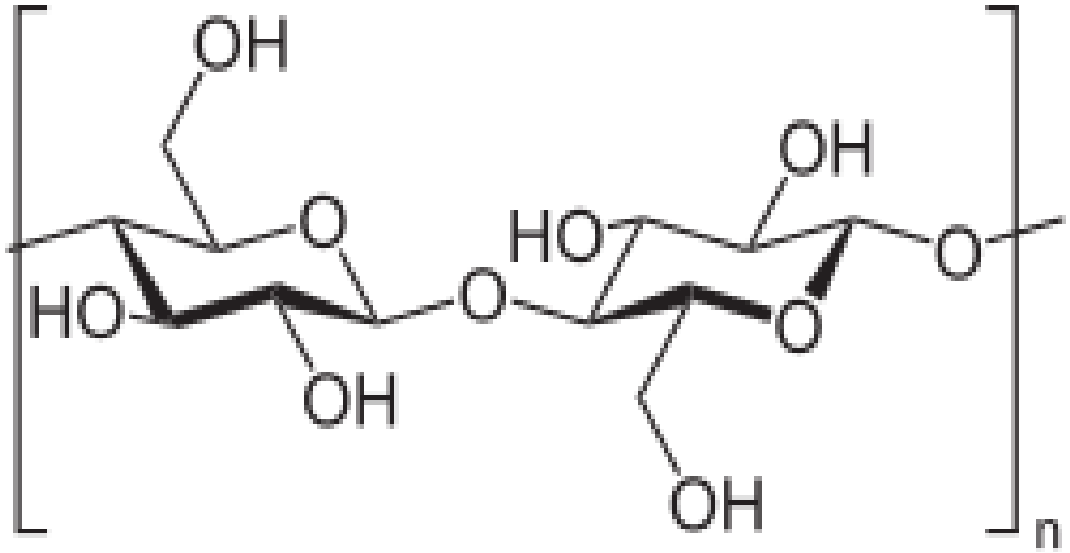


Figure 2: Structure of Cellulose

Lignin:

Lignin is a three dimensional, aromatic and amorphous polymer existing in the thick secondary wall layers embedded with cellulose microfibrils and hemicelluloses. Lignin is present in all layers of woody cell walls but in fibers and tracheids, the thin middle lamella has the highest lignin content (Eriksson *et al.* 1990). Lignin provides mechanical strength in vascular plants and protects the more easily degradable cellulose and hemicellulose polymers from microbial attack. The heterogenic lignin polymer is synthesized from phenylpropanoid precursors i.e. *p*-coumaryl, coniferyl, and sinapyl alcohol in the plant xylem cells. During lignin biosynthesis due to the action of laccases and peroxidases, these monolignols are polymerized to *p*-hydroxyphenyl (H-type), guaiacyl (G-type), and syringyl (S-type) type of lignin subunits respectively (Higuchi 2006). Lignin subunits are joined together by many different carbon-carbon and ether bonds of which the *β*-aryl-ether (*β*-O-4) bond is the most common (Sjöström 1993; Argyropoulos and Menachem, 1997). The composition and amount of lignin varies between softwood and hardwood, and also between plant species. In the softwoods lignin consists mainly of guaiacyl subunits while in hardwood lignin contains both guaiacyl and syringyl subunits (Adler 1977; Higuchi 2006). In grasses, lignin present in cell wall of the xylem element contains *p*-hydroxyphenyl subunits (Eriksson *et al.* 1990). Complete chemical structure of plant lignin is still unknown.

Hemicelluloses:

Hemicelluloses are a group of branched heteropolysaccharides consisting of different hexose sugars, pentose sugar, and sugar acid units. Hemicelluloses plays very important

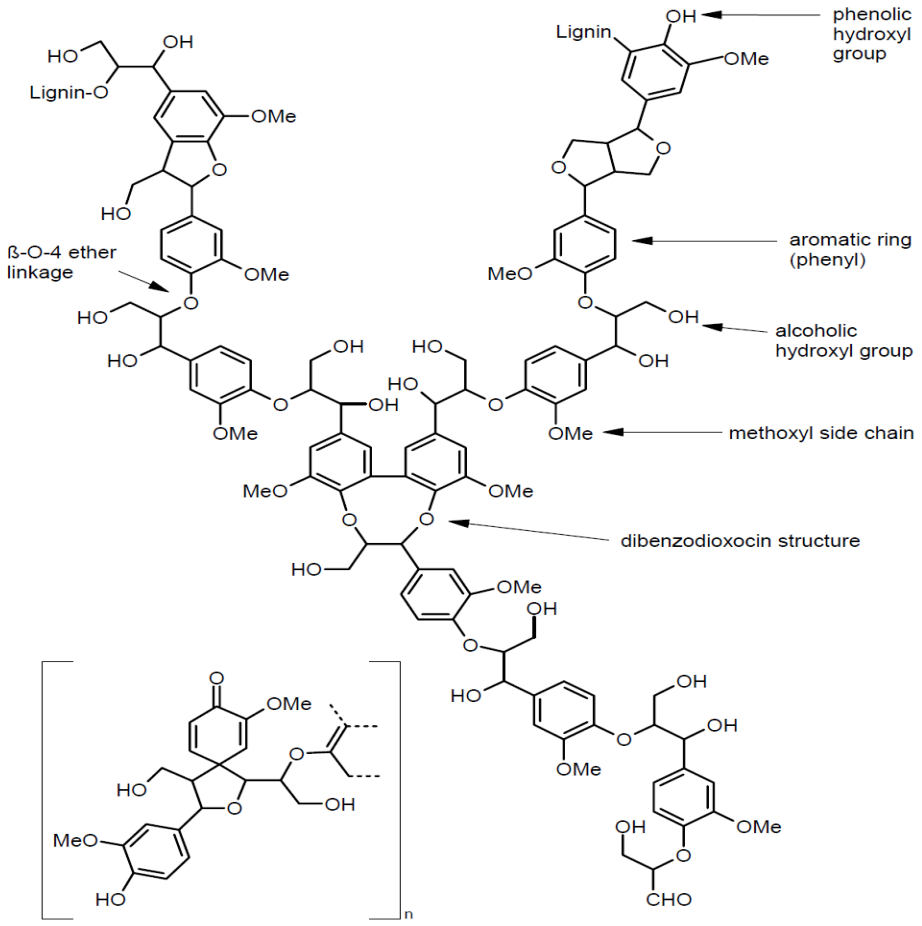


Figure 3: Structural model of lignin by Brunow (2001)

role of a supporting material and comprise 20-30% dry weight of wood (Sjöström 1993). Hemicelluloses are more easily biodegradable than cellulose because they are amorphous and have a moderate degree of polymerization (100-200 units). The composition and structure of hemicelluloses differ in softwood and hardwood: galactoglucomannans are the main hemicelluloses in softwood while glucuronoxylan dominates in hardwood (Eriksson *et al.* 1990). In the wood, hemicelluloses are bound to cellulose microfibrils with hydrogen bonds. Hemicelluloses and lignins are covalently linked to form the lignin-hemicellulose matrix (Fig. 1) (Kuhad *et al.* 1997; Harris and Stone 2008).

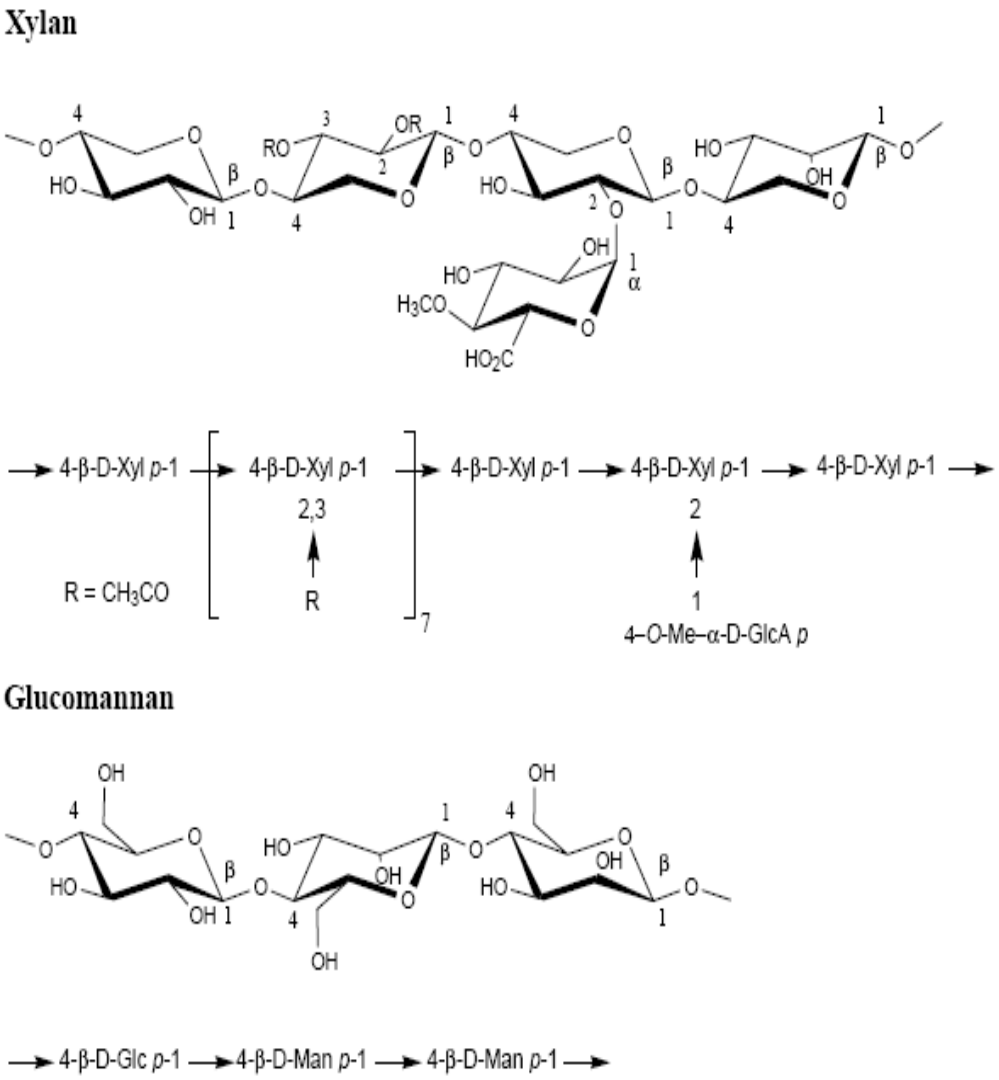


Figure 4: Structure of the major hardwood hemicelluloses

Importance of wood:

Wood is one of the important renewable resources available on the earth used in many ways. From the ancient time the wood is used for burning as fuel for cooking and light. Wood is widely used in construction of buildings, making furniture, window frames, agricultural implements, boxes and plywood.

Though wood is made up of highly integrated matrix of cellulose, hemicellulose, and lignin, which gives the wood strength and resistance to chemical and

microbial attack, many organisms and processes are capable of degrading wood. The wood degrading agents are both biotic and abiotic.

Abiotic degradation

Abiotic factors influencing wood degradation includes heat, strong acids or bases, organic chemicals, mechanical wear, and sunlight (UV degradation). **Heat** degrades both cellulose and hemicellulose, reducing strength and causing the wood to darken. **Strong acids** reduce wood strength by degrading the carbohydrate portion of wood. Wood infected by **strong bases** is bleached and white because the lignin is removed by strong bases. This effect is the base for chemical pulping (Kraft pulping). **Sunlight**, degradation through the ultra- violet light occurs only on few cell layers from the surface and wood color becomes grey to brown. Sunlight also degrades wood by producing free radicals which then degrade the wood polymers.

Biotic factors:

Biotic degradation can occur by many different agents, like insects, bacteria and fungi. Birds and animals can cause mechanical damage of wood in very less amount.

Insects: A number of insects have capacity to degrade wood, like termites (Isoptera), beetles (Coleoptera), and bees and ants (Hymenoptera).

Termites are the most hazardous wood-degrading insects, that causes severe economic losses. They digest everything including cellulose. Termites eat wood generally from the inside to outside, so damage wood is detected after severe damage (Jeffrey 2008).

Bacteria: Bacteria are primitive, single-celled organisms. They are minor degraders of wood. They increase permeability of wood by degrading the pit membranes.

On the basis of the degradation appearance within the various wood cell wall layers. Wood degrading bacteria are divided into three main groups: **erosion, tunneling and cavitation** bacteria (Jurgens *et al.* 2003).

There are also pit degrading bacteria which focuses their attack on bordered pits and scavenging bacteria which degrade remnants of degraded wood. But they are not much studied. (Burnes *et al.* 2000; Singh and Butcher 1991).

Erosion bacteria: Wood degradation caused by erosion bacteria is seen mostly in the marine environments (Singh and Kim 1997). In this type of cell wall degradation starts from cell lumen and moves towards the middle lamella. Bacteria avoids degrading lignin but it degrades cellulose and hemicelluloses (Fig. 5).

Tunneling bacteria: Tunneling bacteria are aggressive organism degrading the secondary wall and middle lamella region containing lignin. They start the decay from the lumen and enters in the secondary wall through bordered pits (Singh and Butcher 1991). Once the cell wall has been penetrated, they degrade all layers of the secondary wall. They form tunnels similar in size to the bacteria and with progress in decay they form concentric bands of residual materials in the cell wall (Fig. 5).

Cavitation bacteria: Cavitation bacteria enter into the cell wall by boring a small hole in the S3 layer from cell lumen just like erosion and tunneling bacteria. They concentrate on causing cavities in the S_2 layer by degrading the cellulose and hemicelluloses, but they do not degrade middle lamella. The cavities are generally angular and diamond shaped (Fig. 6).

Pit degrading bacteria: This type of bacteria focus their attack on bordered pits. They can selectively degrade only the nonlignified, pectin-rich region of pit membrane (Fig. 6) (Burnes *et al.* 2000; Singh 1997).

Scavenging bacteria: Scavenging bacteria are considered as secondary bacteria because they degrade the residual materials left after degradation by tunneling and erosion bacteria (Fig. 5). They are only dependent on degraded cell wall components (Singh and Butcher 1991).

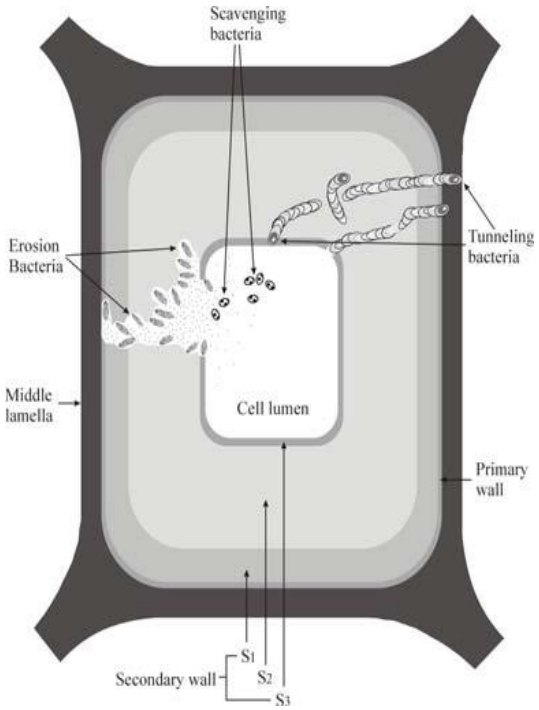


Figure 5: Transverse view of a wood cell and cell wall layers with several types of degradation caused by wood degrading bacteria

(Jurgens *et al.* 2003)

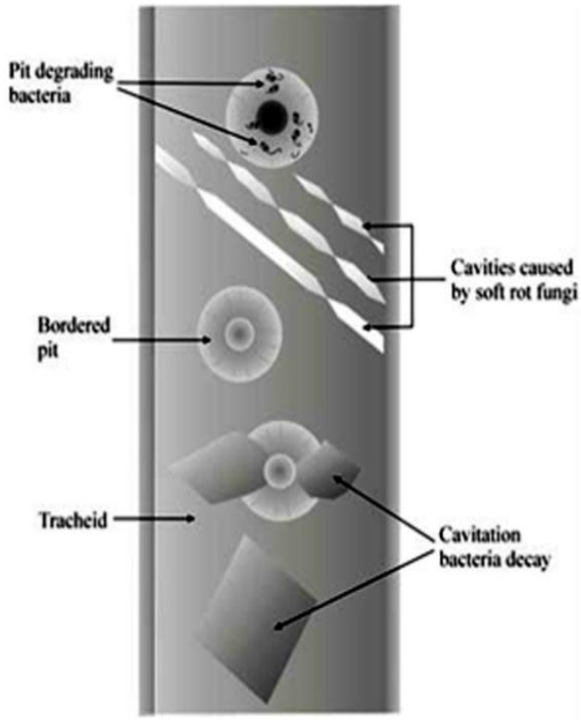


Figure 6: Longitudinal view of a wood cell, with degradation caused by cavitation and pit degrading bacteria and soft rot fungi

(Jurgens *et al.* 2003)

Fungi: Fungal decay is very effectively noticed in the stored and also pretreated wood (Xia *et.al.* 1997). Decay occurs generally in untreated wood which is in direct contact with ground, cement or concrete or exposed to a source of moisture such as rain, seepage, plumbing leaks or condensations. All wood degrading microorganisms have general requirements for survival that include moisture, oxygen, nutrients, favorable temperature, suitable pH and a nontoxic substrate (Blanchette and Hoffman 1994; Eaton and Hale 1993).

Fungi are microorganisms which live on and within wood and slowly digest the cell wall materials leading to softening and decay. Wood decay fungi obtain nourishment by digesting cell walls, thus causing deterioration of wood. The fungus in order to break down the chemical components of the cell wall in the wood produce enzymes capable of attacking these components (Vmezurike 1968). Enzymes are secreted at the tips of hyphae which while its penetration through the wall attack the cell wall components like lignin, cellulose and hemicelluloses and break them into simple sugars making it available for its nutrition.

Members belonging to Basidiomycetes and Ascomycetes are involved in such decay. Basidiomycetes are very important in recycling the carbon stored in wood and they are important in forest ecosystem (Otjen and Blanchette 1985). A lot of changes take place in the wood microstructure during progressive stages of decay. (Blanchette 1984; Luna *et al.* 2004; Lewes and Blanchette 1986).

Usually three groups of wood decay are distinguished. They are a) brown rot b) white rot and c) soft rot. White rot decay is further distinguished into simultaneous rot and selective white rot (Martinez *et al.* 2005).

Brown rot: Wood that has been decayed by a brown rot fungus will have a brown colored appearance that is because of degradation of the cellulose and hemicelluloses. In this type of decay, wood exhibits considerable loss of strength. With the further decay the lignin rich framework of wood cells become wavy in appearance (Fig. 7). In further stages of decay, the wood cells become brittle and fracture into fragments.

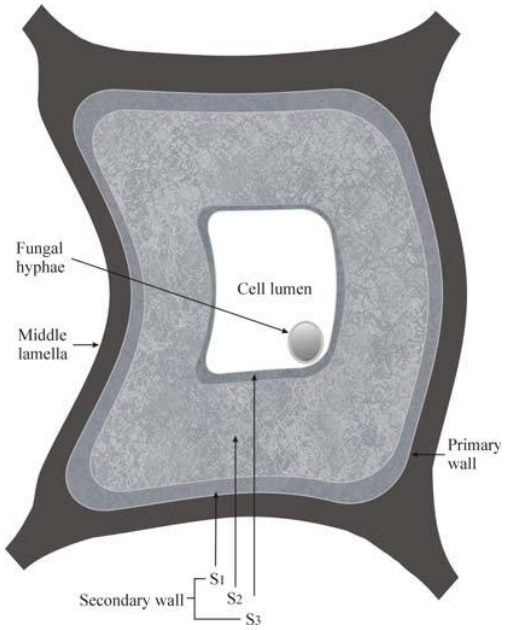


Figure 7: Wood cell wall decayed by a brown rot fungus causing a depolymerization of the cellulose and hemicellulose, leaving a lignin rich, weakened substrate (drawing by J.A. Jurgens, In: Blanchette *et al.* 2010)

White rot: Most of the white rot decay fungi are included in the group Basidiomycetes. They are capable of producing ligninolytic enzymes that initially degrades the darkly colored lignin, leaving the hemicellulose and cellulose, which give the remaining wood a white appearance. The enzymes that cause degradation of the lignin are lignin peroxidase, manganese peroxidase, and laccase, however, several other enzymes also play a role (Cullen and Kersten 2004; Kirk and Farrell 1987). On the basis

of the chemical and morphological characteristics of degraded wood, white rot decay is divided in to 2 forms. There is also a difference in the amount, type and combination of enzymes produced by fungi in these two forms (Blanchette 1991). One type is a simultaneous white rot. In this type of decay all major components within the cell wall are degraded. It causes an erosion and thinning of the wood cell wall (Fig. 8).

The second type is a selective white rot, in which the fungus degrades the lignin components, leaving cellulose and hemicellulose. This type of decay separates the adjacent cells from one another, because of the lignin rich middle lamella which gets degraded (Fig. 9) (Eriksson *et al.* 1990).

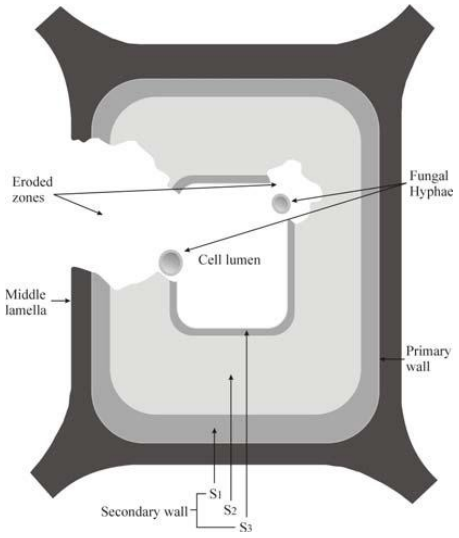


Figure 8: White rot fungal decay of wood cell causing a simultaneous attack of all cell wall layers. (drawing by J.A. Jurgens, In: Blanchette *et al.* 2010)

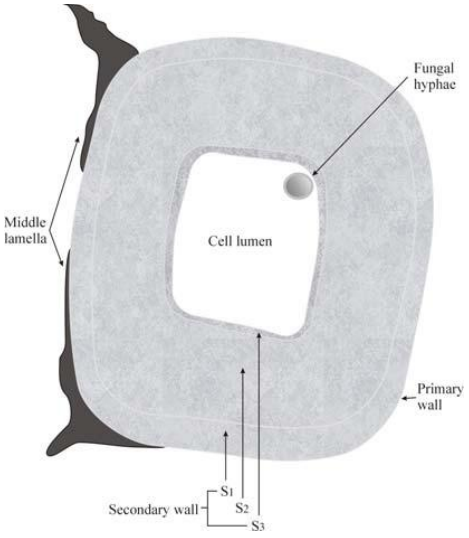


Figure 9: A selective attack of the lignin rich middle lamella, leaving the remaining cellulose and hemicellulose in a wood cell wall is characteristic of one type of white rot decay by fungi (drawing by J.A. Jurgens, In: Blanchette *et al.* 2010)

Soft rot: Fungi that cause soft rot decay in wood belongs to Ascomycota and Deuteromycota. They are divided into two categories type 1 or cavity formation and type 2 or erosion attack, both of which can be observed in hardwoods and softwoods. In type 1 decay a series of angular or diamond shaped cavities are formed in the S_2 layer of the wood cell wall which follows the cellulose microfibril orientation (Fig. 6). In a transverse view, these chains of cavities are seen as round holes of different sizes (Fig. 10). In type 1 decay primarily S_2 and S1 layers are degraded where as S3 layer and middle lamella are not damaged (Eaton and Hale 1993; Khalili *et al.* 2001).

In type 2 form a complete degradation of the secondary wall and slight modification of the middle lamella is observed (Daniel and Nilsson 1997). Cellulose degradation is the primary focus of soft rot decay fungi. In advanced stages of type 2 decay, only a framework of lignin rich middle lamellae will remain.

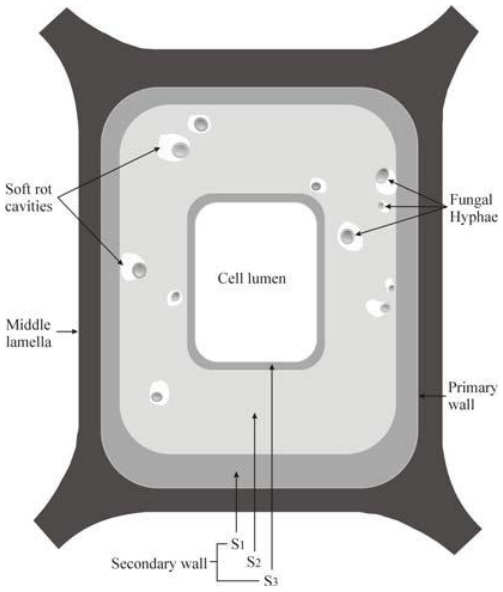


Figure 10: Soft rot decay causing a selective attack of cellulose in the S_2 layer of a wood cell wall (drawing by J.A. Jurgens, In: Blanchette *et al.* 2010)

In the selective delignification, at early stages of decay, lignin is broken down more than cellulose and hemicelluloses. Different anatomical pattern of the selective white rot can be observed under the light microscope. Blanchette (1984) has observed that the middle lamella degrades first with the high degradation of the lignin in the secondary wall and during the later stages of decay all the cells becomes separated from the matrix. Peek (1972) has observed lamellar collapse and an extensive delignification occurs in S_2 layer, which leads to accentuation of radial structures (Schwarze and Engels 1998).

Microbial attacks by some of these organisms are highly beneficial. This is found to be favorable for the pulping process as it removes the lignin preferentially over the cellulose which reduces the energy and chemical requirement during the lignin removal of pulping.

Enzymes responsible for degrading lignin

Wood cell wall is made up of very complex polymers lignin and cellulose, both most abundant polysaccharides on the earth. For degradation of wood the fungus has to penetrate in the wood for which it secretes the enzymes from the tip of the hyphae (Fig. 11). This enzymes attacks the cell wall components and breaks down them into simpler forms which is utilized by the fungus. Although plants and animals are producers of ligninolytic enzymes microorganisms remains an important source of ligninolytic enzymes especially fungi which are the most potent source of lignin degrading enzymes. The saprophytic fungi secrete these enzymes to degrade the lignin polymer. Among fungi, the white rot fungi are the best known producer of these enzymes, followed by the brown rots and the soft rots (Niladevi 2009).

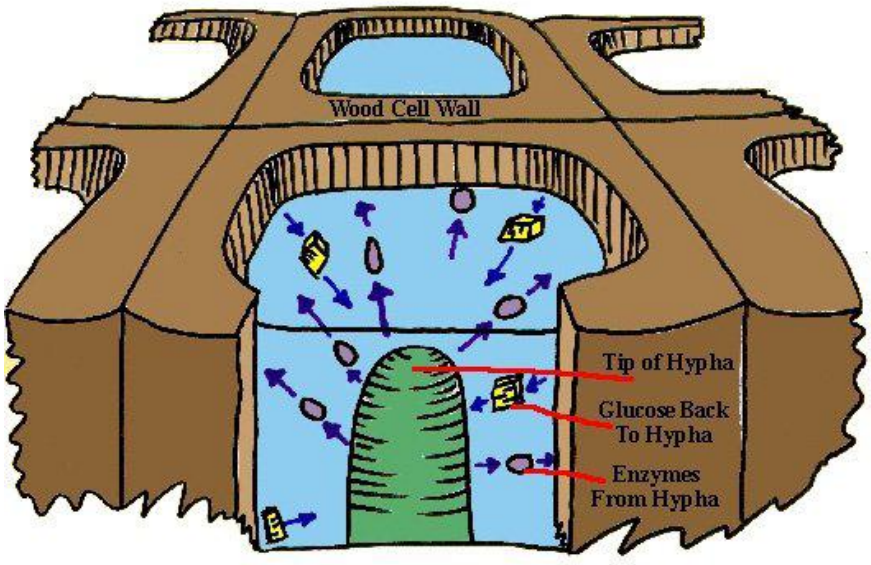


Figure 11: Fungal hyphae degrading the wood (Mohebby 2003)

Lignin is the second major reservoir of the fixed carbon sources in nature, comprising 15% of earth's biomass (Hammel 1992). The structural complexity of lignin requires different oxidative enzymes to be involve in its complete decay. Lignin peroxidase, Manganese peroxidase and Laccase are the major lignin degrading enzymes. (Niladevi 2009). Other than this many other enzymes play role in the lignin degradation such as veratryl alcohol oxidase (Bourbonnais and Paice 1988), Aryl alcohol dehydrogenase, Glyoxal oxidase (Kersten 1990), Quinone oxidoreductase, aromatic acid reductase, vanillate hydroxylase, dioxygenase, catalase (Leisola and Fietcher 1985; Buswell and Eriksson 1988) and aromatic aldehyde oxidase (Deobald and Crawford 1989).

Different ligninolytic enzymes have different mechanisms to degrade lignin. Action of ligninolytic enzymes is dependent on the generation of lignin free radicals which, because of their chemical instability undergoes many different cleavage

reactions (Hammel 1997). Following table shows the reaction mechanism of enzyme, along with their cofactor and mediator (adapted from Hatakka 2001).

Enzyme and Abbreviation	**Cofactor**	**Substrate, mediator**	**Reaction**
Lignin peroxidase, **LiP**	H_2O_2	Veratryl alcohol	Aromatic ring oxidized to cation radical
Manganese peroxidase, **MnP**	H_2O_2	Mn, organic acids as chelators, thiols, unsaturated fatty acids	Mn(II) oxidized to Mn(III); chelated Mn(III) oxidizes phenolic compounds to phenoxyl radicals; other reactions in the presence of additional compounds
Laccase	O_2	Phenols, mediators, e.g., hydroxybenzotriazole or ABTS	Phenols are oxidized to phenoxyl radicals; other reactions in the presence of mediators
Aryl alcohol oxidase, **AAO**	-	Aromatic alcohols (anisyl, veratryl alcohol)	Aromatic alcohols oxidized to aldehydes; H_2O_2 production
Glyoxal oxidase, **GLOX**	-	Glyoxal, methyl glyoxal	Glyoxal oxidized to glyoxal acid; H_2O_2 Production

Now a days in concern to minimizing the environmental problems like pollution, in industries like textile, paper etc. ligninolytic enzymes are used. Wesenberg *et al*. (2003), Gill *et al.* (2002) and Chander and Arora (2007) studied the role of enzyme in textile dye decolourization and degradation of phenolic and non-phenolic aromatic compounds. Kirby *et al.* (2000) reported that laccase from *Phlebia tremellosa*

decolorized eight synthetic textile dyes. Textile dye named Malachite green was successfully transformed by laccase from *G. lucidum* (Murugesan *et al*. 2009)

White rot fungi that produce ligninolytic enzyme are reported for detoxification and decolonization of effluents of various industry like paper and pulp, coal conversion, petrochemical, alcohol distilleries, dyeing and textile industries (Rubilar *et al.* 2008; Raghukumar *et al.* 2008; D'souza *et al.* 2006). Ligninolytic enzymes are also used in denim washing (Pazarlioglu et al. 2005), Cosmetic industry (Aaslyng *et al.* 1996), biosensors (Ferry and Leech 2005; Cordi *et al*. 2007)

In paper industry it is required to separate the lignin in wood. Pretreatment of wood with ligninolytic enzyme may give the delignification (Kuhand *et al.* 1997). In the paper industry chlorine is used in bleaching process to remove the lignin residues which create lots of pollution. Fungal laccase can be applied as biobleaching agent as it degrades the remaining lignin in pulp and decolorize it (Call 1994). Laccase produced by *T. versicolor* has been studied for biobleaching of paper pulp (Wesenberg *et al.* 2003).

Pulping is the process in which wood or non wood fibers are separated and made flexible, either by chemical, mechanical, chemi-mechanical, thermo-mechanical or by solvent treatment, so that a fiber suspension is obtained. Which is converted into a slurry and used in paper- making and other purposes (Zhang et al. 2006). Thus the objective of pulping is to separate cellulose fibers from wood.

Kraft pulping process is frequently used pulping procedure around the world (Damiano *et al*. 2003). Chemical pulping involves use of different chemicals to remove noncellulose wood components and leaving intact the cellulose fibers (Smook 1992;

Biermann 1996). Chemical pulps are made by digesting raw materials, using sulfate and sulfite. In the sulfate process sodium hydroxide (NaOH) and sodium sulfide (Na_2S) are used and in addition to this sodium sulfate is added in the recovery cycle to compensate for chemical losses as chemical recovery is an essential part of the pulp production process (Tran 2007; Vakkilainen 2000; Bajpai 2008; Biermann 1996).

After chemical pulping 5-10% of the lignin residues remains in the pulp, responsible for the brown colour of the pulps. And so bleaching is required, in which remaining fraction of lignin is removed and whitening and brightening of the pulp occurs (Pokhrel & Viraraghavan 2004).

Mechanical pulping is traditionally used but it is high energy consuming. Chemical pulping dissolves lignin from the plant material, it is very efficient technique but this pulping process releases a large amount of hazardous chemicals which are pollutants of the environment.

Biopulping, which involves the treatment of lignocellulosic materials with lignin-degrading fungi, has been shown to result in energy savings and strength improvement (Akthar *et al.* 1993; Shukla, *et al.* 2004). In biopulping the pretreatment of materials with white rot fungi enhances the subsequent pulping step and substantially reduces the refining energy consumption in the pulping (Singh *et al*. 2010). Biopulping is considered to be effective biological methods as promising alternative to the alkali and chemical bleaches and reduces the utilization of chemicals and energy, pollutants and increase the yield and strength of pulp (Keller *et al*. 2003).

Enzymes are known to play important role in the natural delignification so they can be utilized for the degradation of lignin in the paper and pulp industry. In

biopulping process the amount of enzyme required is very high, hence powerful producers of enzyme are required to be selected. The secretion of ligninolytic enzymes is regulated to a large extent by carbon, nitrogen sources and various chemical and natural inducers (Kanwal and Reddy 2011). If by different methods of enhancing activity of lignin modifying enzymes (D'souza-Ticlo *et al.* 2006; Bollag & Leonowicz, 1984) we can enhance the enzyme activity methods of processing the substrate we can obtain more delignification, which would be great use in pulping. Ligninolytic enzymes produced by white-rot fungi were activated during the secondary metabolic phase and affected by nitrogen concentration in medium (Buswell *et al.* 1995), so addition of different substrate can enhance the production of ligninolytic enzymes. Many of these compounds resemble lignin molecules or other phenolic chemicals (Marbach *et al.* 1985; Farnet *et al.* 1999).

Veratryl alcohol is one such aromatic compound which is known to play an important role in the synthesis and degradation of lignin (Barbosa *et al.* 1996). Ethanol, indirectly enhance laccase production (Lee *et al.* 1999). Mansur *et al.* (1997) has used xylidine and veratryl alcohol and observed the enhancement in laccase activity. Yeast extract and peptone is also a well known nitrogen source to enhance the activity of ligninolytic enzymes (Arora and Gill 2000; Mikiashvili *et al.* 2006, Kanwal and Reddy 2011).

Use of white rot fungi and modification of conditions to enhance the enzyme activity would help in reducing the energy and chemical requirement during biopulping and would also help in quality improvement of paper. With this thought two main objectives ware framed for the present study. One was to identify potential alternative

source of raw material for paper industry and other was to identify appropriate delignifying fungi and its optimal conditions which could be utilized for enhanced biopulping process in the paper industries.

The entire work has been broadly categorized into three major aspects:

i) Evaluate and identify alternative sources of raw materials for paper and pulp industry

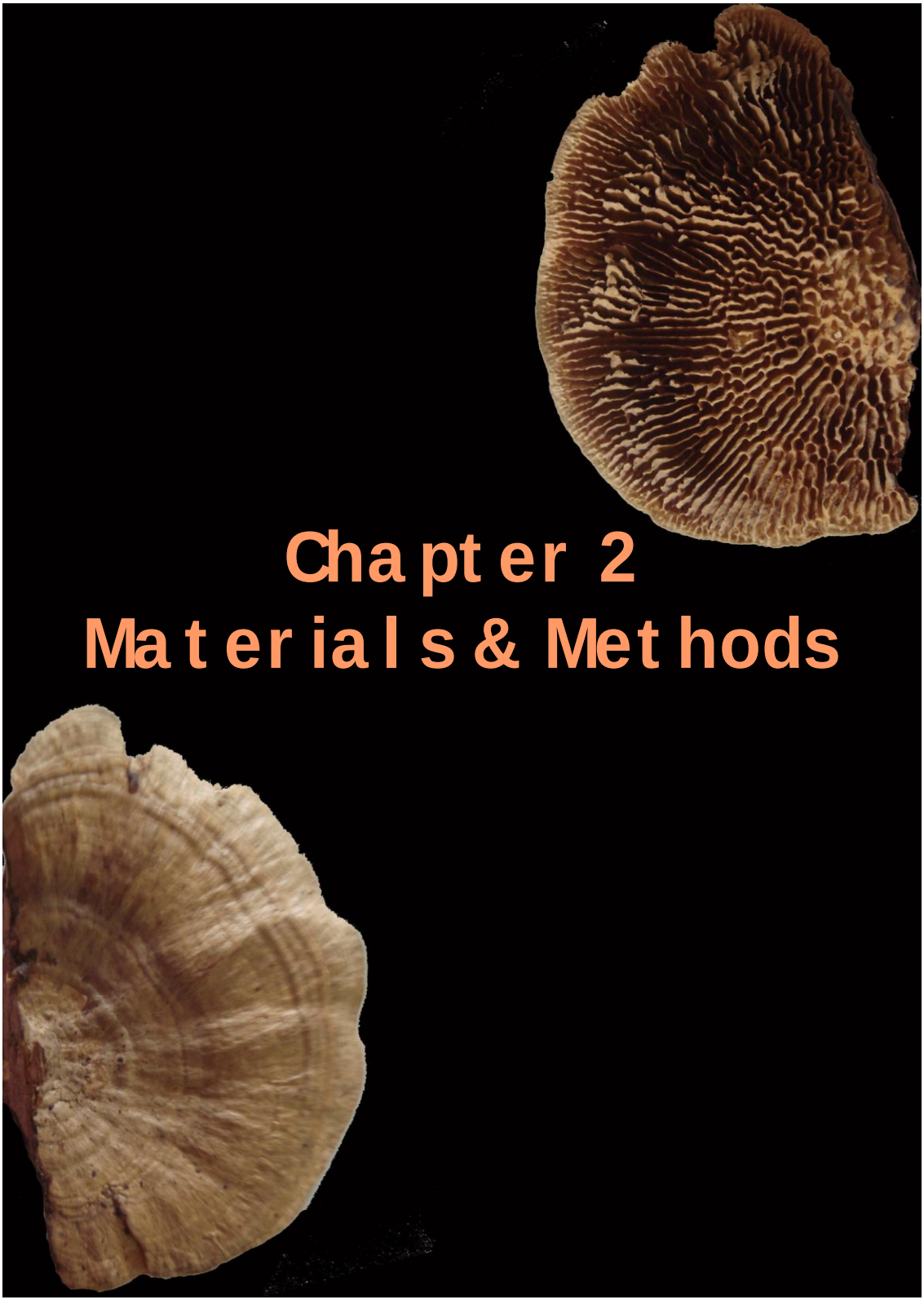
- Survey of forest areas for potential raw materials
- Collection of unpalatable grasses
- Evaluation of fiber properties and comparison of these properties with the determined values of the other raw materials
- Suggest the suitable potential alternative raw material

ii) Identification of selectively delignifying fungal strain and studying the pattern of decay

- Survey of different forest, saw mill and paper industries
- Collection, Identification, Isolation and maintenance of collected fungal strains
- Detection of ligninolytic / cellulolytic activity of collected fungi
- Selection of ligninolytic fungi
- Detect efficiency of the selected fungi to degrade lignin/ cellulose in the different forms of substrate (Biochemical analysis)
- Study of Anatomical and Biochemical alterations in the *in vitro* decayed wood samples.

iii) Experimental studies for enhancement and improvement of biopulping

- Collection of different substrates for the biopulping
- Selection of delignifying fungal strains
- Biochemical evaluation of fungal delignification in the selected alternative material
- Evaluation of optimal condition requirement by selected fungal strains for production of ligninolytic enzymes
- Study the effect of different chemicals and different pretreatments of substrate, on the enhancement of enzyme production and its effect on delignification
- Evaluation of biopulping under the obtained optimal condition with optimal enhancer using the selected fungi.



Chapter 2
Materials & Methods

1) Survey

a) Saw mills:

Survey of 25 different saw mills situated at Gajravadi, Dabhoi road, Sama, Harni, Chaini, Makarpura, Padra, Lakkad pitha in Vadodara were conducted to identify the different species of timber available and sold for different purposes. The list of saw mills visited and the different available wood species sold in the market was prepared.

b) Industry:

Visit to the J K Paper industry and Kalam Khush was conducted to understand the different steps involved in the paper making. In J. K. Paper industry, located at Songudh Dist. Tapi, Gujarat they use kraft pulping technique to make pulp. Pulp made out of wood and bamboo mixture and banana pseudostem was collected from this industry for the present study. In Kalam khush mechanical pulping technique is used for making pulp from the waste left over cloth pieces from tailors.

c) Forest area:

Different forest areas were surveyed for collection of grasses, raw materials and fungal fruiting bodies.

2) Collection

a) Wood materials:

Following the survey of sawmills five different commonly used timber species were selected and collected/purchased in the form of whole wooden logs. The logs were then cut into planks of 3 inch thickness. These planks were oven dried and cut into wooden chips, saw dust (by Electric surface planer Manufactured by Umiya Traders,

Jamkhambhaliya) and wooden blocks (by Jackshow machine) and used for the different experiments.

b) Grasses:

In Gujarat grass lands cover an area of about 8490 Sq. Km. Many of the densely and wildly growing grass species are unpalatable and rich in fiber. Survey of different forest areas were conducted for the collection of the raw materials for experiment. The grasslands selected for survey in the present study falls under two major forest divisions in Baria and Godhra of Gujarat. The two main grasslands visited are Rampur which comes under the Baria forest division of Dahod district and Bandheli which comes under the Godhra forest division of Panchmahal district. Selected grasses were collected and brought to the laboratory. Culm was separated from the leaves and inflorescence, chopped in the size 5 cm to 7 cm and oven dried at 60˚ C. These prepared raw materials were used to evaluate the fiber properties and conduct other pulping experiments.

c) Fungi:

Different forest areas were surveyed for the collection of the fungal fruiting bodies. Places visited to collect fungal fruiting bodies are Rampur grassland in Baria forest division of Dahod district, Bandheli grassland in Godhra forest division of Panchmahal district, Pavagadh forest located close to Vadodara in Panchmahal district. Other than these areas The M.S. University Arboretum, Vadodara and surrounding places were visited for the collection of fungal fruiting bodies. Fungal fruiting bodies were collected in zip-lock plastic bags, and brought to the laboratory and immediately inoculated for isolation and identification of the fungi.

3) Perpetration of Media

For isolation and maintenance of culture mainly PDA and MEA medium were used and in some experiments broth of the same medium (without Agar Agar) were used. Method for preparing medium is given below:

a) PDA (Potato Dextrose Agar) (Stevens 1981)

Potato ……200 gm

Dextrose ……..…20 gm

Agar Agar ……20 gm

Distilled water ……1 lit.

Potato was peeled and cut into small cubes, weighed, and boiled in water until soft. Boiled potatoes were squeezed through a sieve. Agar Agar was added and boiled till it gets dissolved, then dextrose was added and stirred until dissolved, sterilized by autoclaving at 121˚ C temperature and 15 psi pressure for 20 minutes.

b) MEA (Malt Extract Agar) 3 % (Stevens 1981)

Malt extract ……..……30 gm

Agar Agar ……30 gm

Distilled water ……1 lit.

Malt extract was boiled in the water until dissolved. To this agar agar was added and sterilized in autoclave at 121˚ C temperature and 15 psi pressure for 20 minutes.

4) Isolation and Identification

Small pieces of 2mm X 2mm were cut from the collected fruiting body and surface sterilized with 0.1% $HgCl_2$. The pieces of fruiting body were inoculated in petridish containing PDA and MEA medium under asceptic condition and incubated for

7 days. After development of colony these were subcultured in slants made up of PDA and MEA medium and maintained for the experimental studies.

Basidiocarps of collected fungi were dried. A small piece of the fruiting body was packed in locked polythene bags, assigned identification number and sent to Forest Research Institute, Dehradun for confirmation and authentic identification.

5) Screening

a) Screening of fungal isolates for enzyme activity:

Fungal isolates were screened to identify its cellulolytic and ligninolytic ability according Bains *et al*. (2006). This was done by substituting malt extract agar medium (3%) with respective enzyme substrates viz. tannic acid for ligninases and carboxy methyl cellulose for cellulases. The petri plates were inoculated with fungal mycelium and incubated at 28^{o} C for 7 days. Five set of replicates were maintained for all the selected fungi. The enzyme activities were evaluated by observing the zone of clearance if any, formed by flooding the plates with visualizing dye congored for 15 minutes to detect the cellulolytic activity (Teather and Wood 1982) and ligninolytic activity was assessed by observing the dark brown colored zone around the fungal colony.

b) Screening of fungi for their ability to degrade lignin and cellulose:

The selected fungi were experimented for its ability to degrade cellulose and lignin. The raw materials used were wood chips, wood blocks, saw dust and grasses.

Wood blocks, Wood chips and Saw dust were oven dried, weighed, and then soaked in water for 12 hour, autoclaved for 1 hr., and inoculated with fungal culture and incubated for 10, 20, 30 days.

For the **Grass culm** used as raw material water was added, autoclaved for 45 min., inoculated with the fungus and incubated for 3, 5, and 7 weeks.

Banana pseudostem were cut in to small pieces and dried in oven. Four different set of experiments were kept as mentioned below:

a). 5 gm. of Banana pseudostem soaked in water for 24 hrs + 3 % MEA medium.

b). 5 gm. of Banana pseudostem soaked in water for 24 hrs + without medium.

c). 5 gm. of dry Banana pseudostem + 3 % MEA medium.

d). 5 gm. of dry Banana pseudostem + without medium.

All the four set of combinations were sterilized by autoclaving at 121˚ C temperature and 15 psi pressure for 45 minutes. After cooling, it was inoculated with 9 mm disc of 10 days old fungal culture in aseptic condition. The culture was then incubated at 24 ±0.5 ˚ C for the desired incubation period. Material was analyzed for lignin and cellulose content.

Same experiment was conducted with **Banana pseudostem pulp** obtain from the JK Paper Industry. After incubation all the raw materials were removed and freed of mycelium from the surface, oven dried and weighed for measuring weight loss. These samples were then used to analyze lignin (Kalsons lignin) and cellulose (anthrone method).

6) Fiber properties

a) Fiber properties of selected substrates:

Fiber dimensions: Small slivers were obtained of the culm and wood materials, macerated with Jeffrey's solution (Sass 1958). (1:1 mix of 10% aqueous Chromic Acid + 10% aqueous Nitric Acid, Chromic Acid – Potassium dichromate 10% in Sulfuric

Acid). The slivers were then washed, place in small flasks with 50 ml distilled water and the fiber bundles were separated into individual fibers using a small mixture. The macerated fiber was then stained with Safranin and the fiber suspension was placed on a slide by means of medicine dropper. For fiber diameter, lumen diameter and cell wall thickness determination, cross sections were used. All the measurements were made with the help of Leica Qwin Pro image analyzer.

Derived values: Five derivatives values were also calculated using fiber dimensions. The values were then compared with those of other non – wood plant fibers (Ververis *et al* 2004). The following five equations were used to find the derived values which are important criteria for determining fiber qualities in papermaking (Saikia *et al* 1997, Ogbonnaya *et al* 1997 , Kırcı 2006).

Felting rate /Slenderness ratio: Fiber length ÷ Fiber diameter

Elasticity coefficient (%)/ Flexibility coefficient: (Fiber lumen diameter ÷ Fiber diameter) ×100

Rigidity coefficient (%) : (Cell wall thickness ÷ Fiber diameter) ×100

Runkel index/ Runkel ratio: (Fiber cell wall thickness × 2) ÷ Lumen Diameter.

F ratio (%): Fiber length ÷ Cell wall thickness ×100

7) Selection of raw materials

From the visit to paper industries (JK Paper industry, Kalam khush handmade paper) it could be understood that, now a days paper was being produced from the wood and grass as raw materials in the ratio of 70 : 30. Other raw materials have been tried and selection of materials as alternative raw material was done on the basis of above experiments (Screening of fungi for their ability to degrade cellulose &lignin).

8) In vitro decay experiment

a) Weight loss:

Decay chambers were prepared by modified method of Blanchette (1986). Fungi were grown on 3% MEA medium under aseptic condition before 7 days of inoculation of wooden block. From the wooden plank wooden blocks were cut in 1.5 cm X 1.5 cm X 3 cm. Then the wood blocks were placed in distilled water for 1 h to get enough humidity and sterilized at 121^0 C, 15 psi for 1 h in autoclave. The wooden blocks were then inoculated in decay chambers under aseptic conditions, and incubated for 10 days, 20 days, 30 days, 2 months, 3 months, 6 months, 9 months, and 12 months at 25 ± 28˚C.

b) Anatomical:

i) Light microscopy:

After completion of the incubation period wooden blocks were taken out, mycelium was removed from the surface and preserved in FAA (formaldehyde:acetic acid:alcohol, 5:5:90) for the anatomical studies (Blanchette 1986). Sections of 15–20 µm were taken on a sledge microtome (Ernst Leits Wetzlar). Sections from all three dimensions of wood transverse (T.S.), longitudinal (L.S.), radial (R.L.S.) were taken. Selected section were arranged on the slide and tied with cotton thread. Sections were further stained with safranin- fast green, and toludine blue (Johansen 1940) and made permanent by passing through xylene series.

ii) Scanning electron microscopy:

For scanning electron microscopy (SEM), wooden blocks were cut in the size of 5–10 mm pieces exposing one of three sections of wood transverse, radial or tangential and were fixed in the 3% glutaraldehyde in a 0.1 M phosphate buffer (pH

7.2) for 2–3 days at 25 ^{o} C. These wooden blocks were then passed through the dehydration series of 10, 20, 30, 50, 70, 90, 100% ethanol and stored in 100% alcohol. Dried wood samples were directly mounted on stubs and observed using (JEOL) JSM-5610LV SEM (Wilcox and Brier 1987)**.**

c) Biochemical analysis:

i) Cellulose (anthrone method):

Prior to chemical analysis of the wood, both control and decayed wood blocks were ground and passed through a 40-mesh-sized screen. Estimation of cellulose was performed using the method suggested by Yemn and Willis (1954). Acetic–nitric reagent (3 ml) was added to 0.5 g of the sample in a test tube and mixed well. The test tubes were then placed in a water bath at 100°C for 30 min. After cooling, the samples were centrifuged for 15–20 min at 5000 rpm and the supernatants were discarded. The residue obtained was washed with distilled water and 10 ml of 67% sulfuric acid was added to it. Then it was allowed to stand for 1 h. One milliliter of the before mentioned solution was diluted to 100 ml with distilled water. One milliliter of this solution was taken in three different test tubes. Ten milliliters of anthrone reagent was added to it and mixed well. After that, the tubes were heated in boiling water bath for 10 min, cooled, and it was measured at 630 nm using a spectrophotometer (Systronics Spectrophotometer 106). A blank was obtained by using anthrone reagent and distilled water. A standard curve was prepared by using D-glucose (mg/ml) with different concentrations treated with anthrone reagent.

ii) Lignin (Kalson lignin):

Estimation of lignin content was performed using the method suggested by Dill

and Kraepelin (1986). Flasks containing 1 g of ethanol-benzene extracted wood meal and 20 ml of H_2SO_4 (72%) were gently shaken in a water bath at 30◦C for 1 h. The acid was then diluted with H_2O to 4% (wt/vol), and the samples were autoclaved at 121◦C for 30 min. The lignin that settled overnight was quantitatively collected by filtration through a crucible, washed free of acid with hot water, and dried. The lignin content was calculated as a percentage of oven-dried, non-extracted wood meal.

9) Evaluation of enzyme activities

a) Preparation of fungal enzyme sample:

Enzymatic assays were performed in 100 ml of 3% liquid MEA medium. The medium was sterilized by autoclaving at 121˚ C temperature and 15 psi pressure for 20 minutes. After cooling the medium, it was inoculated with 9 mm disc of 10 days old fungal culture in aseptic condition. The culture was incubated at 24 ±0.5˚ C for the desired incubation period. After completion of incubation period the cultures were taken out, mycelium broken with the glass pieces and then filtered through whatman paper No. 1 disc. This filtrate was used as enzyme solution.

b) Enzyme assay:

i) Lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP activity):

Lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP) activities were measured according to the procedure of Castillo *et al.* (1994) using as substrate 0.167 mM 3-methyl-2-benzothiazolinone hydrazone (MBTH) which interact with 2.37 mM 3-(dimethylamino) benzoic acid (DMAB) producing a purple coloured reaction. Substrates were prepared on 0.1 M succinic-lactic acid buffer at pH 4.5. Cuvette (1.5 ml) contained 417.5 µl MBTH, 417.5 µl DMAB, 100 µl $MnSO_4$, 50 µl

supernatant (enzyme) and 15 µl H_2O_2 to measure the enzyme activity. To distinguish between magnesium dependent and independent peroxidases reaction was performed in the presence and absence of 3mM $MnSO_4$. 4mM H_2O_2 was used to initialise the reaction. Reactions were carried out at 37°C and monitored spectrophotometrically at 590 nm during 1 min. The enzyme activity was calculated using as extinction coefficient $\varepsilon = 53000\ M^{-1}cm^{-1}$.

ii) AAO activity:

Aryl alcohol oxidase (AAO) activity was estimated according to the procedure of Guillen *et al.* (1990) following the increase in absorbance due to the vertraldehyde formation from veratryl Aryl alcohol oxidase alcohol (3,4-dimethoxybenzyl alcohol). The reaction mixture contained 5mM veratryl alcohol in a 0.1 mM sodium phosphate buffer pH 6 and 50 µl supernatant (enzyme solution). Reaction was performed at 30°C and visualized spectrophotometrically at 310 nm during 2.5 min and activity expressed in µkat/g dw using $\varepsilon = 9300\ M^{-1}cm^{-1}$as the extinction coefficient of vertraldehyde.

iii) Laccase activity:

Laccase activity was determined by the oxidation of 2, 2'-azino-bis (3-ethylbenziazoline-6-sulphonic acied) (ABTS) by laccase causes by blue discoloration of the substrate (Niku-paavola *et al.* 1988). Enzyme assay were performed with 500 µM ABTS in 50 mM Sodium acetate buffer at pH 4.5. A 20 µl aliquot of enzyme solution was added to 580 µl of the ABTS. Change in the absorbance at 420 nm were observe ($\varepsilon = 36,000\ M^{-1}cm^{-1}$).

10) Parameters affecting enzyme activity

a) Effect of pH:

To estimate the effect of different pH on enzyme activity, all enzyme assays were carried out at different pH in 0.1 M of HCl-KCl buffer, pH 1 and 2; citrate buffer, pH 3 and 4; acetate buffer pH 5 and 6 and phosphate buffer, pH 7 and 8. Then all the enzyme assay were carried out spectrophotometrically (Trivedi 2002).

b) Effect of Temperature:

Enzyme containing supernatants were incubated at 25, 35, 45, 55, 65, 75 ˚ C in water baths for four hours to allow ample time for denaturation. Then all the enzyme assay were carried out.

11) Study on the effect of different chemicals on the enhancement of enzyme activity

3 % MEA medium was prepared, autoclaved and supplemented with different concentration of chemicals (Ethanol, Veratryl alcohol, Xylidine, Yeast Extract, Peptone) and inoculated with a 9 mm disc of 10 days old culture. After completion of incubation period enzyme assays were carried out.

Ethanol and enzyme production:

Effect of different concentration of ethanol on biomass yield and enzyme production was evaluated in the MEA medium. The ethanol was added to media after the media was autoclaved in the concentration of 1%, 2%, 3%, 4%, 5% (Lee *et al*. 1999). A control treatment was included in the experiment that does not contain ethanol.

Veratryl Alcohol and enzyme production:

In the experiment to determine the optimal concentration of veratryl alcohol for induction of biomass yield and enzyme production MEA medium was used. Veratryl alcohol added to MEA media to obtain final concentration of 4 mM, 8 mM, 12 mM, 16 mM, 24 mM. (Barbosa *et al.* 1996) A control treatment was included in the experiment that contained no veratryl alcohol.

Xylidine and enzyme production:

To determine the effect of xylidine 3 % MEA medium was utilized. Xylidine was added to the MEA media to obtain final concentrations of 4 µM, 7 µM, 10 µM, 20 µM, 30 µM. The control treatment did not include xylidine in the MEA medium.

Yeast Extract and enzyme production:

To determine the effect of Yeast Extract it is substituted in the MEA medium. Yeast extract is added in MEA medium in the concentration of 0.1 g/l, 0.2 g/l, 0.4 g/l, 0.6 g/l, and 0.8 g/l. In the control the yeast extract is not added.

Peptone and enzyme production:

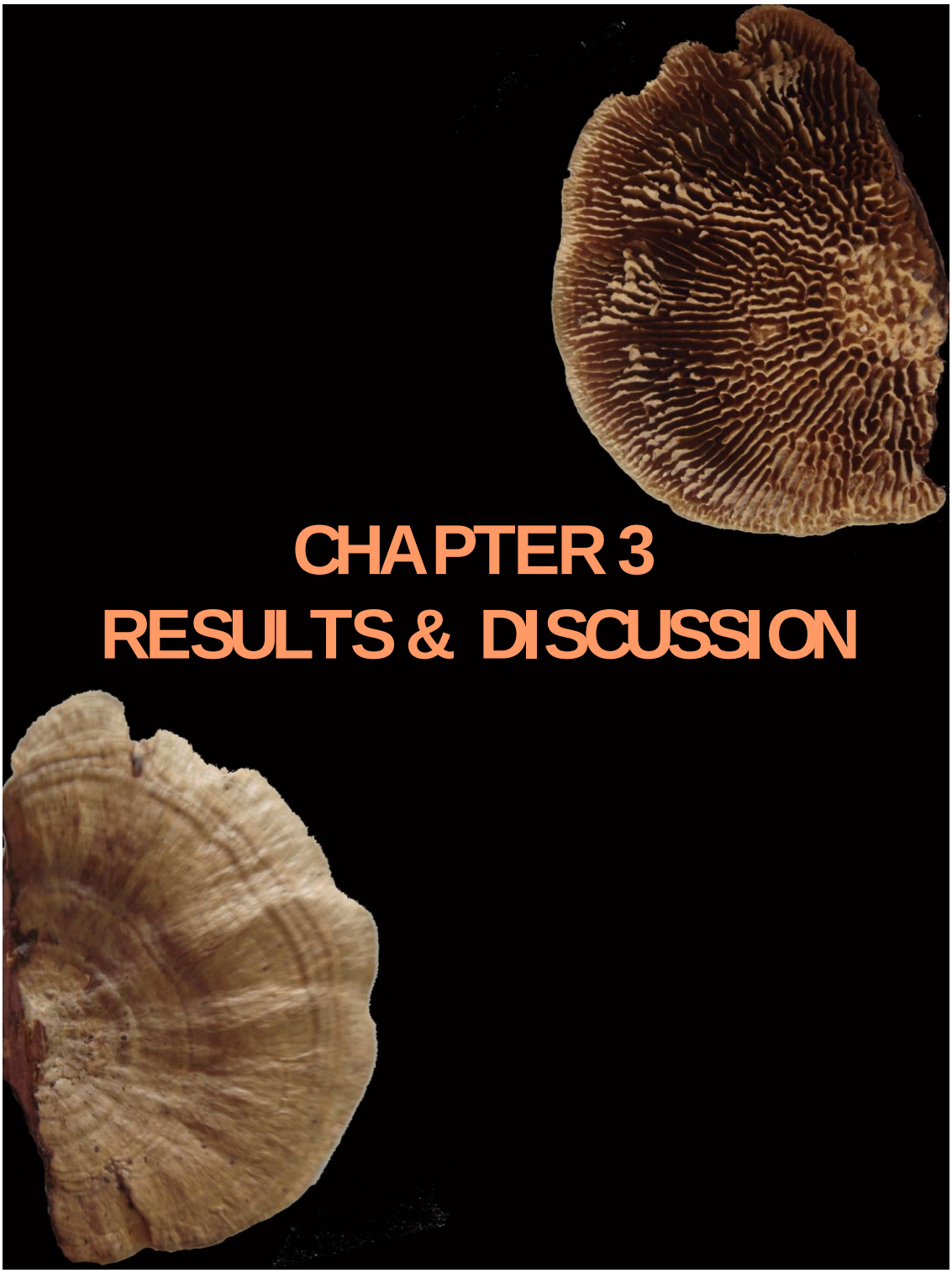
In the experiment to determine the optimal concentration of peptone for induction of biomass yield and enzyme production MEA medium was used. Peptone was added to MEA media to obtain concentration of 0.1 g/l, 0.2 g/l (Kanwal and Reddy 2011), 0.4 g/l, 0.6 g/l, and 0.8 g/l. A control treatment was included in the experiment that contained no peptone.

12) Experimental set up to analyze the effect of enhancement of enzyme activity on biopulping

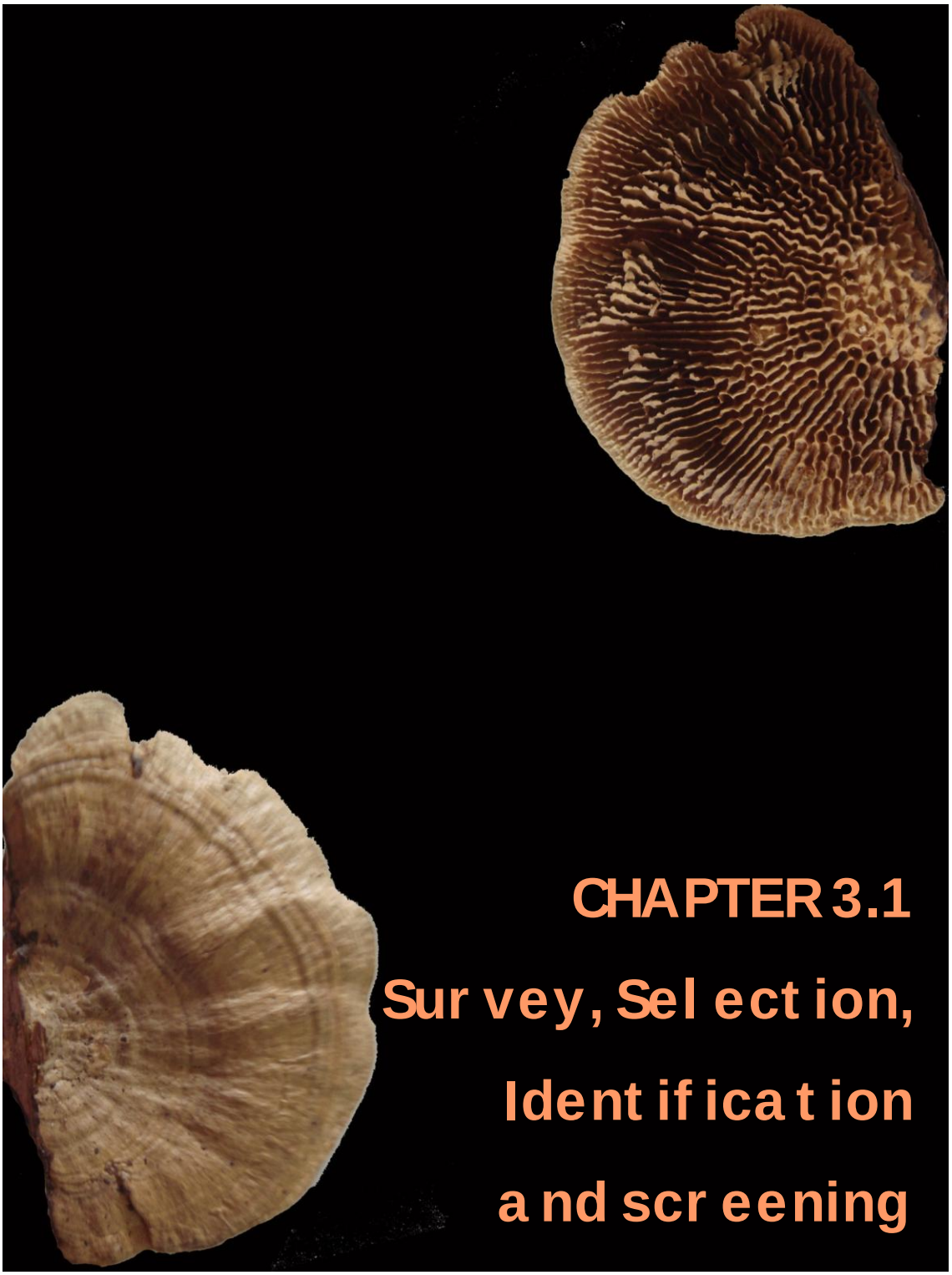
From the above experiment the best concentration of the chemicals and experimental conditions (pH and Temperature) were selected for the experimental set up. On the basis of the enhancement of enzyme activity 1 % of Ethanol, 12 mM and 16 mM of Veratryl alcohol, 30 µM of Xylidine, 0.4 g/l of Yeast Extract, 0.6 g/l of Peptone were used for the further experiment. All these chemicals were added in 3 % MEA medium and all the five selected substrate (*Eucalyptus* wood blocks and wood shaving, *Phragmites karka* culm, pulp, *Sesbania sesban* stem) in 5 % concentration were added. Then this was sterilized by autoclaving at 121˚ C temperature and 15 psi pressure for 20 minutes. After cooling, it was inoculated with 9 mm disc of 10 days old fungal culture under aseptic condition. Then culture was incubated at 24 ±0.5 ˚ C for the desired incubation period. After completion of incubation period the cultures were removed, mycelium broken with the help of glass pieces and then filtered through whatman paper No. 1 disc. These filtrates were used as enzyme solution. The residual sample was used to analyze for lignin and cellulose content.

13) Statistical analysis

Data presented throughout this thesis were analyzed using Microsoft office excel. Results, where appropriate when subjected to an analysis of variance (ANOVA) testing at the P=0.05 level. Standard errors of the means (SE) were calculated.



CHAPTER 3
RESULTS & DISCUSSION



CHAPTER 3.1

Survey, Selection, Identification and screening

I) RESULT

1) Survey and collection

a) Survey of saw mill and selection of wood species

Survey of 25 different saw mills in Vadodara were conducted to identify the different species of wood available and sold for different purposes. Table 1 shows the list of saw mills visited and the different species of wood available and sold in the market.

Sr.No.	Name and Adders of the sawmill	Wood species available in sawmill
1	Jai gopal Sawmill, Gajrawadi, Vadodara.	*Shorea robusta* Roth Nigerian teak, Ghana teak, Panama teak *Pinus longifolia* Roxb. *Mangifera indica* L. *Azadirachta indica* L. *Acacia arabica* (Lam.) Willdv
2	Shiv Sakti Sawmill, Dabhoi road, Vadodara.	*Dryobalanops* sp. *Shorea contorta* Vidal Nigeria teak, Ghana teak, Sudan teak
3	Vishal Sawmil, Dabhoi road, Vadodara.	*Mangifera indica* L. *Tamarindus indica* L. *Tectona grandis* L.f. *Azadirachta indica* L. *Pongamia pinnata* (L.) Pierre
4	Siddarth sawmill, Dabhoi road, Vadodara.	*Pinus longifolia* Roxb. *Pinus rouxbergii* Sargent *Tectona grandis* L.f. Burma teak, Malaysia teak
5	Bhagvati ply and timbr agency, Dabhoi road, Vadodara.	Nigeria teak Nagpuri teak Valsad teak Malaysia teak *Shorea robusta* Roth
6	Swastika sawmill, Nr. Ysmuns mill, Dabhoi road, Vadodara.	Burma teak, Nigeria teak, Ghana teak *Terminalia myriocarpa* van Heurck & Muell. *Cedrus deodara* (Roxb.) G.Don *Shorea contorta* Vidal *Shorea robusta* Roth
7	Meshesh timber mall,	Nigeria teak

	Sama road, Sama, Vadodara.	*Platanus occidentalis* L. *Fagus grandifolia* Ehrh.
8	Shivshakti sawmill, Sama road, Sama, Vadodara.	*Mangifera indica* L. *Acacia arabica* (Lam.) Willdv *Shorea robusta* Roth *Tectona grandis* L.f. Nigeria teak Ghana teak *Pinus longifolia* Roxb.
9	Genesh sawmill, Sama road, Vadodara.	*Tectona grandis* L.f. Nigeria teak Ghana teak African mahagony *Shorea robusta* Roth
10	Sahajanand sawmill, Sama road, Vadodara.	*Tectona grandis* L.f. *Mangifera indica* L. *Acacia nilotica* (L.) Willd. *ex* Delile *Azadirachta indica* L.
11	Bajrang Timber Trading Co. Opp. Sama jakatnaka, Sama, Vadodara.	Nigeria teak, Burma teak, Valsad teak *Shorea robusta* Roth *Pinus longifolia* Roxb. *Ficus carica* L.
12	J.K.S. sawmill, Harni, Vadodara.	*Tectona grandis* L.f. *Dryobalanops* sp.
13	Kalyan sawmill, Harni, Vadodara.	*Azadirachta indica* L. *Prosopis juliflora* (Sw.) DC. *Mangifera indica* L. *Acacia nilotica* (L.) Willd. *ex* Delile
14	Jagdish sawmill, Harni, Vadodara.	*Cedrus deodara* (Roxb.) G.Don *Pinus longifolia* Roxb. *Bombax ceiba* L. *Azadirachta indica* L.
15	Patel timber merchant, Harni, Vadodara,	Ghana teak Nigeria teak *Gmelina arborea* Roxb *Azadirachta indica* L.
16	Datidar sawmill, Harni, Vadodara.	*Tectona grandis* L.f. Nigeria teak Malaysia teak Valsad teak Nagpuri teak *Dalbergia sissoo* Roxb. *Cedrus deodara* (Roxb.) G.Don *Terminalia myriocarpa* van Heurck & Muell. *Pinus longifolia* Roxb.

17	Jivarai sawmill, Station road, Near Shastri Bridge, Vadodara.	*Ficus benghalensis* L. *Tectona grandis* L.f. *Tamarindus indica* L. *Bombax ceiba* L. *Azadirachta indica* L. *Mangifera indica* L. *Peltophorum roxburghii* (G. Don)Degener *Cedrus deodara* (Roxb.) G.Don
18	Ambika sawmill, Chhani road , Chhani, Vadodara.	*Hardwickia binata* Roxb. *Delonix regia* (Boj. ex Hook.) Raf. *Acacia arabica* (Lam.) Willdv *Pinus longifolia* Roxb. *Tectona grandis* L.f. *Cordia myxa* L.
19	Warrior timber merchant, Chhani road, Chhani, Vadodara.	*Pithecellobium dulce* (Roxb.) Benth. *Mangifera indica* L. *Acacia nilotica* (L.) Willd. *ex* Delile *Emblica officinalis* Gaertn.
20	Pragati timber mart, Chhani road, Chhani, Vadodara.	*Tectona grandis* L.f. *Tamarindus indica* L. *Acacia nilotica* (L.) Willd. *ex* Delile *Shorea robusta* Roths
21	Patel timber mart, Chhani road, Chhani, Vadodara.	*Tectona grandis* L.f. Burma teak Nigeria teak *Shorea robusta* Roth *Acacia nilotica* (L.) Willd. *ex* Delile *Mangifera indica* L. *Tamarindus indica* L.
22	Shri mahadev sawmill, Nr Makerpura Road, Vadodara.	*Tectona grandis* L.f. *Eucalyptus globulus* Labill. *Acacia nilotica* (L.) Willd. *ex* Delile
23	Patel sawmill, Lakkad pidha, Vadodara.	*Mangifera indica* L. *Syzygium cuminii* (L.) Skeels. *Azadirachta indica* L.
24	Jalaram sawmill, Padra, Dist. Vadodara.	*Bambusa arundinacea* Schreb. *Pterocarpus marsupium* Roxburgh *Dryobalanops* sp. *Tectona grandis* L.f. *Shorea robusta* Roths *Azadirachta indica* L.
25	Shiv Shakti timbers, Padra, Dist. Vadodara.	*Azadirachta indica* L. *Eucalyptus globulus* Labill. *Acacia nilotica* (L.) Willd. *ex* Delile *Prosopis juliflora* (Sw.) DC.

Table 1: List of saw mills visited and wood species available with them

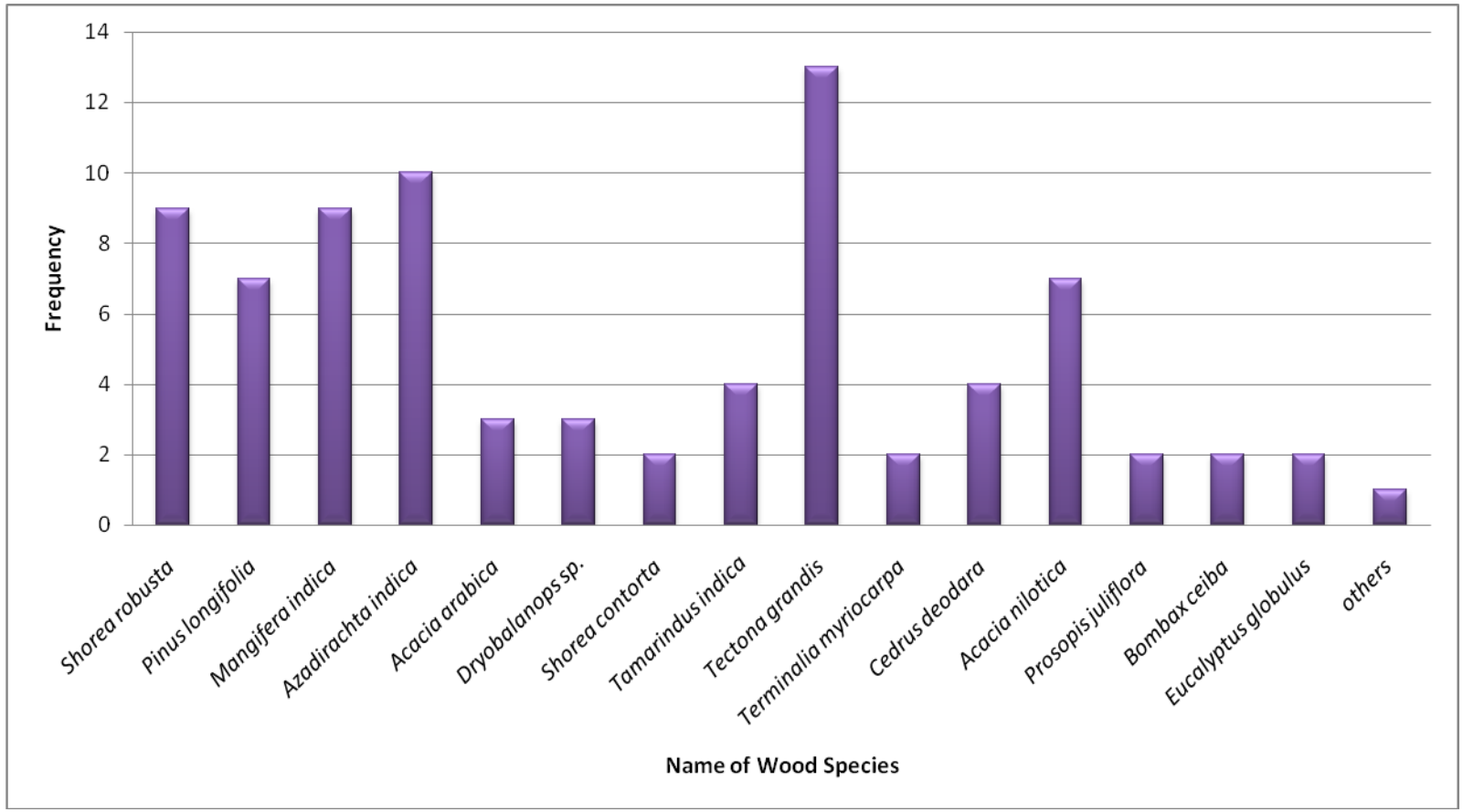


Figure 12: Frequency of availability of wood species in saw mills of Vadodara

Others : *Platanus occidentalis, Syzygium cumini, Bambusa arundinacea, Pterocarpus marsupium, Gmelina arborea, Dalbergia sissoo, Ficus benghalensis***,** *Peltophorum roxburghii, Hardwickia binata, Delonix regia, Cordia myxa, Pithecellobium dulce, Emblica officinalis, Ficus carica, Fagus grandifolia,* African mahagoni, *Pinus rouxbergi.*

From the survey it was found that 41 different wood species were available in different saw mills of Vadodara. In most of the saw mills different varieties of teak were available. As shown in Figure 12 *Shorea robusta,* Nigerian teak, *Mangifera indica, Azadirachta indica and Tectona grandis* were the most popularly available species in saw mills of Vadodara, where as *Acacia arabica, Dryobalanops* sp., *Tamarindus indica*, Burma teak, *Cedrus deodara,* Ghana teak, *Pinus longifolia* and *Acacia nilotica* were the moderately available species in Vadodara. Among the different varieties of teak, Nigerian teak was very commonly sold in the market. All other wood species were available only at one or two places in Vadodara.

Selection of wood species as raw material for the further study was done on the basis of the conducted survey. Wood of *Mangifera indica* and *Syzygium cumini* were found to be widely used for furniture. Wood shavings and waste obtained from the carpenters are normally used for fuel by the local people. Wood of *Pithecellobium dulce* and *Bombax ceiba* are used to make packing boxes which are discarded or burnt for fuel after use. *Eucalyptus globulus* wood is traditionally used in combinations with other species of wood for paper making. The wood species selected for further study were collected from the different saw mills of Vadodara as shown in Table 2.

No.	Name	Family	Local name	Collected from
1	*Mangifera indica*	Anacardiaceae	Mango	Patel saw mill, Lakkad pidha, Vadodara
2	*Syzygium cuminii*	Myrtaceae	Jamun	Patel saw mill, Lakkad pidha, Vadodara
3	*Bombax ceiba*	Malvaceae	Shimlo	Jalaram saw mill, Padra, vadodara.
4	*Eucalyptus globulus*	Myrtaceae	Nilgiri	Shri Mahadev saw mill, Nr Makerpura Road, Vadodara
5	*Pithecellobium dulce*	Fabaceae	Gorasamli	Jalaram saw mill, Padra, Vadodara.

Table: 2 List of wood species along with their family, local name and place of collection

b) Survey of paper industry and collection of pulp

Pulp and paper industry is one of the oldest and major industry in our country. There are two basic steps involved in paper production I) Pulp making II) paper making. Survey of paper industries were conducted to practically understand the process of paper making. Two industries were visited (i) Kalm Khush paper industry, Ahmedabad and (ii) J K Paper industry, Songudh which is very well known paper industry in Gujarat. Shri A. M. M. Murugappa Chettiar Research Center at Taramani in Chennai was also visited, the institute has completed project on hand making paper using technology of bio pulping.

1. Kalm Khush paper industry, Ahmadabad: Gandhiji's philosophies on hand spinning and weaving cotton are well known, but it is perhaps little known that he also encouraged the hand making of paper. Kalm Khush paper industry is a small factory which makes handmade paper using waste products such as textile scraps to create fabulous artisan papers. The industry is located opposite to the Gandhi Ashram in Sabarmati area, on the outskirts of Ahmadabad in Gujarat. It is a part of the Gujart Khadi Gramodhyog mandal. The raw material used in this industry is waste white cotton cloth obtained from the different tailors in Ahmadabad.

The paper making process involves mechanical pulping. The quality of the paper produced here was rough and so used in making cards, covers and paper bags. In this process bleaching of the pulp was avoided, so hazardous chemicals used for bleaching was not required and so it is an ecofriendly process. In this process of paper making chemicals and energy utilized was low but the man power required was found to be very high. Chart given below describes paper making process practiced in this industry.

Step 1: Raw material, Chemicals collected and stored in godown - Cotton cloth is used as raw materials, which is purely waste material obtained from the tailors. Other chemicals required are stored in godown.

↓

Step 2: Cutting and sorting of raw materials - From the raw material stones and other extraneous materials and coloured threads are removed manually and is chopped in to small pieces.

↓

Step 3: Raw material dusting - Raw material is washed with petrol and soap so dust is removed and material becomes white.

↓

Step 4: Pulping in bitter - Bitter is a machine with barrings. In this machine cotton cloth is added with guavar gum, soda and alum and kept for 6-8 hrs. Pulp obtained is soft and white.

↓

Step 5: Lifting coaching wet - It is also a manual process. Big water tanks with wire meshed frame the center is kept and the pulp is poured on the frame and spreaded evenly. The frame is removed from the water and on this evenly distributed pulp, muslin cloth is spread to adhere the pulp on it. The muslin clothes with the evenly spread pulp is stacked one above the other in 50-100 layers.

↓

Step 6: Pressing - These prepared stacks are pressed with a manually operated press, the pressure of which removes the excess water from the pulp.

↓

Step 7: Drying - From these stacks the papers are removed and hanged in shade for drying.

↓

Step 8: Smoothing of the rough paper - It is done in roller press. In this the rough papers are kept inbetween iron sheet 20 papers are kept together and passed from the press for 3 to 4 times.

↓

Step 9: Cutting - Cutting in to required size is done by a sharp cutter.

↓

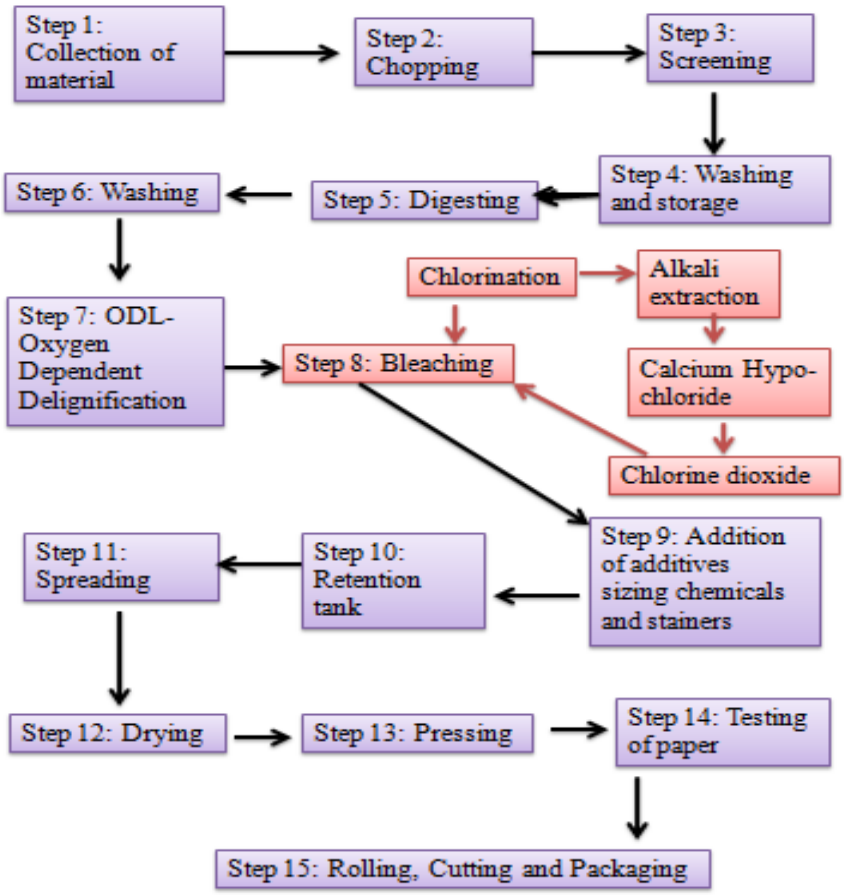
Step 10: Sorting of papers - Papers are sorted out on the bases of their thickness.

↓

The paper are mainly used for making decorative files, lamps, note books etc.

2. J K Paper industry, Songudh

The well known JK Paper industry is situated in Songudh dist. Tapi, Gujarat. JK Paper industry is one of the major industries supplying papers for xerox and printing in Gujarat. JK Paper over the decades has emerged as a manufacturer of best in class international quality papers. It is the market leader in Branded copier and amongst the two top players in coated paper and Hi end packaging boards. The purpose of visiting JK Paper industry was to understand the process of pulping and paper making. Mr. K. Radhamohan, Manager (QC) accompanied and explained the process of paper making. The steps involved were:



Step 1: Collection of material - Bamboo and wood (*Eucalyptus*, Subabul), are the main raw materials used in this industry bamboo and wood used in the ratio of 70 : 30.

↓

Step 2: Chopping - Raw materials are conveyed to the chippers (which are large rotating discs holding four or more long heavy knives) to reduce the wood, to small chips.

↓

Step 3: Screening - Chips are screened on the basis of their size by using vibrating screens. Here the desire product (150 mm) is separated from saw dust and oversize chips. Oversize chips are again submitted to the chippers.

↓

Step 4: Washing and storage - Chips were washed with water and send to the storage tank.

↓

Step 5: Digesting - Digester is big tank which is filled with chips and the cooking white liquor contains sodium sulfide, sodium carbonate and caustic soda and pressure and temperature is raised.

↓

Step 6: Washing - Pulp after separation from cooking liquor is washed. And cooking liquor is send to recovery furnace to recover its chemicals.

↓

Step 7: ODL- Oxygen Dependent Delignification - Washed pulp is now subjected to oxygen dependent delignification.

↓

Step 8: Bleaching - Bleaching of the pulp is done in four stages. 1) Chlorination, 2) Alkali extraction, 3) Calcium Hypo-chloride and 4) Chlorine dioxide

↓

Step 9: Addition of additives sizing chemicals and stainers - In the bleached pulp starch and talcum powder without zinc is added. Methyl violate dye is added so the pulp become pure white in colour.

↓

Step 10: Retention tank - Retention chamber is prepared in which pulp is kept for some time so all the additives and dyes are absorbed properly in the pulp.

↓

Step 11: Spreading - Spreading is done by cylinder field with pulp solution which produce a thin layer of fibers on a end less belt.

↓

Step 12: Drying - This end less belt carries the pulp layer to the series of squeeze rollers and dryers. Due to the heat of the dryers the water is evaporated.

↓

Step 13: Pressing - Then this paper is passed through press rollers where the paper is pressed and now paper become smooth.

↓

Step 14: Testing of paper - The paper after pressing automatically tested for its quality. It is computerized testing.

↓

Step 15: Rolling, Cutting and Packaging - Thus tested papers are rolled and send for cutting. Cutting is done by machine in a desire size and packing is done manually.

In the research laboratory of J.K. Paper industry is trying to find alternative source of raw material for making paper has been evaluated. Research on making paper from the banana pseudostem by the mechanical pulping and chemical bleaching is in process.

Raw materials, unbleached pulp and effluents were collected from the industry to experiment the effect of fungal treatment to increase the efficiency of pulping and to reduce the pollution of hazardous waste. The effluents procured at the different stages of bleaching were found to be very hazardous. Part of effluent is recycled but a major portion of it is released without any kind of treatment.

From the J.K.Paper industry effluents from all four stages of bleaching has been collected and biological pre treatment was given. This study showed appreciable removal of BOD, COD and Chloride with fungal pretreatment and so can be adopted by process industry which is having such kind of characteristics of the effluent. Among the two fungi tested *Daedaleopsis confragrosa* is found to be more effective and applicable for treatment of pulp and paper mill effluent compared to *Phellinus pectinatus*. Addition of glucose as a co-substrate makes the white rot fungi a more effective bioremediator, indicating this extra carbon source to be very important for fungal treatment (Pandya et al. 2012).

3. Shri A.M.M.Murugappa Chettiar Research Center

Shri A.M.M.Murugappa Chettiar Research Center is a non-Govermental voluntary research organization established in1977 at Tharamnai, Chennai, Tamil Nadu. The research center has been working on devices and technologies for rural application ecofriendly technologies to combat pollution.

They have developed concept of biotereatment with phototrophic bacteria for paper pulp. Alternative cellulosic material sources which is Banana pseudostem, grasses and other agricultural wastes have been identified and an absolutely chemical free technology which not only makes pollution free but also helping to conserve large quantity of water has been developed.

c) Survey of forest area and collection of grass species and fungal fruiting bodies and its identification

Survey of different forest areas: Survey of different forest areas were conducted for the collection of the fungal fruiting bodies and raw materials mainly (grasses) for experiment.

In Gujarat grass lands cover an area of about 8490 Sq. Km. Many of the densely and wildly growing grass species are unpalatable and rich in fiber. The grasslands selected in the present study falls under two major forest divisions Baria and Godhra of Gujarat. The two main grasslands visited are Rampur which comes under the Baria forest division of Dahod district and Bandheli which comes under the Godhra forest division of Panchmahal district.

Pavagadh forest located close to Vadodara and coming under the Panchmahal district was also visited to collect fungal fruiting bodies and grass samples.

Other than these areas The M.S. University Arboretum, Vadodara and surrounding places were visited for the collection. From all these places fungal fruiting bodies and grasses were collected.

Collection of grasses: The commonly growing grasses were selected and collected. Information on the palatability and other economic uses of these grasses

were obtained by consulting the local people, and on the basis of the information 24 grasses were collected which are listed in Table 3. Out of the collected grasses *Desmostachya bipinnata, Sorghum halepense, Cymbopogon martinii, Imperata cylindrica, Heteropogon contortus, Vetiveria zizanioides, Heteropogon triticeus* were identified as the most unpalatable grasses. These grasses are cut and disposed. All other grasses were collected as they are available in huge amount. These species are cut and used efficiently when there is a drought condition. When conditions are good and grass available is in plenty they are cut and simply used for fuel. Grasses are known to have good fiber and so it was thought to be evaluated for its fiber property and to be suggested for paper making.

Sr. No.	**Name of species**	**Tribe**	**Common name**	**Source**
1	*Desmostachya bipinnata* (L.) Stapf	Eragrosteae	Dabh	Bandheli
2	*Sorghum halepense* (L.) Pers.	Andropogoneae	Baru	Rampur
3	*Bracharia reptans* (L.) Gard. & C.E. Hubb.	Paniceae	Chaurela	Rampur – kalitali
4	*Themeda triandra* Forsk.	Andropogoneae	Bhathedu	Bandheli
5	*Cymbopogon martinii* (Roxb.) Wats.	Andropogoneae	Rosha	Bandheli
6	*Imperata cylindrica* (L.) P. Beauv.	Andropogoneae	Varanu	Bandheli
7	*Heteropogon contortus* (L.) P. Beauv.	Andropogoneae	Shukli	Bandheli
8	*Sehima nervosum* (Rottl.) Stapf	Andropogoneae	Shaniyar	Rampur
9	*Saccharum spontaneum* L.	Andropogoneae	Dhub	Express highway Ahmadabad
10	*Chionachne koenigii* (Spr.) Thw.	Maydeae	Kaha	Rampur
11	*Ischaemum molle* Hk. f.	Andropogoneae	Valeru	Rampur – Kalitali
12	*Pennisetum setosum* (Sw.) L. C. Rich.	Paniceae	Nepiyar	Rampur – Kalitali

13	*Ischaemum rugosum* Salisb.	Andropogoneae	Valeru	Rampur – kalitali
14	*Dicanthium annulatum* (Forsk.) Stapf	Andropogoneae	Zinzvo	Bandheli
15	*Dicanthium caricosum* (L.) A. Camus	Andropogoneae	Zinzvo	Bandheli
16	*Phragmites karka* (Retz.) Trin. ex Steud.	Arundineae	Narkul	Gandhinagar
17	*Thelepogon elegans* Roth ex R. &S.	Andropogoneae	Ekal	Rampur
18	*Apluda mutica* L.	Andropogoneae	Karedi	Bandheli
19	*Cenchrus ciliaris* L.	Paniceae	Anjan	Bandheli
20	*Vetiveria zizanioides* (L.) Nash	Andropogoneae	Khus	Gandhinagar
21	*Heteropogon triticeus* (R. Br.) Stapf ex Craib.	Andropogoneae	Katrvo	Pavagudh
22	*Ischaemum pilosum* (Klein ex Wild.) Wt.	Andropogoneae	Kaev	Rampur – Kalitali
23	*Coix lachryma-jobi* L.	Maydeae	Kaha	Rampur
24	*Bothriochloa pertusa* (L.) A.	Andropogoneae	Zinzvi	Rampur, Bandheli

Table 3: Grass species collected its tribe, common name and source

Collection of fungal fruiting bodies:

Table 4 lists the fungi collected along with their place of collection. Fruiting bodies sent for identification at Forest Research Institute (FRI) Dehradun are represented in Fig. 13 and 14 along with the Identification Number (ID. No.). The collected fungal fruiting bodies were critically observed for its morphological characters and compared with the description from CBS Aphyllophorales database and Myco bank data bank.

ID.No.	Name of fungi	Collected from
1	*Flavodon flavus*	M.S.University Arboretum
2	*Daedaleopsis confragosa*	Pavagadh
3	*Coriolopsis telfarii*	Bandheli
4	*Flavodon flavus*	Bandheli
5	*Oxyporus cervinogilvus*	Bandheli
6	*Schizophyllum commune*	M.S.University Arboretum
7	*Ganoderma colosseum*	M.S.University Arboretum
8	*Ganoderma lucidum*	M.S.University Arboretum
9	*Ganoderma lucidum*	M.S.University Arboretum
10	*Ganoderma applanatum*	M.S.University Arboretum
11	*Phellinus pectinatus*	M.S.University Arboretum
12	*Phellinus gilvus*	M.S.University Arboretum
13	*Coriolopsis polyzona*	Pavagadh
14	*Trametes roseola*	Rampur
15	*Polyporus grammocephalus*	M.S.University Arboretum
16	*Pycnoporus sanguineus*	Rampur
17	*Microporus xanthopus*	M.S.University Arboretum
18	*Datronia caperata*	Bandheli
19	*Datronia caperata*	Bandheli
20	*Datronia caperata*	Bandheli
21	*Ceriporia mellea*	Bandheli
22	*Coriolopsis floccose*	Bandheli
23	*Coriolopsis floccose*	Bandheli

Table 4: Fungal fruiting body along with their ID. No. and source

***Flavadon flavus* (Klotzsch) Ryvarden (Fig. 13, ID. No. 1, 4)**

Kingdom : Mycetae
Division : Basidiomycota
Class : Basidiomycetes
Order: Polyporales
Family: Steccherinaceae
Genus: *Flavodon*
Species: *flavus*

Fruiting body is gymnocarpous with sporiferous part of the fruit body laid only on the dorsal layer surface extending up to a large length. The hymenophore is unilateral and confined to the upper side. The fruiting bodies are stipeless attached to the substratum (wood) ventrally. The Hymenophore & its over lying hymenial layer is spiniform The basidiocarps are resupinate to effused reflexed reclining over the surface & attached to the substratum. It is effused to 20 cm. It forms crust like layer adpressed to substratum. Pilear surface is cream –yellowish when fresh & becoming brown on drying Margin is distinctly yellow.

Hymenophore is spiniform with tubular hymenia or tube like tramal outgrowth. Hence these are included with polypores. The pores are hexagonal leading to linear tube. Dissepiments (partitions) are thin and entire. Spiniform out growths are concolorous with the same colour. The tip of these are distinctly darker than the remaining portion.

***Daedaleopsis confragosa* (Bolton) J. Schröter (Fig. 13, ID. No. 2)**

Kingdom : Mycetae
Division : Basidiomycota
Class : *Agaricomycetes*
Order: Polyporales
Family: Polyporaceae
Genus: *Daedaleopsis*
Species: *confragosa*

It is a white rot fungus, growing on decaying hardwood logs. Fruiting body is 5-15 cm; broadly convex to more or less flat; fan-shaped or nearly round in outline; dry; pale grayish to brown or reddish brown; typically with zones of color. Pore surface is white, becoming dingy brownish in age; typically with elongated, maze-like pores and fairly thin walls between the pores, but sometimes with more or less round pores, or even with the pores elongated so much that they appear like gills.

Coriolopsis telfarii (KL). Ryv. **(Fig. 13, ID. No. 3)**

Kingdom : Mycetae
Division : Basidiomycota
Class : *Agaricomycetes*
Order: Polyporales
Family: *Polyporaceae*
Genus: *Coriolopsis*
Species: *telfarii*

It is found on dead wood. Fruiting body is annual to biennial, solitary, but in some cases fused laterally. Fruiting body is elongated lobed, reniform or semicircular, applanate to conchate with concave surface, up to 7 cm wide, 10 cm long, 2-5 mm thick, thin and flexible when dry. Pileus variably covered with forked hairs. Pore surface is wood coloured, pores angular, thin-walled, 1-2 per mm, in older specimens becoming lacerate and dentate, as some pores grow stronger than others

Oxyporus cervino-gilvus **(Jungh.) Ryv. (Fig. 13, ID. No. 5)**

Kingdom : Mycetae
Division : Basidiomycota
Class : Agaricomycetes
Order: Hymenochaetales
Family: Schizoporaceae
Genus: *Oxyporus*
Species: *cervino-gilvus*

It is found on deciduous wood. Fruiting body annual, resupinate to effused reflexed with an elongated pileus, single or imbricate, more rarely orbicular with a tapering base, flexible and tough when dry. Pileus soft and finely hairy and cinnamon to pale brown, margin papery thin, often slightly lobed or incised. Pore surface pale yellow to straw-coloured. Context pale cinnamon, fibrous, about 1 mm thick.

***Schizophyllum commune* Fries (Fig. 13, ID. No. 6)**

Kingdom : Mycetae
Division : Basidiomycota
Class : Agaricomycetes
Order: Agaricales
Family: Schizophyllaceae
Genus: *Schizophyllum*
Species: *commune*

The genus name *Schizophyllum* literally means "split leaves" referring to this character. Fruiting bodies shrivel in dry weather & appear light grey to brown .The marginal proliferation distinguishing feature of the genus is very distinctly noticed in the collected sample.

The fruiting bodies are scattered or clustered on hardwood logs &branches. Fruiting body is gymnocarpous with the sporiferous part of the fruiting body restricted or limited only to the underside of the cap. The hymenophore is confined to the lower side.

The fruiting body is 1-4 cm wide laterally attached to substratum stipeless or irregular to shell shaped. It is attached above the substratum. The upper surface is covered with small white to grayish hairs. Hymenophore is gilled. They are distinct on the under surface, folded & split down the middle. Hence this fungus is also designated as split fungi or split gilled fungi. The split is shallow & resembles a groove.

***Ganoderma colossus* (Fr.) Baker. (Fig. 13, ID. No. 7)**

Kingdom : Mycetae
Division : Basidiomycota
Class : Agaricomycetes
Order: Polyporales
Family: Ganodermataceae
Genus: *Ganoderma*
Species: *colossus*

Fruiting body annual, sessile, semicircular to elongated in shape, soft when fresh, light of weight and context cheesy in consistency when dry. Pileus glabrous, yellow to pale brown, cuticle present, margin of lighter colour than the basal part. Pore surface white to cream when fresh, pores 3-4 per mm, quite thick-walled. Context soft and punky when fresh, cheesy to chalky and easily crumbled with a nail.

***Ganoderma lucidum* (Curtis) P. Karsten (Fig. 13, ID. No. 8, 9)**

Kingdom : Mycetae
Division : Basidiomycota
Class : Agaricomycetes
Order: Polyporales
Family: Ganodermataceae
Genus: *Ganoderma*
Species: *lucidum*

Fruiting body is perennial, Stipate, Stalk lateral or central, reniform in shape. Fruiting body is corky, becomes woody latter on. Upper surface shiny with laccate crust, reddish brown, smooth context brown. Hymenial surface whitish or creamish, turning brown later. Pores are small brown. Hymenial layer made up of hyphae.

***Ganoderma applanatum* (Persoon) Patouillard (Fig. 13, ID. No. 10)**

Division : Basidiomycota
Class : Agaricomycetes Kingdom : Mycetae
Order: Polyporales
Family: Ganodermataceae
Genus: *Ganoderma*

Species: *applanatum*

Fruiting body is perennial, sessile. Fruiting body is corky, applanate, soon becomes hard and woody. Sometimes very large. Upper surface dull brown to blackish zoned, uneven, crusty. Hymenial surface white when fresh, turning light yellow or brown. Hymenial layer made up of hyphae.

***Phellinus pectinatus* (Klotzsch) Quélet (Fig. 14, ID. No. 11)**

Kingdom : Mycetae
Division : Basidiomycota
Class : Agaricomycetes
Order: Hymenochaetales
Family: Hymenochaetaceae
Genus: *Phellinus*
Species: *pectinatus*

It is found on deciduous wood. Fruiting body is perennial, larger, margin entire, woody hard and quite heavy when dry. This species is recognized by its numerous sulcate zones and its duplex consistency with a thin black line below the tomentum. Typically the pore surface is glancing and often receding with an irregular development, especially in more compound fruiting bodies. The pore surface has a distinctly yellowish-brown colour.

***Phellinus gilvus* (Schw.) Pat. (Fig. 14, ID. No. 12)**

Kingdom : Mycetae
Division : Basidiomycota
Class : Agaricomycetes
Order: Hymenochaetales
Family: Hymenochaetaceae
Genus: *Phellinus*
Species: *gilvus*

Basidiocarps perennial, sessile to slightly effused-reflexed; pilei solitary or imbricate, upper surface reddish to yellowish brown, warted or even hispid in parts,

tomentose to glabrous, often rugose, zonate or azonate; margin concolorous; pore surface dark purplish brown, context bright yellowish brown, zonate.

***Coriolopsis polyzona* (Pers.) Ryv. (Fig. 14, ID. No. 13)**

Kingdom : Mycetae
Division : Basidiomycota
Class : *Agaricomycetes*
Order: Polyporales
Family: *Polyporaceae*
Genus: *Coriolopsis*
Species: *polyzona*

It is found on dead angiosperms of all kinds. Fruiting body is annual to perennial, sessile, reniform. Pileus yellowish when fresh, in old specimens frequently with green tints due to algae in the tomentum, tomentose to slightly hispid in numerous sulcate to flat, concentric zones, margin thin, undulating, lobed and incised. Pore surface cream golden-brown, pores angular to round.

***Trametes roseola* Pat. & Har. (Fig. 14, ID. No. 14)**

Kingdom : Mycetae
Division : Basidiomycota
Class : *Agaricomycetes*
Order: Polyporales
Family: *Polyporaceae*
Genus: *Trametes*
Species: *roseola*

It grows on living and dead deciduous trees. Fruiting body annual to perennial, solitary, elongated to semicircular. Consistency soft corky when fresh, drying to tough and corky. Pileus convex, upper surface finely velvety tomentose, with age more glabrous, but not glossy. Colour first white-greyish, later darker buff or more pale dirty

brown, margin obtuse, thick, even to slightly lobed. Pore surface pink to buff, when old more dirty brownish, pores round to slightly angular.

***Polyporus grammocephalus* Berk. (Fig. 14, ID. No. 15)**

Kingdom : Mycetae
Division : Basidiomycota
Class : *Agaricomycetes*
Order: Polyporales
Family: *Polyporaceae*
Genus: *Polyporus*
Species: *grammocephalus*

It is found on deciduous wood. Fruiting body annual, solitary, pileate, and laterally attached with a stipelike contracted base. Pileus glabrous, tan or pale brown with numerous fine radial lines becoming more tufted towards the base which often can be covered with raised tufts of agglutinated hyphae. Margin thin and deflexed in dried specimens. Stipe as such usually absent, but in a few specimens there is a short stipe present, sterile on the lower side between the pores and the mycelial pad by which the fruiting body is attached to the substratum. Pore surface straw coloured, tan to pale brown in old specimens, pores thin walled and angular, sometimes slightly split.

***Pycnoporus sanguineus* (Fr.) Murr. (Fig. 14, ID. No. 16)**

Kingdom : Mycetae
Division : Basidiomycota
Class : *Agaricomycetes*
Order: Polyporales
Family: *Polyporaceae*
Genus: *Pycnoporus*
Species: *sanguineus*

It is found on standing and fallen trunks of almost every kind of deciduous wood, especially common in open and sunny localities. Fruiting body annual, solitary,

narrowly attached to the substrate, but also semistipitate or contracted into a stemlike base. Consistency coriaceous when fresh, quite hard when dry. Pileus with lighter and darker zones, first orange then red, later often intensively red-orange, Young pilei velvety to warted, older ones more smooth and shiny. Margin acute, entire or somewhat incised, often lighter than the rest of the pileus. Stipe rarely present, with the lateral pileus. Pore layer red-orange. Pores circular.

***Microporus xanthopus* (Fr.) Kunt. (Fig. 14, ID. No. 17)**

Kingdom : Mycetae
Division : Basidiomycota
Class : *Agaricomycetes*
Order: Polyporales
Family: *Polyporaceae*
Genus: *Microporus*
Species: *xanthopus*

It is found on dry deciduous wood, often in open habitats like savanna. Fruiting body is annual, solitary or in small groups, centrally or laterally stipitate and usually infundibuliform, sometimes two or more fruiting bodies may grow together to form more complicated fruiting bodies. Pileus up to 10 cm in diameter and 1-3 mm thick, glabrous and shiny when fresh, more dull when dry, yellowish-brown in numerous narrow concentric zones, often with alternating dark and light colours, margin thin and wavy. Stipe round, glabrous, covered with a thin, light yellowish to light brown cuticle, Pore surface cream to pale buff, almost pure white towards the margin, pores entire and very minute. Context pure white, very thin and covered with a distinct cuticle.

***Datronia caperata* (Berk.) Murr. (Fig. 14, ID. No. 18, 19, 20)**

Kingdom : Mycetae
Division : Basidiomycota
Class : *Agaricomycetes*
Order: Polyporales
Family: *Polyporaceae*
Genus: *Datronia*
Species: *caperata*

It is found on angiosperms of all kinds. Fruiting body annual, sessile, applanate, contracted base, Pileus with numerous, narrow concentrically arranged zones and frequently when dry also with radial striae or streaks, Pore surface cinnamon to deep chocolate-brown, pores variable, medium to small, round to angular.

***Ceriporia mellea* (Berk. & Br.) Ryv. (Fig. 14, ID. No. 21)**

Kingdom : Mycetae
Division : Basidiomycota
Class : Agaricomycetes
Order: Polyporales
Family: Meruliaceae
Genus: *Ceriporia*
Species: *mellea*

It is found on dead wood. Fruiting body is annual, resupinate, usually of small dimension in small patches or more effused, rarely above 10 cm long and wide and 1.5 mm thick, consistency soft when fresh, brittle when dry. Margin sterile. Pore surface cream-yellow to even greenish, dull pores irregular round to angular.

***Coriolopsis floccosa* (Jungh.) Ryv. (Fig. 14, ID. No. 22, 23)**

Kingdom : Mycetae
Division : Basidiomycota
Class : Agaricomycetes
Order: Polyporales
Family: Polyporaceae
Genus: *Coriolopsis*
Species: *floccosa*

Basidiocarp annual, pileate, sessile to a stipelike base, single or laterally fused, up to 4 cm wide and 11 cm long in fused fruiting bodies, up to 1 cm thick at the base, margin entire, lobed or dentate, undulating and sharp. Pileus deep hazel brown frequently grayish brown, tomentose, distinctly to indistinctly zonate. Pore surface grayish brown a distinct blue ashy grey tint, pores round to angular, entire. Context tobacco brown, shiny fibrous and floccose.

2) Isolation of fungal isolates

All the 23 fruiting bodies were inoculated for isolation of fungal culture, but only 7 pure cultures could be obtained successfully. The seven successfully isolated culture were of *Flavodon flavus, Schizophyllum commune, Daedaleopsis confragosa, Ganoderma lucidum, Ganoderma applanatum, Ganoderma colossum and Phellinus pectinatus.* All the cultures were maintained on the PDA medium. Figure 15 shows the fungal fruiting body and cultures isolated from them.

Figure 13: Collected and identified fungal fruiting bodies with their respective ID Number

1: *Flavodon flavus*

2: *Daedaleopsis confragosa*

3: *Coriolopsis telfarii*

4: *Flavodon flavus*

5: *Oxyporus cervinogilvus*

6: *Schizophyllum commune*

7: *Ganoderma colossum*

8: *Ganoderma lucidum*

9: *Gonoderma lucidum*

10: *Ganoderma applanatum*

Figure 13

Figure 14: Collected and identified fungal fruiting bodies with their respective ID Number

11: *Phellinus pectinatus*

12: *Phellinus gilvus*

13: *Coriolopsis polyzona*

14: *Trametes roseola*

15: *Polyporus grammocephalus*

16: *Pycnoporus sanguineus*

17: *Microporus xanthopus*

18: *Datronia caperata*

19: *Datronia caperata*

20: *Datronia caperata*

21: *Ceriporia mellea*

22: *Coriolopsis floccose*

23: *Coriolopsis floccose*

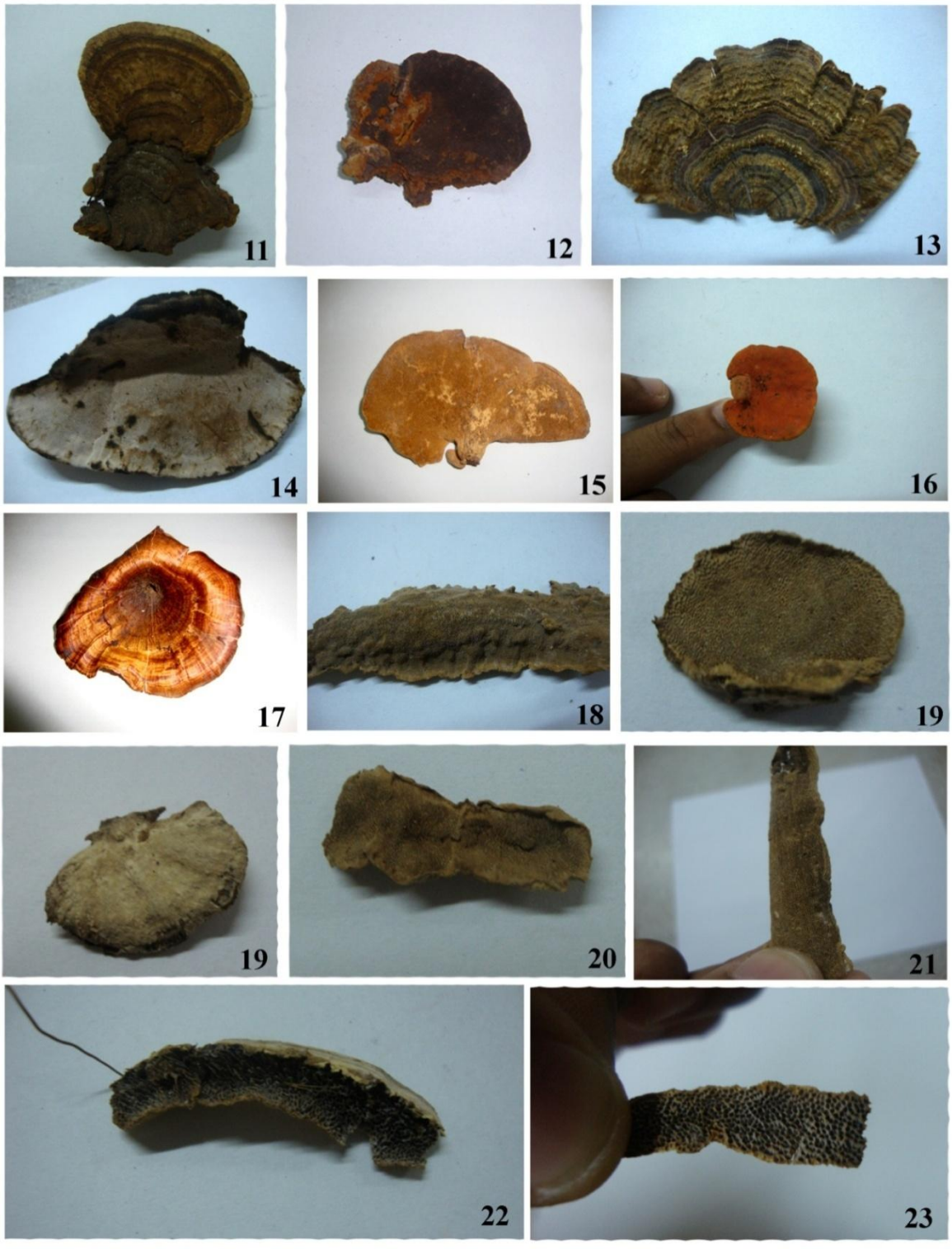


Figure 14

Figure 15: Fruiting body and respective isolated cultures of the fungi

A: Fruiting body of *Daedaleopsis confragosa*

B: Isolated culture of *Daedaleopsis confragosa*

C: Fruiting body of *Ganoderma lucidum*

D: Purified culture of *Ganoderma lucidum*

E: Fruiting body of *Flavodon flavus*

F: Isolated culture of *Flavodon flavus*

G: Fruiting body of *Schizophyllum commune*

H: Isolated culture of *Schizophyllum commune*

I: Fruiting body of *Phellinus pectinatus*

J: Purified culture of *Phellinus pectinatus*

Figure 15

3) Screening

a) Screening of fungal isolates for cellulolytic and ligninolytic enzymes by plate method

In identifying an unknown fungus one of the first questions is whether this fungus belongs to the white rot or the brown rot. This is usually determined by the well known Bavendamn test. Bavendamn (1928) was the first who differentiated between the white rot and brown rot fungi on the basis of their oxidative enzymes.

D. confragosa in Fig. 16 B showed the absence of cellulolytic enzyme as the red colored zone was not formed in the plate with CMC. It showed brown colored zone at the bottom of the petri plate substituted with the tannic acid (Fig. 16 D). Same result was observed in the screening with *P. pectinatus* (Fig. 16 E to H).

Table 5 shows the enzyme activity of different fungi. *F. flavus* and *S. commune* showed positive ligninolytic and cellulolytic enzyme activity. All other fungi (*D. confragosa, G. lucidum, G. applanatum, G. colossum* and *P. pectinatus*) showed positive ligninolytic enzyme activity and negative cellulolytic activity.

Sr. No.	Name of fungi	Enzymatic activity
1	*Flavodon flavus*	Ligninolytic –cellulolytic
2	*Schizophyllum commune*	Ligninolytic –cellulolytic
3	*Daedaleopsis confragosa*	Ligninolytic
4	*Ganoderma lucidum*	Ligninolytic
5	*Ganoderma applanatum*	Ligninolytic
6	*Ganoderma colosseum*	Ligninolytic
7	*Phellinus pectinatus*	Ligninolytic

Table 5: Enzyme activity of different fungi

Figure 16: Enzyme activity of different fungal species

A: Control (Only MEA medium) of *D. confragosa*

B: Plate with MEA + CMC showed no red colored zone at the back side of petridish with *D. confragosa*.

C: Control (Only MEA medium) of (*D. confragosa)* without any colored zone.

D: Plate with MEA + Tannic Acid with (*D. confragosa)* brown colored zone at the bottom of petridish .

E: Control (Only MEA medium) of (*P. pectinatus*) without any colored zone.

F: Plate inoculated with (*P. pectinatus*) showing absence of colored zone in the presence of CMC. G: Plate showing absence of colored zone in control (Only MEA medium) of *P. pectinatus*

H: Plate showing brown colored zone with addition of tannic acid by *P. pectinatus*.

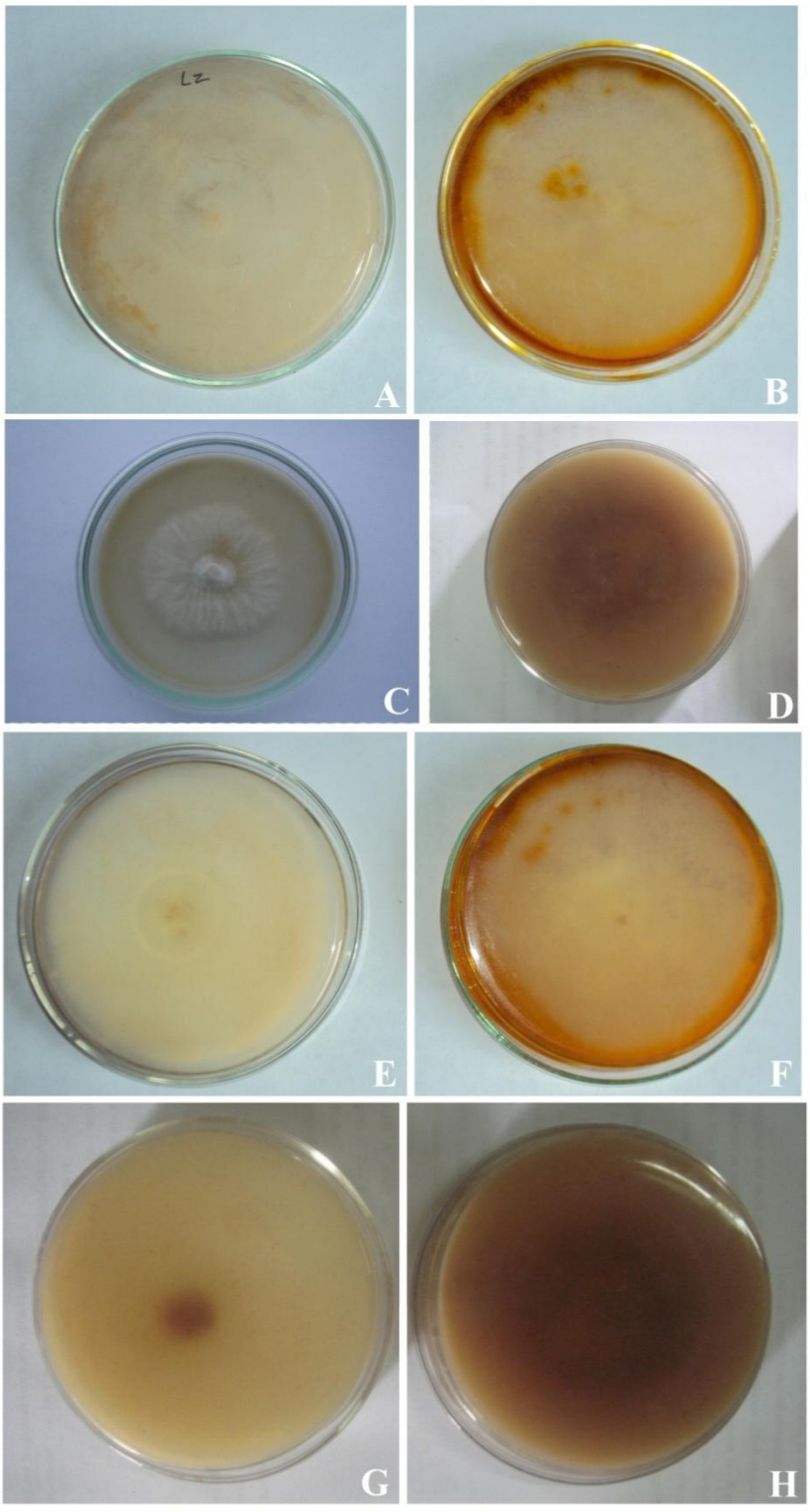


Figure 16

b) Screening of fungi for their ability to degrade cellulose and lignin

This experiment was conducted to identify the best suitable form of the raw material for selected fungi to degrade cellulose and lignin. Both the selected fungi were experimented for their ability to degrade cellulose and lignin in different forms of raw material. All the selected wood species were taken in form of wood blocks, wood shavings and saw dust. All the selected grasses species were inoculated with both of the fungus and incubated for 3, 5 and 7 weeks. Banana pseudo stem and its pulp (obtain from the J. K. Paper industry) were experimented in different combinations as explained in materials and methods with *D. confragosa.*

i) Different forms of wood (wood blocks, wood chips and saw dust)

Figure 17 represents the different forms of the raw material used for the experiment. Figure 17 A shows the method by which 3 wooden blocks have been placed on the Malt Extract Agar medium and incubated as control (without fungal inoculum). Figure 17 B shows the wooden blocks inoculated with fungi after the incubation period, completely covered by the fungal mycelium.

The other forms of the raw material i.e. wood in the form of wood chips (Fig. 17 C) and saw dust (Fig. 17 E) used as control and treated (Figs. 17 D and E respectively) are also shown. Grass culm used for the experiment, both control and fungal pretreated were inoculated in poly-propylene bags of 6 X 8 inch size (Figs. 17 G, H).

The raw materials after desired incubation period were analyzed for weight loss, lignin loss and cellulose loss results of which are given in Table 6, 7 and 8.

Figure 17: Different forms of substrate (control and treated)

A: Wood blocks incubated as control (without any fungus)

B: Wood blocks inoculated with the fungus and completely covered with mycelium

C: Wood shavings inoculated as control

D: Wood shavings inoculated with the fungus covered with mycelium

E: Saw dust inoculated as control

F: Saw dust inoculated with the fungus showing growth of the fungus

G: Grass culm inoculated as control

H: Grass culm inoculated with the fungus showed mycelium grown on culm (After 3 weeks of incubation)

Figure 17

Weight loss experiment: The results of weight loss experiments are presented in Table 6. *M. indica* inoculated with *D. confragosa* showed highest weight loss in wood shavings. But in all other wood species saw dust showed highest weight loss, moderate in wood shavings and the least in wood blocks. Only in *B. ceiba* wood with *D. confragosa* showed higher weight loss after 20 days of incubation period.

Wood of *M. indica* showed the maximum weight loss in the form of wood shavings which is significantly high in comparison to wood blocks infected with *P. pectinatus*. *S. cuminii* degraded by *P. pectinatus* showed highest weight loss in saw dust condition. *B. ceiba* wood shaving and saw dust infected with *P. pectinatus* showed almost the same amount of degradation.

Name of Wood species	Period of incubi-tion	*Daedaleopsis confragosa*			*Phellinus pectinatus*		
		Wood block	Wood shavings	Saw dust	Wood block	Wood shavings	Saw dust
Mangifera indica	10 days	4.29	9.5	8	2.88	9.5	8.5
	20 days	5.27	11	10	3.24	12	10.5
Syzygium cuminii	10 days	1.43	9.5	11	2.45	7.5	11
	20 days	2.78	12.5	15	3.05	9.5	12
Bombax cieba	10 days	1.83	5.5	10	5.98	9.5	10
	20 days	11.35	9.5	13	8.68	12	12
Eucalyptus globules	10 days	3.08	11.5	11	2.07	7.5	11
	20 days	7.09	13	14	3.17	10	13
Pithecellobium dulce	10 days	2.76	10	10.5	3.00	10	11.5
	20 days	4.32	12.5	13	4.20	11	12.5

Table 6: Percentage weight loss of wood blocks, shavings and saw dust infected by *Daedaleopsis confragosa* and *Phellinus pectinatus*

In *E. globulus* wood infected with *P. pectinatus*, saw dust showed the highest amount of degradation, wood shavings showed moderate and wood blocks

showed the least amount of degradation. Same was the case observed with *P. dulce* infected with *P. pectinatus*.

Lignin analysis: Table 7 represents the results of percentage lignin loss. *M. indica* wood in the form of saw dust showed maximum lignin loss when treated with *D. confragosa* while in *P. pectinatus* pretreatment wood shavings showed greater lignin loss.

Name of Wood species	Period of incubi-tion	*Daedaleopsis confragosa*			*Phellinus pectinatus*		
		Wood block	Wood shavings	Saw dust	Wood block	Wood shavings	Saw dust
Mangifera indica	10 days	4	5	10	3	10	10
	20 days	9	10	15	6	14	12
Syzygium cuminii	10 days	8.33	8.33	8.33	5	8.33	8.33
	20 days	16.67	16.67	16.67	8.33	25	16.67
Bombax cieba	10 days	8.33	8.33	8.33	8.33	8.33	8.33
	20 days	16.67	9	8.33	16.67	19.17	16.67
Eucalyptus globulus	10 days	10	5	3	4	10	10
	20 days	20	12	10	10	12	20
Pithecellobium dulce	10 days	8.33	8.33	8.33	8.33	8.33	8.33
	20 days	16.67	8.33	16.67	9.67	8.33	8.33

Table 7: Percentage lignin loss of wood blocks, shavings and saw dust infected by *Daedaleopsis confragosa* and *Phellinus pectinatus*

In *S. cuminii* wood, all the three forms (shavings, blocks, saw dust) showed same amount of lignin loss in 20 days incubation period when pretreated with *D. confragosa* while when pretreated with *P. pectinatus* the loss was more in wood shavings.

B. ceiba and *E. globulus* showed more or less same results when treated with *D. confragosa.* Maximum loss of lignin was found when the wood was pretreated in the form of wood blocks. But with *P. pectinatus* wood shavings and saw dust form of *B. ceiba* and *E. globulus* wood respectively showed an effective loss of lignin. *D.*

confragosa showed maximum amount of lignin loss (16.67 %) in *P. dulce.* Wood blocks and saw dust showed maximum lignin loss when treated with *D. confragosa* while *P. pectinatus* wood blocks showed the maximum loss of lignin. Overall the maximum loss of lignin obtained in 20 days incubation period with both fungi was found to be 16.67 %. Maximum loss of lignin was found in wood shaving treated with *P. pectinatus* (19.17 %)

Cellulose Analysis: Table 8 represents the percentage cellulose loss in wood blocks, shavings and saw dust infected by *Daedaleopsis confragosa* and *Phellinus pectinatus.*

Name of Wood species	Period of incubi-tion	*Daedaleopsis confragosa*			*Phellinus pectinatus*		
		Wood block	Wood shavings	Saw dust	Wood block	Wood shavings	Saw dust
Mangifera indica	10 days	0	11.11	11.11	0	3.33	8.89
	20 days	4.44	11.11	22.22	11.11	11.11	7.78
Syzygium cuminii	10 days	10	0	10	10	6	10
	20 days	20	10	20	16	10	20
Bombax cieba	10 days	9.09	0	0	9.09	18.18	13.64
	20 days	27.27	18.18	9.09	15.45	27.27	25.45
Eucalyptus globulus	10 days	10	10	10	10	9	7
	20 days	20	20	20	20	16	15
Pithecellobium dulce	10 days	9.09	18.18	18.18	9.09	0	0
	20 days	12.73	27.28	27.28	11.81	14.54	9.09

Table 8: Percentage cellulose loss of wood blocks, shavings and saw dust infected by *Daedaleopsis confragosa* and *Phellinus pectinatus*

M. indica wood in wood block forms showed minimum cellulose loss when pretreated with both the fungus. *S. cuminii* wood in the form of wood blocks and saw dust showed good and same amount of cellulose loss which is greater than wood shavings when pretreated with *D. confragosa*, while when pretreated with *P. pectinatus* cellulose loss is lowest in the form of wood shavings.

B. ceiba wood in form of blocks showed highest cellulose loss (27.27 %) and saw dust showed the lowest (9.09 %) loss of cellulose when treated with *D. confragosa* while when pretreated with *P. pectinatus* wood blocks showed lowest cellulose loss (15.45 %), wood shavings and saw dust showed approximately same cellulose loss (27.27 % and 25.45 % respectively).

In the wood of *E. globulus* all the forms (wood blocks, wood shavings, saw dust) showed equal amount of the cellulose loss when pretreated with *D. confragosa* while when pretreated with *P. pectinatus* loss was more in wood block forms (20 %).

P. dulce wood in the form of wood blocks showed lowest cellulose loss (12.73%) when treated with *D. confragosa* for 20 days while saw dust form of wood pretreated *with P. pectinatus* showed lowest cellulose loss (9.09 %).

ii) Grass culm degradation experiment

All the 24 collected grasses were inoculated with both the selected fungi to observe the ability of the fungus to degrade the lignin and cellulose. Figure 17 G shows the control experiment of the grass culm and figure 17 H shows the experimented inoculated grass culm. The results of weight loss, lignin loss and cellulose loss is given in the Table 9, 10 and 11 respectively.

Weight loss experiment in grasses:

All the 24 collected grass samples were subjected to weight loss experiment table 9 represents the results of the experiments. All the grasses infected with the fungi *D. confragosa* showed a significant and gradual increase in the weight loss with an increase in the incubation period.

Desmostachya bipinnata, Themeda triandra, Saccharum spontaneum, Apluda mutica, Heteropogon triticeus and *Coix lachrymal-jobi* showed the highest weight loss with the *D. confragosa*. But all other grasses also showed significant weight loss (20-30 %).

With the white rot fungus *P. pectinatus* a gradual increase in the weight loss with an increase in the incubation period was noted. Here few grasses showed less weight loss and a few showed very high weight loss.

C. ciliaris, S. halepense, B. reptans, D. annulatum, D. caricosum, T. elegans, V. zizanioides, H. triticeus, I. pilosum, and *B. pertusa* showed very high weight loss, while *D. bipinnata, T. triandra, C. martinii, H. contortus,* and *S. spontaneum* showed lesser weight loss.

No.	Grass species	*Daedaleopsis confragosa*			*Phellinus pectinatus*		
		Percentage wt. loss after 3 wk.	Percentage wt. loss after 5 wk.	Percentage wt. loss after 7wk	Percentage wt. loss after 3 wk.	Percentage wt. loss after 5 wk.	Percentage wt. loss after 7wk
1	*Desmostachya bipinnata* (L.) Stapf	21.2	24.4	31.2	9.6	10	12
2	*Sorghum halepense* (L.) Pers.	14	18.6	25	19.8	30.8	34
3	*Bracharia reptans* (L.) Gard. & C.E. Hubb.	24	26.2	29.2	13	26.8	32.8
4	*Themeda triandra* Forsk.	16	26.2	37	7.6	8.6	11.2
5	*Cymbopogon martinii* (Roxb.) Wats.	19.2	21	25	9.2	15	13.4
6	*Imperata cylindrica* (L.) P. Beauv.	23.8	25	26.6	11	14.4	20
7	*Heteropogon contortus* (L.) P. Beauv.	25.4	26	28	7.2	9.2	11.8
8	*Sehima nervosum* (Rottl.) Stapf	22.8	24	26.8	14	14.2	18
9	*Saccharum spontaneum* L.	24	24.8	32.8	8.4	8.8	12.4
10	*Chionachne koenigii* (Spr.) Thw.	19.6	22	23.8	9	10.6	14.8
11	*Ischaemum molle* Hk. f.	24.2	25.2	28.2	11.8	13.2	16
12	*Pennisetum setosum* (Sw.) L. C. Rich.	22.6	25.2	32.2	15.4	17.6	24.4
13	*Ischaemum rugosum* Salisb.	18.2	22	25.2	16.6	28.8	31.2
14	*Dicanthium annulatum* (Forsk.) Stapf	19.2	25	26.4	14.6	30	42.4
15	*Dicanthium caricosum* (L.) A. Camus	23.8	24.8	27.8	14.2	28.6	36.8
16	*Phragmites karka* (Retz.) Trin. ex Steud.	22	24.8	27.8	21.2	27.6	31
17	*Thelepogon elegans* Roth ex R. &S.	19.8	23	25.2	12.8	35.8	42.8
18	*Apluda mutica* L.	18.8	28.8	34.6	8.4	10.4	31
19	*Cenchrus ciliaris* L.	20.6	24	28.6	15.6	18.2	37
20	*Vetiveria zizanioides* (L.) Nash	17.2	17	26.4	13.8	26.6	35.6
21	*Heteropogon triticeus* (R. Br.) Stapf ex Craib.	21.8	28.2	31.6	27.2	30.6	32.6
22	*Ischaemum pilosum* (Klein ex Wild.) Wt.	14.6	19	21.4	26	28.8	33.2
23	*Coix lachryma-jobi* L.	16.2	28.4	37.4	21	26.2	27.6
24	*Bothriochloa pertusa* (L.) A.	19.2	20.2	25	7.2	19.4	42.6

Table 9: Percentage weight loss of grass samples infected with *Daedaleopsis confragosa* and *Phellinus pectinatus*

Percentage Lignin loss:

Analysis of lignin was done using the standard method (Kalsons lignin). Percentage lignin loss in different grasses is given in the table 10. Grasses infected with both the fungi showed significant amount of lignin loss.

All the grasses infected with *D. confragosa* showed significant amount of lignin loss (greater than 20 %). Except *C. lachrymal – jobi* (11.11 %) and *I. rugosum* (20 %) all other grasses showed higher lignin degradation.

With *P. pectinatus,* only *D. bipinnata, S. nervosum* and *H. triticeus* showed very less lignin loss in comparison to all other grasses.

Percentage Cellulose loss:

Analysis of cellulose was performed using the standard method (anthrone method). The results obtained are presented in table 11.

All the grasses infected with both the fungi showed gradual cellulose loss. Minimum amount of cellulose loss was observed after 3 weeks of incubation which goes on increasing till 7 weeks.

Within a incubation bperiod of 7 weeks the least amount of cellulose degradation with the fungus *D. confragosa* was observed in *B. pertusa, V. zizanioides, C. ciliaris, A. mutica, C. koenigii,* and *I. cylindrical* (less than 20 %). While with the fungus *P. pectinatus* lesser amount of cellulose loss was noticed in *C. ciliaris, A. mutica, D. bipinnata, T. triandra, B. pertusa, V. zizanioides* and *T. elegans* (less than 25 %).

No.	Grass species	*Daedaleopsis confragosa*			*Phellinus pectinatus*		
		Percentage lignin loss after 3 wk.	Percentage lignin loss after 5 wk.	Percentage lignin loss after 7 wk.	Percentage lignin loss after 3 wk.	Percentage lignin loss after 5 wk.	Percentage lignin loss after 7 wk.
1	*Desmostachya bipinnata* (L.) Stapf	9	18	27	10	12	20
2	*Sorghum halepense* (L.) Pers.	13.64	26.36	36.36	18.18	27.27	27.27
3	*Bracharia reptans* (L.) Gard. & C.E. Hubb.	10	20	30	10	20	30
4	*Themeda triandra* Forsk.	9.09	18.18	36.36	27.27	36.36	45.45
5	*Cymbopogon martinii* (Roxb.) Wats.	13	23	40	20	24	30
6	*Imperata cylindrica* (L.) P. Beauv.	39.03	48.78	60.97	23.07	30.76	38.46
7	*Heteropogon contortus* (L.) P. Beauv.	9.09	16.36	27.27	9.09	18.18	36.36
8	*Sehima nervosum* (Rottl.) Stapf	12	20	32	5	10	14
9	*Saccharum spontaneum* L.	10	15	30	10	18	26
10	*Chionachne koenigii* (Spr.) Thw.	16.67	33.33	41.67	8.33	33.33	41.67
11	*Ischaemum molle* Hk. f.	18.18	23.64	40.91	9.09	27.27	36.36
12	*Pennisetum setosum* (Sw.) L. C. Rich.	11.11	15.55	33.33	11.11	22.22	33.33
13	*Ischaemum rugosum* Salisb.	7.78	11.11	20	11.11	22.22	26.67
14	*Dicanthium annulatum* (Forsk.) Stapf	13.33	22.22	30	3.33	11.11	22.22
15	*Dicanthium caricosum* (L.) A. Camus	11.11	20	28.89	11.11	22.22	24.44
16	*Phragmites karka* (Retz.) Trin. ex Steud.	10	20	27	20	20	30
17	*Thelepogn elegans* Roth ex R. &S.	11.11	15.55	33.33	4.44	11.11	22.22
18	*Apluda mutica* L.	10	20	30	10	20	30
19	*Cenchrus ciliaris* L.	9.09	12.73	27.27	18.18	27.27	36.36
20	*Vetivaria zizanioides* (L.) Nash	9.09	17.27	25.45	22.72	27.27	36.36
21	*Heteropogon triticeus* (R. Br.) Stapf ex Craib.	10	16	24	2	10	19
22	*Ischaemum pilosum* (Klein ex Wild.) Wt.	10	20	30	10	20	40
23	*Coix lachryma-jobi* L.	2.22	6.67	11.11	11.11	22.22	33.33
24	*Bothriochloa pertusa* (L.) A.	10	20	24	10	20	25

Table 10: Percentage lignin loss of grass samples infected with *Daedaleopsis confragosa* and *Phellinus pectinatus*

No.	Grass species	*Daedaleopsis confragosa*			*Phellinus pectinatus*		
		Percentage cellulose loss after 3 wk.	Percentage cellulose loss after 5 wk.	Percentage cellulose loss after 7 wk.	Percentage cellulose loss after 3 wk.	Percentage cellulose loss after 5 wk.	Percentage cellulose loss after 7 wk.
1	*Desmostachya bipinnata* (L.) Stapf	11.54	23.07	26.92	7.69	15.38	20.83
2	*Sorghum halepense* (L.) Pers.	17.39	26.08	30.43	8.69	26.08	30.43
3	*Bracharia reptans* (L.) Gard. & C.E. Hubb.	16.67	25	33.33	12.5	16.67	37.5
4	*Themeda triandra* Forsk.	13.63	27.27	36.36	9.09	13.64	22.73
5	*Cymbopogon martinii* (Roxb.) Wats.	12	20	24	12	20	28
6	*Imperata cylindrica* (L.) P. Beauv.	8.69	13.04	17.39	9.09	22.73	31.82
7	*Heteropogon contortus* (L.) P. Beauv.	16.67	16.67	20.83	8.33	12.5	25
8	*Sehima nervosum* (Rottl.) Stapf	13.33	16.67	23.33	16.67	30	36.67
9	*Saccharum spontaneum* L.	14.81	22.22	29.63	11.11	25.93	33.33
10	*Chionachne koenigii* (Spr.) Thw.	10	13.33	16.67	13.33	23.33	33.33
11	*Ischaemum molle* Hk. f.	11.54	23.07	26.92	11.53	23.07	34.62
12	*Pennisetum setosum* (Sw.) L. C. Rich.	13.79	20.68	31.03	13.79	22.41	31.03
13	*Ischaemum rugosum* Salisb.	13.04	17.39	26.08	8.69	17.39	30.43
14	*Dicanthium annulatum* (Forsk.) Stapf	12.5	20.83	37.5	16.67	25	25
15	*Dicanthium caricosum* (L.) A. Camus	10	15	20	20	25	25
16	*Phragmites karka* (Retz.) Trin. ex Steud.	12.5	20.83	25	16.67	29.17	33.33
17	*Thelepogn elegans* Roth ex R. &S.	4.54	18.18	31.81	4.54	18.18	22.73
18	*Apluda mutica* L.	4.76	14.28	14.28	4.76	9.52	14.28
19	*Cenchrus ciliaris* L.	4.76	19.04	19.04	4.76	9.52	19.05
20	*Vetivaria zizanioides* (L.) Nash	9.09	13.63	18.18	9.09	13.64	22.73
21	*Heteropogon triticeus* (R. Br.) Stapf ex Craib.	16.67	25	37.5	20.83	33.33	41.67
22	*Ischaemum pilosum* (Klein ex Wild.) Wt.	12.5	25	33.33	8.33	16.67	29.17
23	*Coix lachryma-jobi* L.	12	20	36	12	24	32
24	*Bothriochloa pertusa* (L.) A.	4.54	9.09	13.63	9.09	18.18	22.73

Table 11: Percentage cellulose loss of grass samples infected with *Daedaleopsis confragosa* and *Phellinus pectinatus*

iii) Banana pseudostem experiment

Banana pseudostem was inoculated with the *D. confragosa* and incubated for 25 days after that they were removed and analyzed for its percentage weight loss. Percentage lignin loss and percentage cellulose loss are shown in the table 12 and 13.

Banana pseudostem	Percentage Weight loss	Percentage Lignin loss	Percentage cellulose loss
MEA + not soaked	30.2±1.2	13.33±0.5	19.23±0.9
MEA + soaked	34.2±1.3	23.33±0.7	29.17±1.2
Without MEA + not soaked	7.2±0.9	9.99±0.7	3.33±0.56
Without MEA + soaked	24.2±0.78	13.33±1.2	6.89±0.7

Table 12: Percentage weight, lignin and cellulose loss of banana psudostem infected with *D. confragosa*

Banana pseudostem pulp	Percentage Weight loss	Percentage Lignin loss	Percentage cellulose loss
MEA + not soaked	13±0.9	5.26±0.65	14.29±0.98
MEA + soaked	15.2±0.95	10.53±0.74	11.11±0.22
Without MEA + not soaked	5.2±0.7	5.26±0.55	2.56±0.6
Without MEA + soaked	20.6±0.6	5.26±0.83	5.26±0.56

Table 13: Percentage weight, lignin and cellulose loss of banana pseudostem pulp infected with *D. Confragosa*

Banana pseudostem showed highest weight loss in the presence of MEA medium and soaked condition (30.2 %). And least amount of weight loss is observed without MEA medium and in un-soaked condition (7.2 %). Lignin and cellulose loss was also observed highest in the MEA medium with the soaked condition and lowest lignin and cellulose loss is observed without MEA medium and in un-soaked condition.

Banana pseudostem pulp obtained from the J. K. Paper Industry showed higher weight loss without MEA medium in the soaked condition (20.6 %). But the percentage

lignin loss was higher with the MEA medium and soaked condition (10.53%). Cellulose loss was found to be highest with the MEA medium and un-soaked condition (14.29 %).

4) Fiber properties

The fiber dimensions and their derived indices of wood species and grass species derived were compared with other hardwoods and pruning and is presented in Table 14, 15 and Graph 18, 19, 20 and 21. Fiber length in studied grass species were found to be more compared to the wood species (Fig. 18).

Fiber length among the woods is highest in *E. globulus* (1.74 mm). In *S. cuminii* it is 1.63 mm which is very near to the traditionally used raw material. In *B. ceiba* (1.19 mm) it is moderate. Lowest fiber length among wood is found in *M. indica* (0.945 mm) and *P. dulce* (0.868 mm). Among the grasses *P. karka* (2.85 mm), *V. zizanioides*(2.64 mm), *S. spontaneum*(2.08 mm), *I. cylindrica* (2.0 mm), *T. triandra* (1.9 mm) and *S. halepense*(1.765 mm) are having considerably long fibers.

Fiber dimension	Length Mm	Diameter µm	Lumen diameter µm	Cell wall thickness µm	Literature
Arundo donax (giant reed)	1.22± 0.07	17.3±2.4	8.5±2.4	4.4±0.8	Ververis *et al*, (2004)
Panicum virgatum (switch grass)	1.15±0.10	13.1±2.8	5.8±3.9	4.6±0.9	Ververis *et al*, (2004)
Wheat straw *(T. durum L)*	0.74	13.2	4.0	4.6	Deniz *et al*. (2004)
Corn stalk	1.32	24.3	10.7	6.8	Usta *et al*. (1990)
Tobacco straw	1.07	26.8	16..3	5.3	Erolu *et al.* (1992)
Cotton stalk	1.32	29.3	23.0	3.6	Erolu *et al.*(1992)
Rye straw	1.15	14.7	4.2	1.1	Usta and Eroglu (1987)
Hibiscus cannabinus (kenaf)	1.29	22.1	12.7	4.3	Ververis *et al*, (2004)
Eucalyptus grandis	1.06	19.21	12.20	3.20	Dutt and Tyagi. (2011)
Coniferous	2.7-4.6	32-43	-	-	Atchison. (1993)
Deciduous	0.7-1.6	20-40	-	-	Atchison. (1987)
Desmostachya bipinnata	1.468	13.93	8.96	2.49	Determination
Vetiveria zizanioides	2.64	15.28	7.7	3.79	Determination
Themeda triandra	1.9	19.9	8.05	5.93	Determination
Saccharum spontaneum	2.08	18.9	10.23	4.34	Determination
Cymbopogon martini	0.775	20.95	12.1	4.43	Determination
Phragmites karka	2.85	16.6	9.3	3.65	Determination
Imperata cylindrica	2	17.5	8.7	4.4	Determination
Sorghum halepense	1.765	19.53	6.6	6.456	Determination
Dryobalanops spp. (kapur wood)	1.55	27.7	14.6	6.55	Determination
Mangifera indica	0.945	14.13	6.15	3.99	Determination
Syzygium cuminii	1.631	20.34	7.54	6.4	Determination
Bombax ceiba	1.196	22.5	12.5	5.0	Determination
Eucalyptus globulus	1.745	10.63	5.7	2.46	Determination
Pithecellobium dulce	0.868	17.4	6.75	5.32	Determination

Table 14: Fiber dimensions of the selected raw materials

Species	Slenderness ratio	Flexibility coefficient	Runkel ratio	Rigidity coefficient	Literature
Arundo donax (giant reed)	70.5	49.2	0.91	23.90	Ververis *et al*, (2004)
Panicum virgatum (switch grass)	87.7	44.2	1.5	35.12	Ververis *et al*,(2004)
Wheat straw *(T. durum L)*	53.13	33.04	2.23	34.02	Tutus *et al*, (2010)
Corn stalk	54.32	44.03	1.27	27.98	Tutus *et al*, (2010)
Tobacco straw	39.92	28.60	2.57	36.10	Tutus *et al*, (2010)
Cotton stalk	32.60	63.30	0.57	18.10	Tutus *et al*, (2010)
Rye straw	37.90	60.80	0.65	19.80	Tutus *et al*, (2010)
Hibiscus cannabinus (kenaf)	58.3	57.5	0.67	19.45	Ververis *et al*,(2004)
Eucalyptus grandis	55.18	63.5	0.52	16.65	Tutus *et al*, (2010)
Desmostachya bipinnata	105.38	64.32	0.56	17.85	Determination
Vetiveria zizanioides	172.77	50.39	0.98	24.8	Determination
Themeda triandra	95.48	40.45	1.47	29.79	Determination
Saccharum spontaneum	148.15	54.17	0.85	22.96	Determination
Cymbopogon martini	36.99	57.75	0.73	21.15	Determination
Phragmites karka	171.69	56.02	0.78	21.99	Determination
Imperata cylindrical	114.29	49.71	1.01	25.14	Determination
Sorghum halepense	90.37	33.79	1.896	33.05	Determination
Dryobalanops spp. (kapur wood)	55.91	52.96	0.89	23.64	Determination
Mangifera indica	66.87	43.52	1.29	28.24	Determination
Syzygium cuminii	85.82	37.06	1.69	31.46	Determination
Bombax ceiba	72.49	55.56	0.8	22.22	Determination
Eucalyptus globulus	81.74	53.62	0.86	23.14	Determination
Pithecellobium dulce	68.72	38.79	1.58	30.57	Determination

Table 15: Derived value (indices) of the selected raw materials

In *B. ceiba* lumen diameter is 12.5 µm which is more in comparison to cell wall thickness 4.85 µm. Also in *E. globules* lumen diameter is more than cell wall thickness, but in all other wood cell wall thickness is much higher. Cell lumen in *P. karka* is 9.3 µm and in *S. spontaneum* it is 10.23 µm which is greater than cell wall thickness which is 7.3 µm and 8.68 µm respectively. In *V. zizanioides, I. cylindrical, S. halepense* lumen and cell wall thickness is approximately same (Fig. 19).

Among the wood species slenderness ratio of *S. cuminii* (85.82), *B. ceiba* (72.49) and *E globules* (81.74) is good and in *M. indica* (66.87) and *P. dulce* (68.72) is poor. In *P. karka* (171.69), *V. zizanioides* (172.77), *S. spontaneum* (148.15), *I. cylindrica* (114.29) and *S. halepense* (90.37) have very good slenderness ratio among the grasses while slenderness ratio of *C. martini* (36.99) is very low (Fig. 20).

Flexibility coefficient in *B. ceiba* (55.56) and *E. globulus* (53.62) in wood and *V. zizanioides* (50.39), *S. spontaneum* (54.17), *C. martini* (57.75), *P. karka* (56.02) in grasses is in the range of 50-70. But in all other wood and grasses it is lower than this.

Runkel ratio was lower than 1 in three wood species *B. ceiba* (0.8), *Dryobalanops* (0.88) and *E. globules* (0.86) while in studied grass species except *T. triandra* (1.47) and *I. cylindrica* (1.01) all the other grass species had a runkel ratio lower than 1.

Rigidity coefficient was found to be in the range of 16 to 33 for all the studied species. Lowest rigidity coefficient among the wood species was observed in *B. ceiba* (22.22), *Dryobalanops* (23.64) and *E. globulus* (23.14) while among the grass species lowest rigidity coefficient was observed in *D. bipinnata* (17.85), *P. karka* (21.99) and *C. martini* (21.15) (Fig. 21).

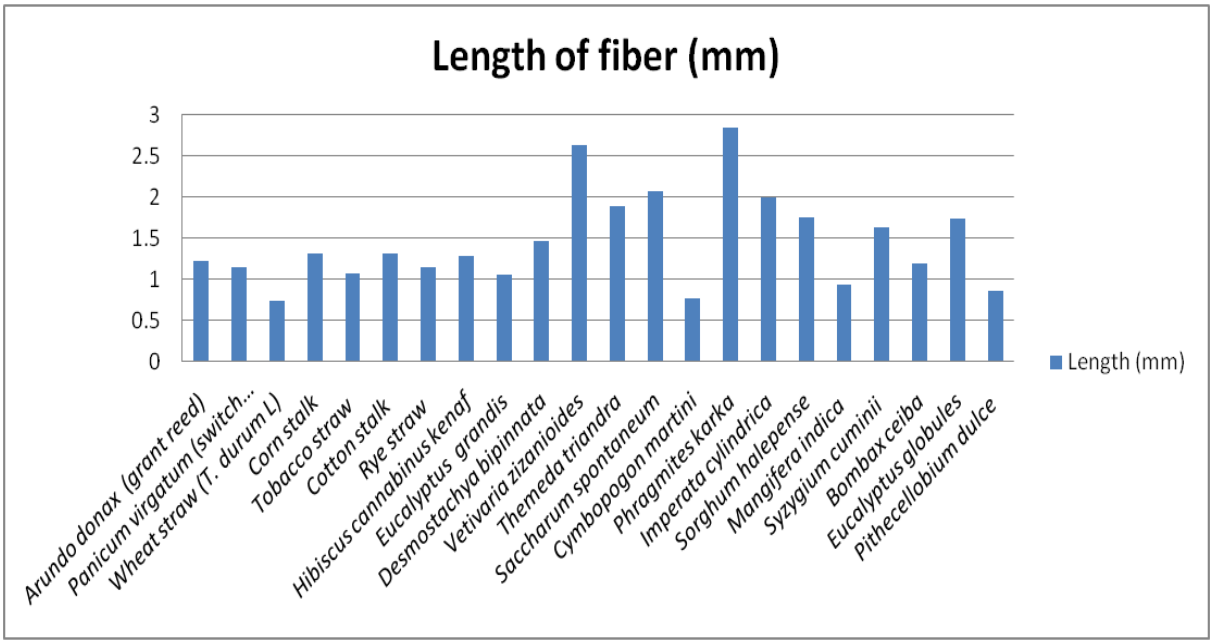


Figure 18: Length of the fiber in the studied species

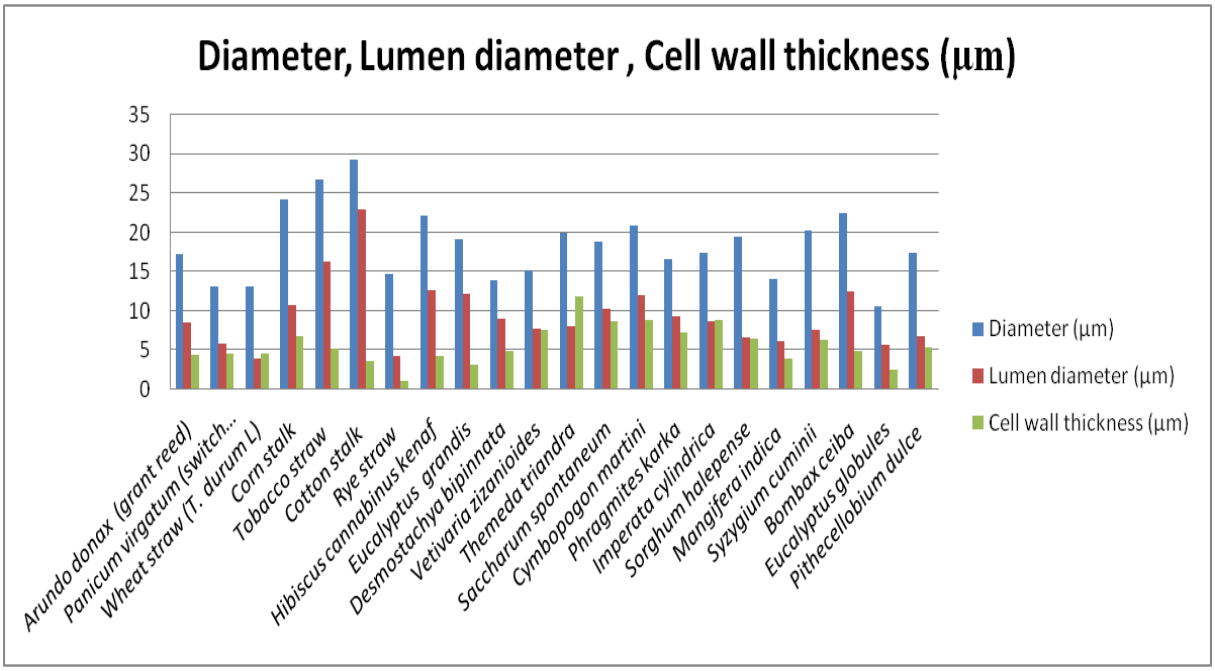


Figure 19: Diameter, lumen diameter, cell wall thickness of the fibers in the studied species

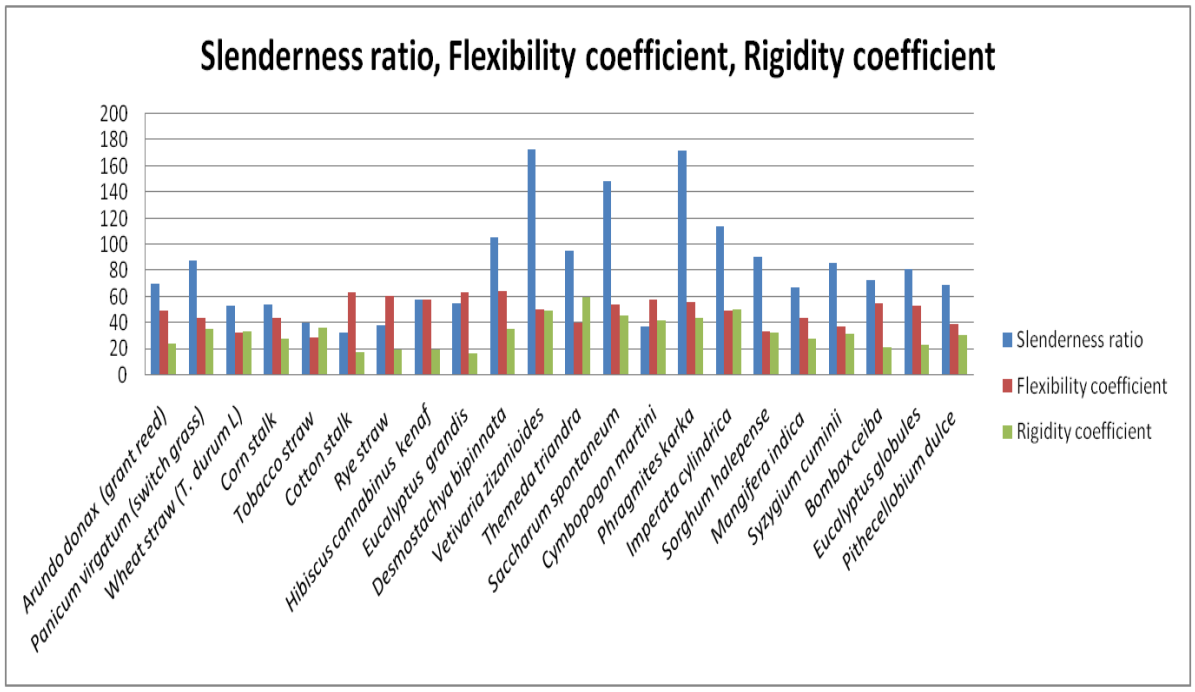


Figure 20: Slenderness ratio Flexibility coefficient, Rigidity coefficient of fibers in the studied species

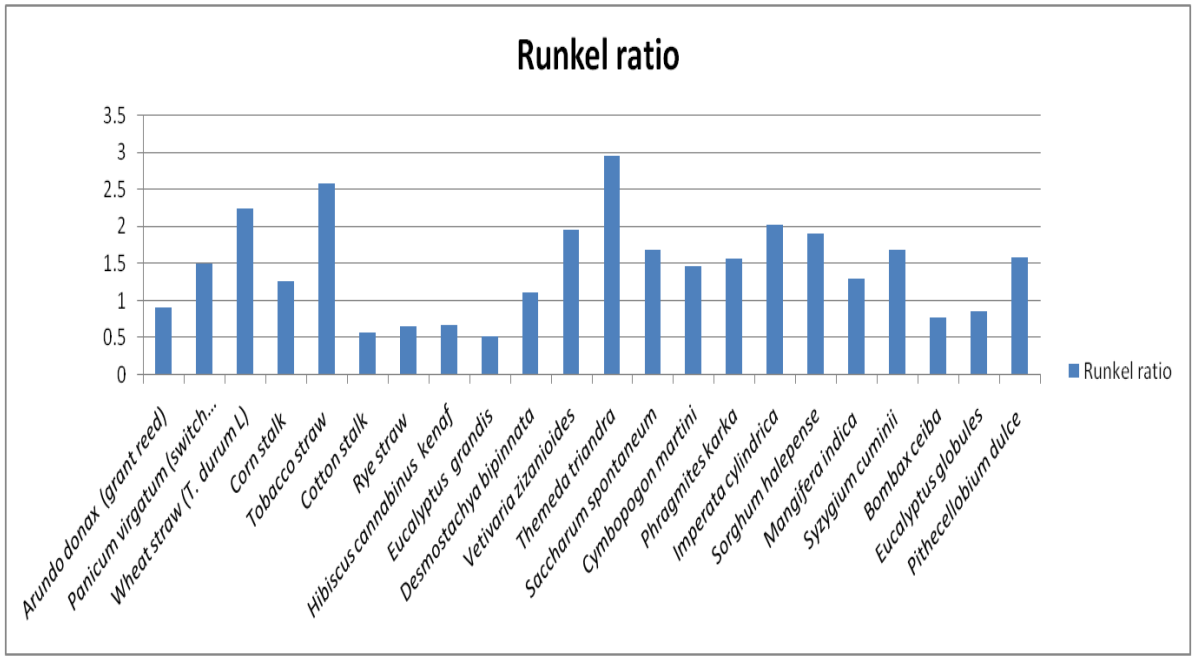


Figure 21: Runkel ratio of fibers in the studied species

II) DISCUSSION

From survey of the saw mills it was very well understood that 42 different varieties of wood was found in the market of Vadodara. Teak (*Tectona grandis*) is one of the most common wood species available in the saw mills. There are many different varieties of teak available in the market like Nigerian teak, Ghana teak, Burma teak, Nagpuri teak, Valsad teak, Sudan teak, Panama teak. On the basis of the uses and availability five different wood species were selected for present study. Five selected species were *Mangifera indica, Syzygium cuminii, Pithecellobium dulce, Bombax ceiba* and *Eucalyptus globulus.* There are varied uses of these woods. But the wood shavings/remnants of carpentry is mostly used as fuel or thrown off. So it was thought worthy to evaluate them for its use in the pulp and paper industry.

Kalam Khush is making pulp by mechanical pulping from waste cotton cloth without any use of paper making machine. It is handmade paper. In Shri A.M.M. Murugappa Chettiar Research Center completed a DST financed a project on the biopulping which helped us with some aspects of biopulping. They have developed concept of biotereatment with phototrophic bacteria for paper pulp. Alternative cellulosic material sources which is Banana pseudostem, grasses and other agricultural wastes have been identified and an absolutely chemical free technology which not only makes pollution free but also helping to conserve large quantity of water has been developed. Visit to very well known J. K. Paper Industry helped to understand the normal kraft process of making paper. These two industries and research center mainly initiated the thought of utilizing lignocellulosic waste like remaining of carpentry work and grasses, for biopulping and use in paper industry it would be to an extent

ecofriendly by reducing use of harsh chemicals. Selectively delignifying fungi would be appropriate for biopulping as they facilitate in loosing and separation of cells which is the first step in pulping where traditionally harsh chemicals under high pressure and temperature are used to delignify the raw materials.

Of the collected fruiting bodies many fungi belonged to family Polyporaceae (*Daedaleopsis confragosa, Coriolopsis telfarii, Coriolopsis polyzona, Trametes roseola, Polyporus grammocephalus, Pycnoporus sanguineus, Datronia caperata, Microporus xanthopus* and *Coriolopsis floccose*).

Selection of the fungi for the further study was done on the basis of experiment performed for screening of fungal isolates for cellulolytic and ligninolytic enzymes by plate method. According to Atri and Sharma (2012) this test provides the basic information for the presence of enzymes in mushrooms. And they have studied five different species of *Pleurotus* for their ligninolytic and cellulolytic activity by plate method.

Bains *et al.* (2006) studied the qualitative evaluation of cellulolytic and ligninolytic enzymes of wood degrading enzymes of white rot fungi (*Ganoderma lucidum, Pleurotus florida, Polyporus volvatus, Polyporus* sp1, *Polyporus* sp2 and *Hymenochaete sp*.). Bains *et al.* 2006 found that the *Ganoderma lucidum* is ligninolytic enzyme secreting fungi which agrees with the present study.

On the basis of the results of enzyme activity two ligninolytic fungi were selected for the further study. *Flavodon flavus* and *Schizophyllum commune* were found to be simultaneous white rot fungi which degrade the lignin and the cellulose simultaneously so they were not selected. Fungi *Daedaleopsis confragosa* and *Phellinus pectinatus* were

selected for the further study as they secrete only ligninolytic enzyme and not the cellulolytic enzyme.

The strains of *F. flavus* showed strong positive reactions to tannic acid. All strains of *F. flavus* produced lignin-modifying enzymes to degrade the lignin in *M. indica* and *S. cuminii* woods. The isolates of *F. flavus* possessed high lignin-degrading capacity whereas isolates of *S. commune* possessed low lignin-degrading capacity (Amee *et al.* 2010).

Nagadesi and Arya (2012) qualitatively estimated Lignocellulolytic enzymes produced by 21different fungal species. *Ganoderma lucidum* showed strong positive reaction to tannic acid, which was in accordance with the result obtained in the present study.

It is very essential to find out the ability of the fungus to degrade lignin and cellulose before conducting the biopulping experiment. To observe the ability of fungi to degrade lignin and cellulose, both the fungi were inoculated with three different forms of selected wood species (Wood blocks, Wood shavings and saw dust), 24 different grass species, Banana pseudostem and pulp made from the banana pseudostem (Obtain from the J.K. Paper industry).

Maximum weight loss with pretreatment of both fungi was obtained in the saw dust. Wooden blocks infected with *D. confragosa* showed a higher weight loss compared to wooden blocks infected with *P. pectinatus*. Similarly saw dust of all the wood species pretreated with *D. confragosa* showed greater weight loss compared to the saw dust pretreated the *P. pectinatus*. Saw dust of *S. cuminii* and *E. globulus* inoculated with the

D. confragosa showed a maximum of 15% and 14% weight loss respectively. The saw dust form of all the wood was more susceptible to both fungi.

In all the stages of decay both the fungi showed significant lignin loss. *Syzygium cuminii* pretreated with *D. confragosa* showed equal amount of lignin and cellulose loss in all the three forms of wood (wood blocks, wood shavings and saw dust). In *Pithecellobium dulce* and *Bombax ceiba* 10 days incubation period with *D. confragosa* showed 8.33% loss of lignin in all the three forms of wood but in 20 days incubation period loss of lignin increased in *Bombax ceiba* pretreated wood blocks and shavings but it remained same in saw dust (8.33%). In *Pithecellobium dulce* pretreated with *D. confragosa* the loss of lignin in wood blocks and shavings increased to 16.67 % but it remained the same in wood shavings. Similarly *P. pectinatus* pretreated different forms of *Bombax ceiba* and *Pithecellobium dulce* showed an equal amount of lignin loss (8.33 %) within 10 days of incubation period. In 20 days incubation period loss of lignin increased in *Bombax ceiba* with maximum being in wood shavings and with a same percentage of 16.67% in saw dust and wood blocks. In *Pithecellobium dulce* after 20 days further loss of incubation period no further loss of lignin after 10 days was obtained in saw dust and wood shavings but in the wood blocks there was a double fold loss of lignin *M. indica* and *E. globulus* showed an increase in loss of lignin with an increase in the incubation period in all the different forms of wood inoculated with both *D. confragosa* and *P. pectinatus.* In general the loss of lignin during the first 10 days of incubation period appeared to be the same when pretreated with both *D. confragosa* and *P. pectinatus* showing a variation only during the further period of incubation.

In all the different substrate forms the cellulose loss was observed. Wood blocks of *M. indica* inoculated with *D. confragosa* and *P. pectinatus* showed no loss of cellulose during the first 10 days incubation period. Wood shavings and saw dust inoculated with *D. confragosa* showed an equal percentage (11.11 %) loss of lignin, an amount of cellulose which was lost in wood blocks and wood shavings inoculated with *P. pectinatus* and incubated for 20 days period. Wood shavings of *S. cuminii* and *B. ceiba* and wood saw dust of *B. ceiba* showed no loss of cellulose in the initial period of 10 days incubation when pretreated with *D. confragosa.* Similarly wood shavings and saw dust of *P. dulce* inoculated with *P. pectinatus* showed no loss of cellulose during the first 10 days period of incubation. *E. globulus* inoculated with *D. confragosa* showed an equal amount of cellulose loss in all three forms of wood during the various incubation periods (i.e. 10 and 20 days). Loss of cellulose in all the three different forms of wood pretreated with both fungi showed an increase in 20 days incubation period. Moreover also the loss of lignin was more in first 10 days incubation period compare to loss of cellulose which was more compared to the loss of lignin in 20 days incubation period.

In pulp and paper industry loss of lignin is required to be more than cellulose because in the process of pulping in paper production cellulose fibers are extracted while lignin is removed from the wood material. From the above observations of the present study it could be concluded that an incubation period of 10 days with both the fungi would be appropriate to have the loss of lignin and no loss or minimum loss of cellulose in all the three different forms of wood substrate.

Sherief *et al.* (2010) compared lignin biodegradation and levels of enzyme production and activities in *Pleurotus* treated rise straw and saw dust. Though fruiting of *Pleurotus* was found to be earlier on rice straw enzyme production and activity and lignin biodegradation was more in saw dust indicating saw dust to be a better substrate for the activity. In the present study also ligninolytic enzyme activity and lignin loss was found to be higher in saw dust compared to other forms of the substrates (wood blocks and wood shavings).

Belewu (2006) evaluated the influence of fungus treatment on biochemical composition and degradation pattern of saw dust and cotton plant by products by *Pleurotus sajorcaju* and found that the process efficiency was highest for the treated saw dust than the treated cotton plant by product

Lignin loss in present study is found to be lesser, except *S. cuminii* infected with both the fungi agrees with results of the present study, *B. ceiba* infected with *D. confragosa* and *P. dulce* infected with *P. pectinatus* showed similar results for cellulose loss. Arora and Sandhu (1987) found 6 % weight loss and 14% lignin loss in 60 days incubation of angiospermic wood saw dust with *Pleurotus ostreatus*, which is very less in comparison to the present study.

5 % lignin degradation in wood shaving of *M. indica* and *E. globulus* infected with *D. confragrosa* was observed, which agrees with the wood shavings treated with *Ceriporiosis subvermispora* in lignin degradation (Fackler *et al.* 2006).

Otjen and Blanchette (1985) has worked on selective delignification of Aspen wood blocks with white rot basidiomycetes and he found weight loss ranging from 3 to 11 % after 4 weeks of incubation which is in accordance with all our results.

Wood shavings and saw dust of *B. ceiba* and *P. dulce* inoculated with *D. confragosa* and *P. pectinatus* respectively showed no loss of cellulose in the first 10 days of incubation period but a loss of 8.33 % lignin which was a good indicator to be used potentially for the process of pulping. However evaluating the different forms of the substrate it was observed that, in the saw dust the fibers of the wood were broken and small fragments of the fibers were formed. Saw dust is susceptible to the fungus and very fast to degrade. In the case of wood shavings the fibers were not broken in the small fragments like that in the saw dust. Only in the wood blocks forms it was possible to get intact fibers which is one of the major prerequisite for a good quality pulp. Wood in form of wood block is appropriate for a good quality pulp, though the process efficiency is slow compared to saw dust and wood chips. For the fungal pretreatment the wood substrate in the form of saw dust is more efficient to have a high enzyme activity and greater loss of lignin. But from the point of view of efficiency of fungal pretreatment in pulp and paper industry the use of wood chips as pretreatment substrate is more applicable. The reason for this is the breakage of fibers when the substrate is utilized in form of saw dust. The fiber property will be reduced bringing about a poor pulp and paper quality. Wood blocks of *M. indica* pretreated with both fungi shows absolutely no loss of cellulose in the first 10 days of incubation period but a loss of 3-4 % lignin was obtained. Also wood shavings of *B. ceiba, S. cuminii* and *P. dulce* showed no loss of cellulose but significant loss of lignin as distinctly observed in table 7 and 8. This indicates that for efficient biopulping *M. indica, B. ceiba, S. cuminii* and *P. dulce* wood can be used in the form of wood blocks or wood shaving and pretreated with

efficient lignin degraders *D. confragosa* and *P. pectinatus* for an for an incubation period of 10 days.

Bor (1960) reported *Saccharum spontaneum, S. bengalense, Arundo donax, Phragmites karka, Themeda arundinacea and T. villosa* to yield excellent fibers for paper pulp. All these grasses can be used in admixture with bamboo pulp. In the present study other unpalatable fast growing grasses were evaluated for its potentiality to be used in pulp and paper industry. Further the potential grasses were experimented by pretreatment with *D. confragosa* and *P. pectinatus* and detecting the amount of lignin and cellulose loss.

Akin *et al.* (1995) has used Bermuda grass (*Cynodon dactylon*) to observe the alteration in structure, chemistry and biodegradability of grass lignocellulose treated with white rot fungi. Many workers has experimented on the wheat straw with different fungi to degrade lignocelluloses (Thakur *et al.* 2012; Berrocal *et al.* 2000; Hossain and Anantharaman 2008).

Rice straw infected with the *Pleurotus ostreatus* after incubation for the 60 days showed 25 % weight loss (Taniguchi *et al.* 2005) which completely agrees with our findings with the fungi *D. confragosa* and *P. pectinatus*. In almost all the grass species an equal amount of weight loss was observed, except a few. Hatakka (1983) pretreated wheat straw with different white rot fungi for 35 days, In this study *Ischnoderma benzoinum* showed 20.2 %, *Phanerochate sordid* showed 42.3%, *Phlebia radiate* showed 45.2 %, *Pleurotus ostretus* showed 23.6 %, *Polyporus versicolor* showed 30.8 % and *Pycnosprous cinnabarinus* showed 35.6 % loss in the weight. Wheat straw showed very high variation in the weight loss with the different fungal species. Thakur

et al. (2012) studied the degradation of wheat straw with *Pleurotus ostreatus* and they got the 40 % dry matter loss only after 32 days but in the present study with the *D. confragosa* and with *P. pectinatus* 40 % weight loss after was not obtained even in 35 days of incubation period. Around 42 % weight loss was obtained in *D. annulatum, T. elegans* and *B. pertusa* pretreated with *P. pectinatus* and incubated for a period of seven weeks time.

Lignin loss is one of the very important factor for biopulping. Here both the selected fungi showed significant amount of the lignin loss. Thakur *et al.* (2012) demonstrates that the fungal pretreatment methods were a remarkable approach for massive utilization of lignocellulosic biomass for higher sugar yield during saccharification and eventually to bioethanol production. *Plerotus ostreatus* was found to possess a good ligninolytic and lignin degrading ability. Lignin removal over the incubation period of five to seven weeks obtained in the present study was found to be in the range of previously reported values of lignin degradation of plant residue by *Phanerochaete flavido-alba* (Lopez *et al.* 2006). Lignin degradation was found to be maximum in *Imperata cylindrica* pretreated with *D. confragosa* and inoculated for 5 weeks (48.78 %) which increased to 60.97 % in 7 weeks incubation period time. A range of 33-41 % loss of lignin in 7 weeks period was found in *D. confragosa* pretreated *Chionachne koenigii, Ischaemum molle, Cymbopogon martinii, Sorghum halepense, Themeda triandra, Pennisetum setosum* and *Thelepogn elegans.*

Rice straw degraded by *Pleurotus ostretus* showed 41 % lignin loss after an incubation period of 60 days (Taniguchi *et al.* 2005). In present study approximately the same results were observed in *T. triandra*, *C. koenigii* and *I. pilosum* infected with *P.*

pectinatus. An increase in dry matter loss along with lignin, cellulose and hemicellulose removal has been reported by various authors (Shrivastava *et al.* 2011; Arora and Sharma 2009; Hatakka *et al.* 2001). In the similar context as rightly indicated by Hatakka *et al.* (2001) the increase in lignin degradation with an increase in incubation period (7 weeks) observed in the present study has probably occurred due to secondary metabolism and nitrogen starvation condition or it could also be attributed to the mycelial autolysis and release of mycelia bound enzyme as reported by Shrivastava *et al.* (2011).

Bak *et al.* (2009) pretreated rice straw with *Phanerochaete chrysosporium* and got 21 % lignin degradation within 15 days of incubation period. Zang *et al*. (2007) obtained 48.6 % lignin loss in Bamboo culm degraded with white rot fungus *Corious versicolar.*

In the lignocellulosic substrates lesser amount cellulose degradation is preferable for its application in the biopulping. Here we found that the cellulose loss in *Apluda mutica* infected with both the fungus showed lowest among all the grass samples which were observed to be only 14 %. *Heteropogon triticeus* infected with *Phellinus pectinatus* and *Dicanthium annulatum* infected with *Daedaleopsis confragosa* showed the highest cellulose loss. In many of the grass samples cellulose loss was found to be more than lignin loss in 7 weeks incubation. Suggesting them not to be suitable raw material for biopulping.

Thakur *et al.* (2012) got only 10.4 % cellulose loss in wheat straw degraded with *Pleurotus ostreatus* in 32 days of degradation, a result which supported our study. Only

in the case of *Bothrichloa pertusa* infected with the *D. confragosa*, while in all the other grasses loss of cellulose was found to be more.

Loss of cellulose up to 3 weeks of incubation period with both the fungi were found to be less which increased after 3 weeks. Rice straw infected with *Phanerochaete chrysosporium* showed 19.8 % cellulose loss within 15 days only (Bak *et al.* 2009) while rice straw is degraded by *Pleurotus ostretus* showed only 17 % cellulose loss after incubation of 60 days (Taniguchi *et al.* 2005) which is in accordance with the results obtained in the case of *I. cylindrica, C. koenigii, A. mutica, C. ciliaris, V. zizanioides* and *B. pertusa* infected with *D. confragosa*, and *A. mutica* and *C. ciliaris* infected with *P. pectinatus*. All the other grasses infected with both the fungi showed higher loss of cellulose. Result show that different fungi show different level of cellulose and lignin loss in same incubation period. The difference in the degradation pattern of substrate by these fungi could be attributed to the different enzyme profile.

In another experiment conducted on the banana pseudostem and its pulp from the J. K. Paper industry interesting results were obtained. Different parts of banana like peels, leaves, pseudostem, stalk and inflorescence were used in different applications like thickening agent, coloring and flavor, source for macro and micronutrients, nuturaceuticals, livestock feed, natural fibers and bio fertilizers (Padam *et al.* 2012). Goswami *et al.* 2005 stated that the pulp obtained from the mixture of banana pseudostem (*Musa paradisiaca*) and bamboo showed increased burst index, tensile index, tear index and oil resistibility. In the present study banana pseudostem and its pulp were evaluated for its potential use as a substrate in the paper industry.

Results of degradation of banana pseudostem degraded with *D. confragosa* is comparable with that of Thakur *et al.* 2012.

Banana pseudostem and pulp infected with *D. confragosa* showed good degradation in presence of MEA medium. Soaking of banana pseudostem and pulp before the pretreatment was found beneficial for the fungal degradation, because the weight loss and lignin loss were found to be more compared to the unsoaked pseudostem and pulp. As the result of which, the combination of presence of MEA medium and soaking of the raw material proved the best for the degradation of lignin and cellulose by the two selected fungi. Addition of MEA to the raw material (Banana pseudostem and pulp) would fasten the process of biopulping.

From pulp and paper makers point of view wood characteristics such as basic density, fiber length, chemical composition and whiteness are of importance for the paper industry (Senisterra *et al.* 2000). Many researchers have studied the fiber properies of different lignocellulosic wastes *Arundo donax, Hibiscus cannabinus* and *Panicum virgatum* (Ververis *et al.* 2004), Wheat straw (Deniz *et al*. 2004), Corn stalk and Tobacco straw (Erolu *et al.* 1992), *Eucalyptus grandis* (Dutt and Tyagi 2011), Rye straw (Usta and Eroglu 1987) which in present study is compared with the selected raw materials.

Ververis *et al.* (2004) studied the fiber properties of 4 non woody plants and 3 agricultural wastes. According to them Kenaf and Reed, fibers are both good source for paper making. Olive tree and Almond tree pruning's have shorter and thicker fibers producing relatively poor index values. Pulp from these species are expected to have relatively low mechanical strength suitable only for replacing hardwood pulps in low or

moderate proportions to produces news print or tissue paper. Long fibers with thin walls are preferred in paper industry. Long fibers have extensibility of the bonding sites increasing strength properties especially tear resistance to the paper sheet. Short and thick fibers do not produce good surface contact and fiber to fiber bonding (Ogbonnaya *et al.* 1997). In the present study, Fiber length of *Eucalyptus globulus* is highest among all the wood fibers. Among the grasses length of the fibers is more in *Phragmites karka, Vetiveria zizanioides, Saccharum spontaneum, Imperata cylindrica* and *Sorghum halepense.* Similarly fibers with large lumen and thin walls tend to flatten to ribbons during papermaking with enhanced interfiber bonding between fibers and consequently have good strength characteristics.

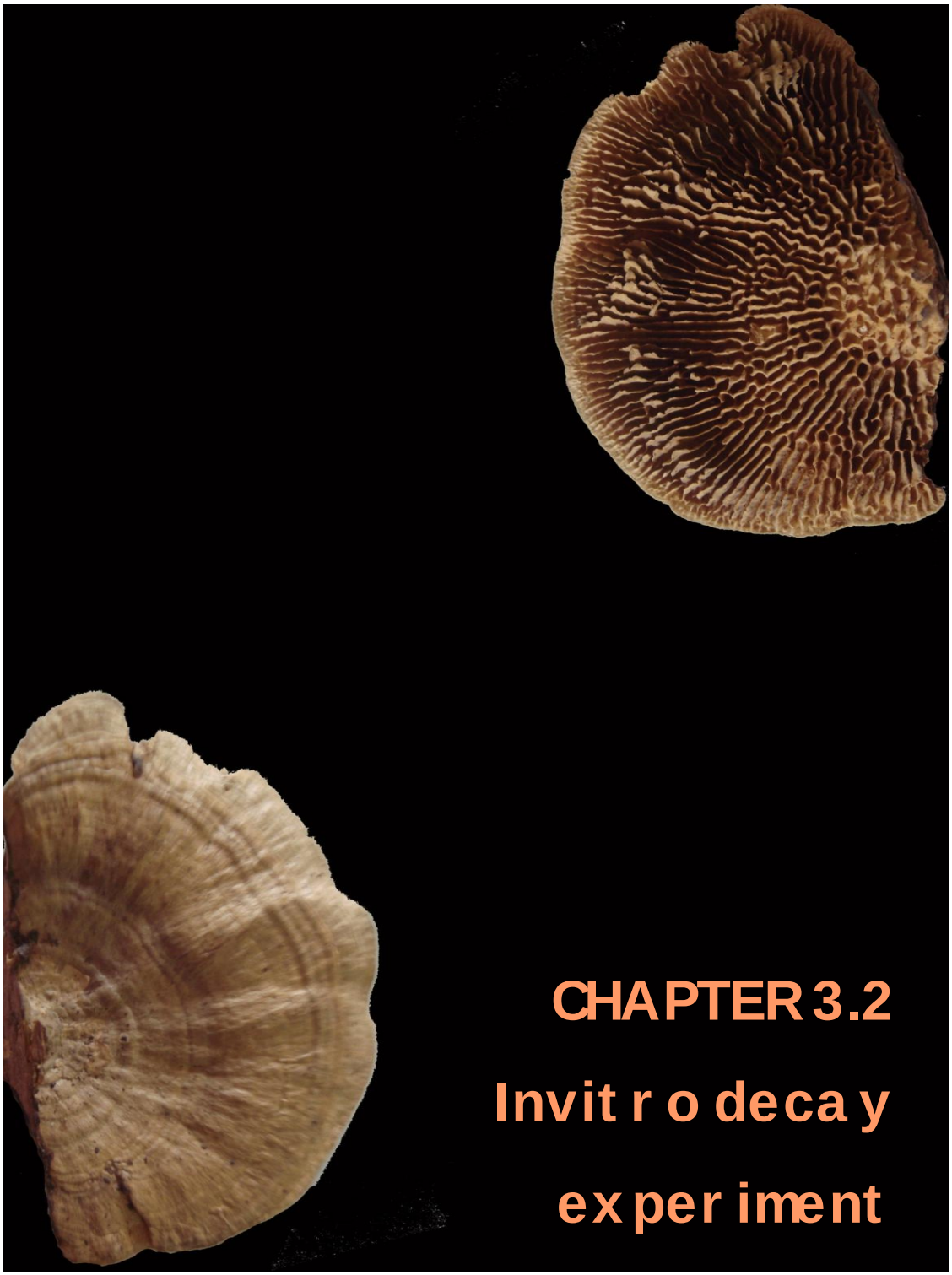
Among wood species *Bombax ceiba* and among grass species *Phragmites karka* and *Saccharum spontaneum* have large lumen with small wall thickness. *Imperata cylindrica, Vetiveria zizanioides* and *Sorghum halepense* have same lumen diameter and wall thickness. Slenderness ratio >70 results in satisfactory pulp tear indices and bursting strength for printing and writing purposes (Cappelletto *et al.* 2000; Law *et al.* 2001).

Among the wood species, *Bombax ceiba, Eucalyptus globulus* and grass species *Phragmites karka, Vetiveria zizanioides, Saccharum spontaneum, Imperata cylindrica* and *Sorghum halepense* have very good derived values especially the slenderness ratio.

Flexibility coefficient of a fibrous material if lower than 70 is valuable for quality pulp and paper production (Bektas *et al.* 1999). Elasticity coefficient if between 0-70 this kind of fibers easily can be flat and give good paper with high strength properties. Higher rigidity coefficient gives lower paper strength properties especially

lower burst, tear and tensile indexes (Bektas *et al.* 1999). The lower the Runkel ratio, the better the material or paper making (Lessard and Chouinard 1980). Low rigidity coefficient and low runkel ratio are found in: *Bombax ceiba, Eucalyptus globulus* among the wood species, and *Phragmites karka, Vetiveria zizanioides* and *Saccharum spontaneum* among the Grass species.

So most suitable raw material based on the fiber properties and their derived values are: Wood species:- *Eucalyptus globulus, Bombax ceiba* and Grass species: *Phragmites karka, Vetiveria zizanioides, Saccharum spontaneum.*



I) RESULT

1) Biochemical analysis

a) Weight loss

All the five selected wood species were subjected to invitro decay experiment inoculating it with two white rot fungi *D. confragosa* and *P. Pectinatus.* Percentage weight loss of these wood species after a desired period of incubation is given was given in Table 16 (*D. confragosa*) and Table 17 (*P. Pectinatus*). Results of percentage weight loss obtained by treating with the two white rot fungi is also given in the form of graphic representations in Figure 22 A (*D. confragosa)* and Figure 22 B (*P. pectinatus*).

Incubation Period	*E. globulus*	*M. indica*	*S. cuminii*	*P. dulce*	*B. ceiba*
10 days	3.08±0.88	4.29±0.91	1.43±0.35	2.76±1.6	1.83±0.44
20 days	7.09±0.65	5.27±0.82	2.78±0.96	4.32±0.7	11.35±1.68
30 days	8.37±1.8	8.66±1.06	4.12±0.5	7.1±0.92	13.68±1.19
2 months	16.48±0.9	15.72±1.5	8.45±1.5	15.14±1.6	21.7±2.01
3 months	21.3±0.8	21.48±1.6	18.18±1.11	17.4±0.68	25.3±1.36
6 months	24.14±1.63	23.7±1.57	20.84±1.8	21.43±0.73	29.5±1.69
9 months	28.73±1.8	27.94±2.01	25.69±0.6	32.4±0.96	46.84±2.4
12 months	66.51±1.5	67.05±1.8	42.73±1.7	46.41±1.79	84.62±2.1

Table 16: Percentage weight loss of wood blocks infected by *Daedaleopsis confragosa*

Incubation Period	*E. globulus*	*M. indica*	*S. cuminii*	*P. dulce*	*B. ceiba*
10 days	2.07±0.64	2.88±0.4	2.45±0.78	3.0±0.4	5.98±1.5
20 days	3.17±0.95	3.24±0.82	3.05±0.62	4.2±0.6	8.68±0.9
30 days	4.32±0.85	4.06±0.59	5.11±0.85	4.65±0.7	10.27±1.5
2 months	11.74±1.67	7.1±0.95	7.16±0.82	5.89±0.9	11.2±1.07
3 months	23.96±1.17	16.26±1.02	12.41±0.9	6.26±1.1	13.23±1.8
6 months	27.36±1.41	21.21±1.4	21.47±0.9	9.68±0.45	17.36±1.3
9 months	35.5±2.5	28.14±0.73	29.21±1.7	10.29±1.38	21.08±1.8
12 months	40.21±1.8	46.06±0.9	39.37±1.3	13.58±0.9	38.18±1.8

Table 17: Percentage weight loss of wood blocks infected by *Phellinus pectinatus*

In all the wood samples weight loss was enhanced with progress in decay. *Eucalypus globulus* wood blocks infected with *D. confragosa* showed gradual increase in weight loss, till 2 months but it enhanced up to 66.51% after 12 months of incubation period (Table 16) while with *P. pectinatus* after 12 months of incubation period the weight loss was only 40.21 % (Table 17).

The percentage weight loss in *M. indica* wood was found to be up to 30 days incubation period, and a maximum of 67.05 % weight loss was observed after 12 months of incubation with *D. confragosa* (Table 16). While with *P. pectinatus* less amount of weight loss was observed up to 2 months, and after 12 months incubation period 46.06 % weight loss was observed (Table 17).

S. cuminii wood infected with *D. confragosa* showed very little weight loss till 2 months and it enhanced up to 42.73 % after 12 months of incubation period (Table 16). With *P. pectinatus* weight loss was very low up to 3 months incubation period, and at 12 months of incubation period it was 39.37 % (Table 17).

Same is the case with *P. dulce* where the wood infected with the *D. confragosa* showed very less decay up to 2 months incubation period and after completion of 12 months it showed 46.41 % weight loss (Table 16). While with the *P. pectinatus* the decay was very slow, exhibiting very less amount of weight loss in all the stages of decay. Even after 12 months only 13.58 % weight loss was observed (Table 17).

B. ceiba wood was found to be highly degraded with *D. confragosa* after 12 months of incubation period (84.62 %). Up to 30 days of incubation period it showed less weight loss (Table 16). *B. ceiba* wood infected with the *P. pectinatus* was less

degraded up to 6 months of incubation period and after 12 months it shows 38.18 % weight loss (Table 17).

b) Lignin loss

Percentage loss in lignin content of all five wood species subjected to invitro decay experiment is given in the Table 18 (*Daedaleopsis confragosa*) and Table 19 (*Phellinus pectinatus*). Results of percentage lignin loss by *D. confragosa* has been represented in the form of graphic representation in Figure 23 A and *P. Pectinatus* in Figure 23 B.

Incubation Period	*E. globulus*	*M. indica*	*S. cuminii*	*P. dulce*	*B. ceiba*
10 days	10±0.7	4±0.2	8.33±0.9	8.33±0.43	8.33±0.4
20 days	20±0.76	9±0.4	16.67±0.9	16.67±0.45	16.67±0.92
30 days	24.8±0.7	14±0.55	18.25±0.9	20.83±0.9	20.58±0.9
2 months	31±0.9	19.5±0.67	21.5±1.1	19.17±0.98	29.47±0.5
3 months	35.2±1.08	24.6±0.9	23.33±0.5	24.17±0.87	40.47±0.5
6 months	38.5±1.2	29.5±0.9	25±0.8	27.25±0.67	44.17±0.8
9 months	42±1.6	37.8±1.6	31.08±1.2	36.25±0.5	53.33±0.9
12 months	48.8±1.4	49.3±1.5	39.58±1.3	42.33±1.5	63.42±1.2

Table 18: Percentage lignin loss of wood blocks infected by *Daedaleopsis confragosa*

Incubation Period	*E. globulus*	*M. indica*	*S. cuminii*	*P. dulce*	*B. ceiba*
10 days	4±0.4	3±0.6	4.99±0.43	8.33±0.4	8.33±0.5
20 days	10±0.5	6±0.6	8.33±0.56	9.67±0.54	16.67±0.6
30 days	11.7±0.52	9.7±0.5	10±0.3	10.83±0.67	20.58±0.54
2 months	19.9±0.7	11.4±0.7	12.5±0.5	14.17±0.9	18.58±0.65
3 months	24.9±0.9	19.3±0.3	16.67±0.57	15±0.98	23.75±0.64
6 months	30.6±0.93	29.5±0.9	19.17±0.6	19.17±0.5	27.5±0.33
9 months	35.5±0.9	33.8±0.92	20.25±.9	20.83±0.78	36.67±0.43
12 months	39.6±1.2	37.3±1.5	24.67±0.98	21.17±0.9	46.75±0.9

Table 19: Percentage lignin loss of wood blocks infected by *Phellinus pectinatus*

E. globulus with both white rot fungi showed gradual increase in the lignin loss with an increase in the incubation period. After 12 months of incubation lignin loss was 48.8 % by *D. confragosa* (Table 18) and 39.6 % by *P. pectinatus* (Table 19).

In the wood of *M. indica* lignin loss with incubation of both the white rot fungi was found to be lesser initially but it enhanced after 2 months of incubation. After 12 months of incubation period, the percentage lignin loss with *D. confragosa* was 49.3 % (Table 18) and with *P. pectinatus* it was 37.3 % (Table 19). *S. cuminii* wood was found to be a very resistant wood as it did not show much lignin loss with both the fungi. With *D. confragosa* it showed 39.58 % lignin loss (Table 18) and with *P. pectinatus* it showed 24.67 % lignin loss (Table 19).

P. dulce wood was also sound like *S. cuminii*. It did not show much loss in lignin content with both the fungi. It showed 42.33 % lignin loss with *D. confragosa* (Table 18) and 21.17 % lignin loss with *P. pectinatus* (Table 19) after 12 months of incubation.

B. ceiba wood was found to be very much susceptible to decay with both the fungi. It showed a very high amount of lignin loss with both the fungi. With *D. confragosa* 63.42 % lignin loss was observed (Table 18) and with *P. pectinatus* 46.75 % lignin loss was observed (Table 19).

c) Cellulose loss

All the selected wood species infected with *D. confragosa* and *P. pectinatus* were analyzed for its cellulose content after completion of each incubation period. Percentage loss in the cellulose content is very important parameter for the biopulping.

Incubatiion Period	*E. globulus*	*M. indica*	*S. cuminii*	*P. dulce*	*B. ceiba*
10 days	10±0.9	0	10±0.51	9.09±0.5	9.09±0.5
20 days	20±0.6	4.45±0.4	20±0.7	12.73±0.7	13.64±0.4
30 days	20.5±0.46	12.22±0.8	22±0.7	13.64±0.9	16.82±0.7
2 months	21.8±0.4	15.56±0.9	22.4±0.5	16.36±0.9	21.82±0.3
3 months	21.1±0.6	19.67±0.7	22.9±0.4	19.09±0.87	25.45±0.5
6 months	23.3±0.9	23.11±0.9	23±0.6	24.55±1.1	27.91±0.9
9 months	24.9±1.2	27.11±0.94	23.8±0.7	25.46±0.95	28.55±0.9
12 months	26.9±1.4	30±1.1	25.7±0.5	27.27±1.5	30.09±1.3

Table 20: Percentage cellulose loss of wood blocks infected by *Daedaleopsis confragosa*

Incubation Period	*E. globulus*	*M. indica*	*S. cuminii*	*P. dulce*	*B. ceiba*
10 days	10±0.5	0	10±0.5	9.09±0.5	9.09±0.3
20 days	20±0.6	11.11±0.4	16±0.7	11.82±0.7	15.46±0.6
30 days	21.8±0.6	12.22±0.5	16.8±0.6	14.55±0.8	21.82±0.8
2 months	23.5±1.2	15.56±0.8	17.2±0.5	15.46±1.2	25.45±0.9
3 months	25.6±1.2	21.11±0.9	19±0.9	17.27±0.4	27.91±0.9
6 months	27±0.9	26.67±0.4	20.8±0.9	18.18±0.5	28.55±1.1
9 months	27.9±0.9	28.89±0.9	21.6±0.97	19.09±1.2	30±1.5
12 months	28.9±1.5	31.11±1.5	24.4±1.5	21.91±1.5	32.18±1.6

Table 21: Percentage cellulose loss of wood blocks infected by *Phellinus pectinatus*

Percentage loss in the cellulose content with *D. confragosa* is represented in Table 20, and with *P. pectinatus* is represented in Table 21. Figure 24 A shows the graphical representation of loss in cellulose with *D. confragosa* and Fiber 24 B shows graphical representation of cellulose content with *P. pectinatus*.

Percentage cellulose loss in *E. globulus* with *D. confragosa* was very less up to 3 months of incubation and after 12 months of incubation it showed 26.9 % loss (Table 20). While with *P. pectinatus* the cellulose loss increased gradually and after the 12 months of incubation. 28.9 % cellulose loss was observed (Table 21).

In the *M. indica* wood infected with the *D. confragosa* and *P. pectinatus* showed gradual increase in the cellulose loss. And till 3 months of incubation with both the fungi shows very less cellulose degradation. After 12 months of incubation 30 % cellulose loss was observed with *D. confragosa* (Table 20) and 31.11 % cellulose loss with *P. pectinatus* (Table 21).

S. cuminii wood with both the fungi showed the less degradation of cellulose. Here the highest loss in cellulose was observed after 12 months of incubation with *D. confragosa* (25.7 %, Table 20) and with *P. pectinatus* (24.4 %, Table 21).

In *P. dulce* the cellulose loss was gradual and lesser in *P. pectinatus*. After 12 months of incubation *D. confragosa* showed 27.27 % loss of cellulose (Table 20) while with *P. pectinatus* showed only 21.91 % degradation of cellulose (Table 21).

As observed in lignin and weight loss *B. ceiba* wood is most susceptible to both the fungi among all the wood species it showed greater loss in cellulose. After 12 months 30.09 % loss in cellulose content is observed with *D. confragosa* (Table 20) and 32.18 % loss with *P. pectinatus* (Table 21).

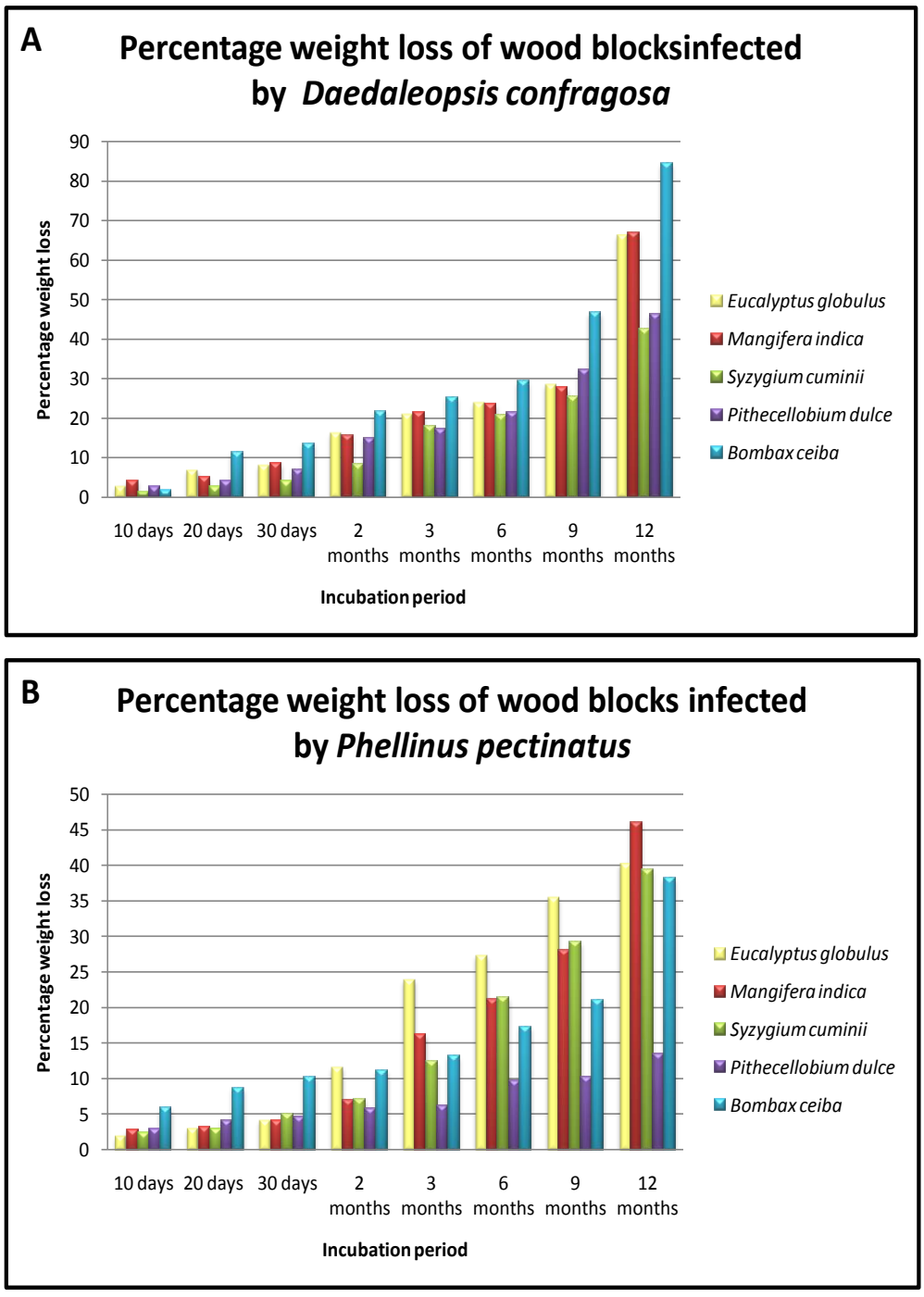

A
Percentage weight loss of wood blocksinfected by Daedaleopsis confragosa
Percentage weight loss
90
80
70
60
50
40
30
20
10
0
10 days
20 days
30 days
2 months
3 months
6 months
9 months
12 months
Incubation period
Eucalyptus globulus
Mangifera indica
Syzygium cuminii
Pithecellobium dulce
Bombax ceiba
B
Percentage weight loss of wood blocks infected by Phellinus pectinatus
Percentage weight loss
50
45
40
35
30
25
20
15
10
5
0
10 days
20 days
30 days
2 months
3 months
6 months
9 months
12 months
Incubation period
Eucalyptus globulus
Mangifera indica
Syzygium cuminii
Pithecellobium dulce
Bombax ceiba

Figure 22

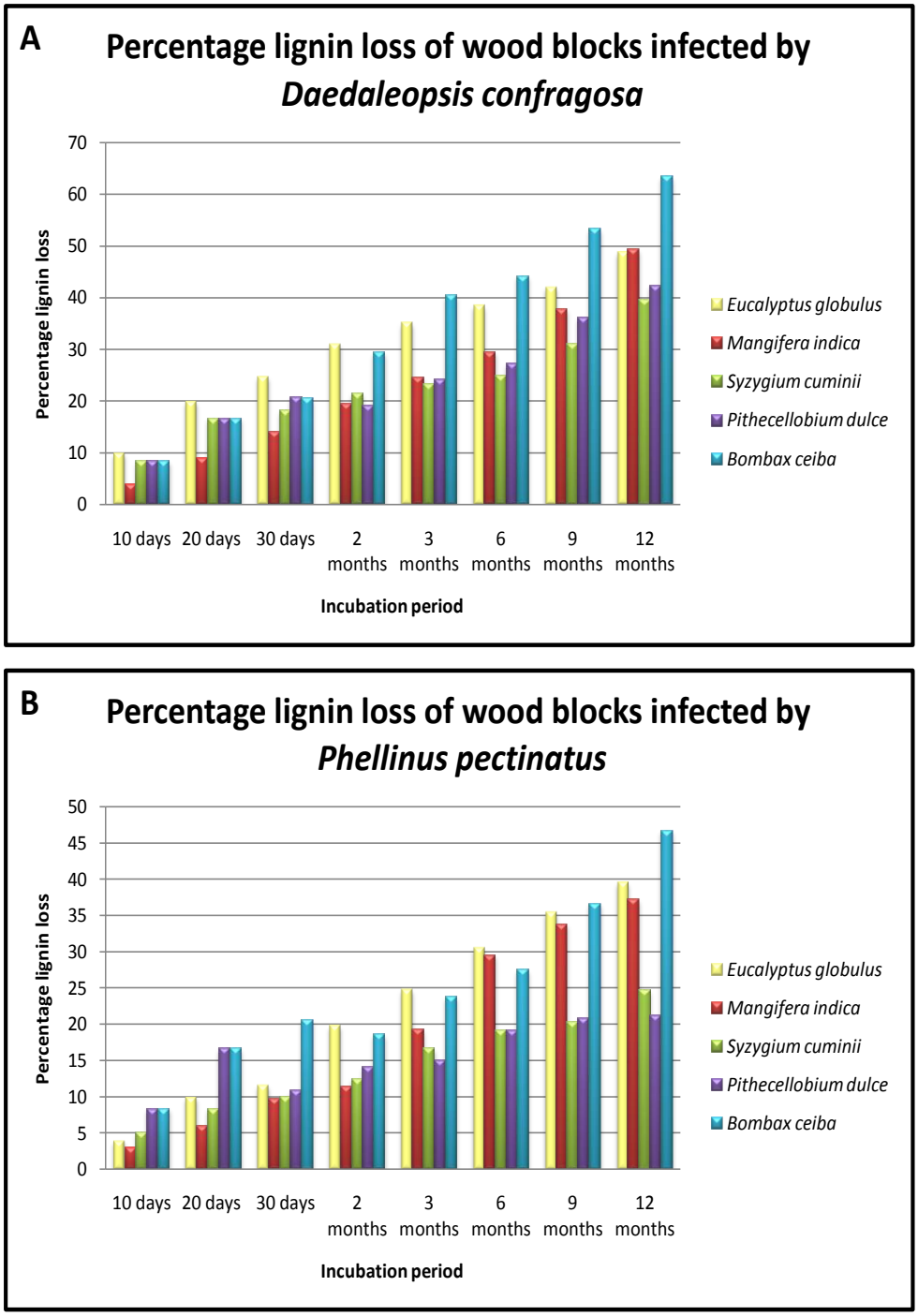

A
Percentage lignin loss of wood blocks infected by
Daedaleopsis confragosa
Percentage lignin loss
70
60
50
40
30
20
10
0
10 days
20 days
30 days
2 months
3 months
6 months
9 months
12 months
Incubation period
Eucalyptus globulus
Mangifera indica
Syzygium cuminii
Pithecellobium dulce
Bombax ceiba
B
Percentage lignin loss of wood blocks infected by
Phellinus pectinatus
Percentage lignin loss
50
45
40
35
30
25
20
15
10
5
0
10 days
20 days
30 days
2 months
3 months
6 months
9 months
12 months
Incubation period
Eucalyptus globulus
Mangifera indica
Syzygium cuminii
Pithecellobium dulce
Bombax ceiba

Figure 23

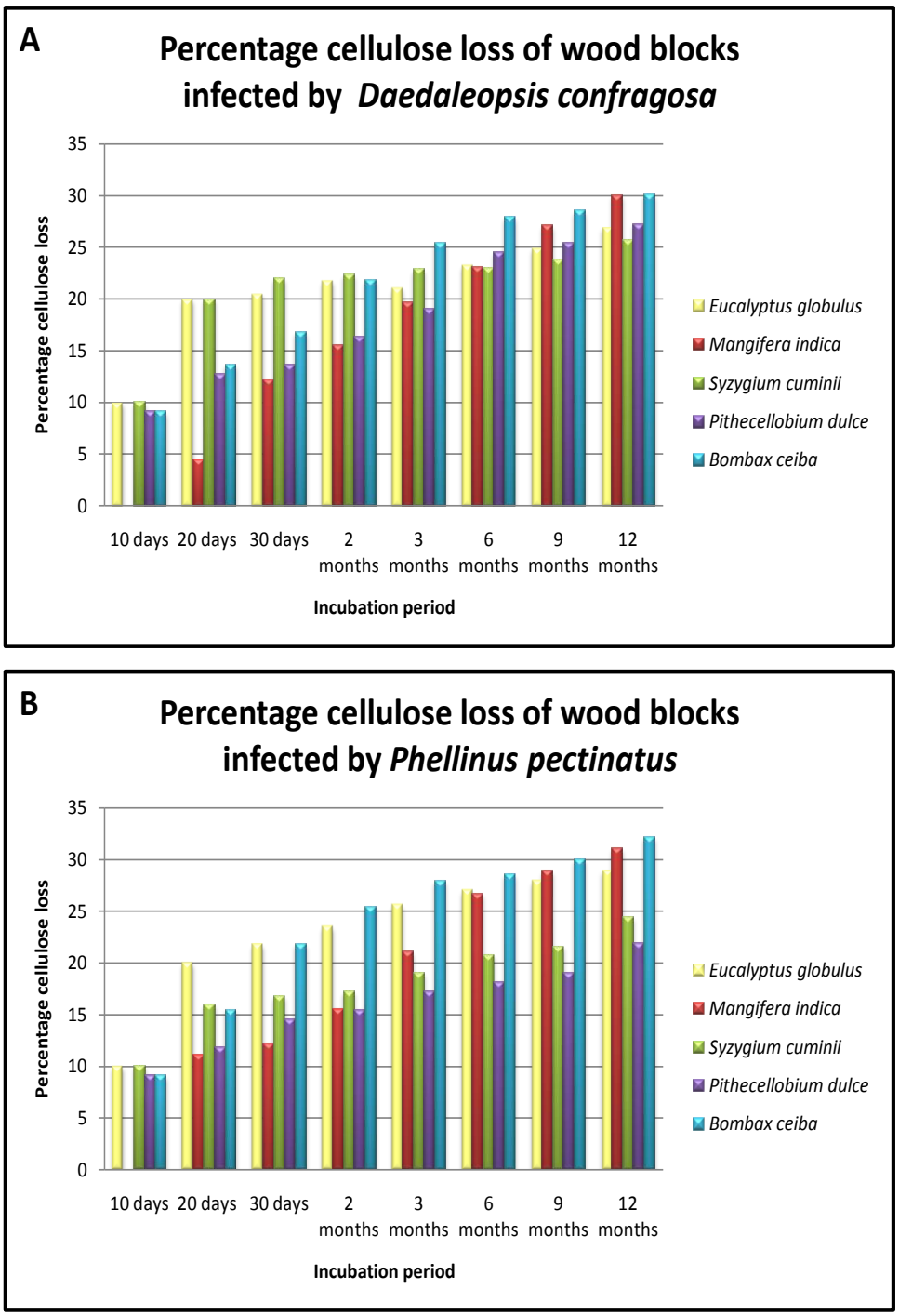

A
Percentage cellulose loss of wood blocks infected by Daedaleopsis confragosa
Percentage cellulose loss
35
30
25
20
15
10
5
0
10 days
20 days
30 days
2 months
3 months
6 months
9 months
12 months
Incubation period
Eucalyptus globulus
Mangifera indica
Syzygium cuminii
Pithecellobium dulce
Bombax ceiba
B
Percentage cellulose loss of wood blocks infected by Phellinus pectinatus
Percentage cellulose loss
35
30
25
20
15
10
5
0
10 days
20 days
30 days
2 months
3 months
6 months
9 months
12 months
Incubation period
Eucalyptus globulus
Mangifera indica
Syzygium cuminii
Pithecellobium dulce
Bombax ceiba

Figure 24

2) Anatomical observations

All the wood species selected for the present study were observed to characterize the normal features of the wood elements and the alterations occurred due to the fungal degradation. The anatomical description of the normal uninfected wood is according to In Side wood NCSU Libraries.

Normal wood structure of *Syzygium cuminii*

Plate 1 shows the photomicrographs of normal wood structure of *S. cuminii*. Growth ring boundaries indistinct. Wood is diffuse porous. Vessel elements arranged in diagonal to radial pattern. Vessel solitary or in radial multiples of 2 common but at times 3 (Plate 1 A). Solitary vessel outline is circular to oval in shape. Fibers with simple to minutely bordered pits .They are unseptate and with thin-thick walled.

Apotracheal and paratracheal axial parenchyma present. Axial parenchyma scanty paratracheal (occasionally) parenchyma cells associated with the vessel or incomplete sheath of parenchyma (Plate 1 B). Around the vessels.

Apotracheal parenchyma diffuse in aggregates at times forming short discontinuous tangential bands (Plate 1 C). Sometimes the axial parenchyma is unilateral paratracheal (paratracheal forming semicircular hoods or cap on one side of the vessels and which can extend tangentially or obliquely in a confluent pattern).

Ray cells are uni- multiseriate, mostly triseriate (Plate 1 D). Axial parenchyma cells in strands (Plate 1 D). They show bordered pitting. The bordered pits are scattered (Plate 1 E).

Body ray cells procumbent with mostly 2-4 row and upright or square marginal cells (Plate 1 F). Vessel ray pits with much reduced borders apparently simple and

horizontally arranged (Plate 1 G). SEM study plate 7 H to L shows the normal wood characteristics which is as described above.

Pattern of decay in *Syzygium cuminii* wood infected with the *Daedaleopsis confragosa*

S. Cuminii wood blocks infected with *D. confragosa* showed initial signs of decay only after 20 days of incubation period. With 30 days of incubation period bore holes begin to appear in the ray parenchyma and medullary ray cells. Fibers remain intact without any alterations upto 6 months of incubation period. Plate 2 and 3 shows the pattern of decay with *D. confragosa* in the wood of *S. cuminii*.

In the initial stages of degradation in a transverse view initiation is observed from the parenchymatous cells surrounding the vessel element. Axial parenchyma cells showed bores holes (Plate 2 A), the number of it increasing during the later stages of decay (Plate 3 B). Scanning electron microscopic view at later stage showed adjacent medullary ray cells also with bore holes (Plate 3 A). Fungal hyphae traversed through a pit branched in the lumen and further again traversed through pit aperture (Plate 2 B). Secondary wall layers of parenchyma cells were dissolved and the cells showed buckling due to loosening and separation of the secondary wall from the primary wall (Plate 2 C; 3 C arrow).

In a longitudinal view also it is distinctly observed that the hyphae initially penetrates through the ray parenchyma cells, which further penetrates in vessel wall through pits. Fungus *D. confragosa* appears to preferentially degrades the middle lamella between ray parenchyma cells (Plate 2 H arrow; 3 G arrow head). The cell wall of the ray parenchyma cells showed bore holes (Plate 2 H arrow head, M). Under

scanning electron microscopic view the ray cells are seen separated due to preferential degradation of middle lamella and the cell wall showed mycelial penetration (Plate 3 I). In the magnified view of the ray cells, microfibrils of the cell wall layers are also observed to be separated (Plate 3 I arrow head). With further stages of degradation the bore holes in the ray parenchyma cells fused with each other (Plate 2 K; 3 G, arrow), simultaneously dissolving the middle lamella and completely separating the ray cells leaving behind only their outline (Plate 2 I; 3 G, H). Radial view showed cracks in the cell wall (Plate 2 O) indicating hyphal pathway and some of it is completely filled with the fungal hyphae (Plate 2 P) and hyphae are observed to passing through the bore holes (Plate 2 Q). Discoloration of the wall layers of ray parenchyma cells could also be depicted in the transverse view of some cells. Discoloration originated in S_2 layer leaving behind the S_3 layer close to the lumen (Plate 2 D) same feature is observed under scanning electron microscopy (Plate 3 D). All these features could be confirmed with the scanning electron microscopic study (Plate 3 L). During the later stages of decay within 9 months incubation period adjacent bore holes of ray parenchyma coalesce forming larger bore holes degrading the parenchyma wall (Plate 3 M).

Vessel elements showed fungal colonization with 30 days of incubation period. Initially fungus through the pits penetrated into the vessel element colonizing them. In longitudinal and radial view initially wood vessel, element very clearly showed the penetration of hyphae through pits (Plate 2 J). Under the scanning electron microscope it is very clearly observed that hyphae from the ray parenchyma cell penetrate in to vessel element colonizing there (Plate 2 N; 3 J). Plate 3 K shows the magnified view of the fungal hyphal penetration. The hyphae after penetrating through the pit grow

branching in different directions longitudinally and at right angles to the longitudinal axis of the vessel element (Plate 3 J, K, N). With scanning electron microscopic view it was distinctly observed that due to the degradation, cell wall layers of vessel element is separated the degradation being concentrated on S_2 layer of cell wall (Plate 3 E). Middle lamella between adjacent vessel element gets degraded separating them. During the later stages of degradation hyphae completely colonize the vessel lumen and degrade the vessel wall (Plate 3 N). At this stage of decay cell wall of the ray parenchyma cells exhibit clear discoloration (Plate 2 H).

In the initial stage of degradation fibers remains unaffected up to 3 months of incubation period. Selective degradation of the middle lamella in the fiber region begins within 6 months incubation period, later on separating the fibers forming longitudinal clefts or channels (Plate 2 E). Further the fungus affects the S_2 layers of the fibers. The transverse view shows round to oval elongated cavities in secondary wall of fiber (Plate 2 F arrow; 3 F), which after sometimes extend up to other cell wall layers (Plate 2 G arrow). S_2 layer gets broken down first. Numerous fine cracks are formed running perpendicular to the middle lamella, and extending from the inner to the outer secondary wall (Plate 2 F asterix). Numerous clefts are observed in the tangential plane in the cell walls of the fibers (Plate 2 E, F arrow head). In longitudinal and radial view fibers showed bore holes during later stages of degradation (Plate 2 K, L arrow head) which become larger with further decay (Plate 2 R; 3 H arrow).

Pattern of decay in *Syzygium cuminii* wood infected with the *Phellinus pectinatus*

In *S. cuminii*, *P. pectinatus* showed the same pattern of decay as observed by *D. confragosa* but growth and colonization of *P. pectinatus* is slower than that of *D.*

confragosa. Here the middle lamella is not much affected but it mainly affects the S_2 layer of the fiber cells. Till the 30 days of incubation period anatomical alteration is not observed. Plate 4 and 5 shows the pattern of decay as observed in the different views under light and scanning electron microscopy observation.

S. cuminii wood blocks incubated with *P. pectinatus* showed all the wood elements intact during the first 30 days of incubation period. Bore holes begin to appear in ray and parenchyma within 2 months incubation period. At some instance vessel elements at this stage appeared to be scantly colonized by fungal hyphae. Fiber cells appear to be highly resistant to the fungus with its cell wall remaining unaltered for period of nine months of incubation.

Initiation of degradation begins from the parenchymatous tissue of the wood, that is medullary rays and axial parenchyma cell (Plate 4 A; 5 A) which at the final stages of degradation showed bore holes prominently. The bore holes were smaller than that of *D. confragosa* indicating *P. pectinatus* to have fungal hyphae of smaller diameter.

Longitudinally and radially the fungus started colonizing in ray parenchyma cells (Plate 5 E, J; 4 L) and started dissolving the middle lamella (Plate 4 J; 5 E). Due to separation of middle lamella ray parenchyma cells separated forming zig-zag shaped channel (Plate 4 H). Under scanning electron microscopic study it was observed that all the ray calls were completely filled with the fungal hyphae and during the later stages of degradation the ray parenchyma cells started showing the bore holes (Plate 4 G, K; 5 I). In tangential view middle lamella between ray parenchyma cells especially at the corners appear to be completely dissolved (Plate 5 F arrow), separating them. Walls of

the ray cells also appears to be degraded with remnant of wall observed in the lumen (Plate 5 F arrow head).

Very less colonization of hyphae was observed in the vessel element (Plate 5 K). Hyphae penetrated in the vessel element through the pits (Plate 4 I arrow; 5 G). Colonization of hyphae could not be much observed in a transverse view, although separation of vessel wall is clearly observed (Plate 4 B; 5 A). Within 3 months of incubation period the secondary wall layers especially S_2 layers started getting degraded separating the cellulose microfibrils and the wall losses its rigidity. Due to the degradation of the S_2 layer preferentially all the cell wall layers are prominently seen in vessel element (Plate 5 B arrow), which is very clearly observed in the magnified view (Plate 5 C arrow). Individual lamellae of the cellulosic get fibrils concentrically separated and at this stage disfiguring of the vessel wall is also clearly observed.

Fibers remained unaffected till the 9 months of incubation period (Plate 4 G; 5 B, H). Only at the 12 month of incubation period in transverse view fiber cells show crack in the S_2 layer of the cell wall (Plate 4 C). The cracks extended from lumen to the outer wall. Under SEM also, fiber cells showed small oval shaped bore holes in the S_2 layer (Plate 5 B). In the fiber region rather than degrading s_3 layer and middle lamella, the fungus preferentially degrades S_2 layer, due to which the fiber cells become loosened (Plate 4 D, E, F) with clefts clearly noticed (Plate 5 D, F arrow). In the scanning electron microscope study the separation of cell wall layer in the fiber cell is very clear (Plate 5 D). Thin fungal hyphae running perpendicularly through fibers (Plate 5 L) could also be noticed.

Plate 1: Normal wood structure of *Syzygium cuminii*

Magnification bar: A to G - 10 µm

A-G: Light microscopic view of the different elements

H- I: SEM view of different wood elements in different views

A: Vessel elements arranged in radial pattern.

B: Incomplete sheath of parenchyma around xylem vessel element.

C: Apotracheal parenchyma in short discontinuous band.

D: TLS showing bi and multiseriate rays.

E: Pits in axial parenchyma.

F: RLS showing procumbent ray cells.

G: Intervessel ray pits elongated and opposite.

H: Tangential longitudinal view of wood showing multiseriate rays

I: Transverse view of wood under SEM showing parenchyma cells

J: Tangential longitudinal view of wood showing vessel element

K: RLS showing ray cells

L: RLS showing vessel element

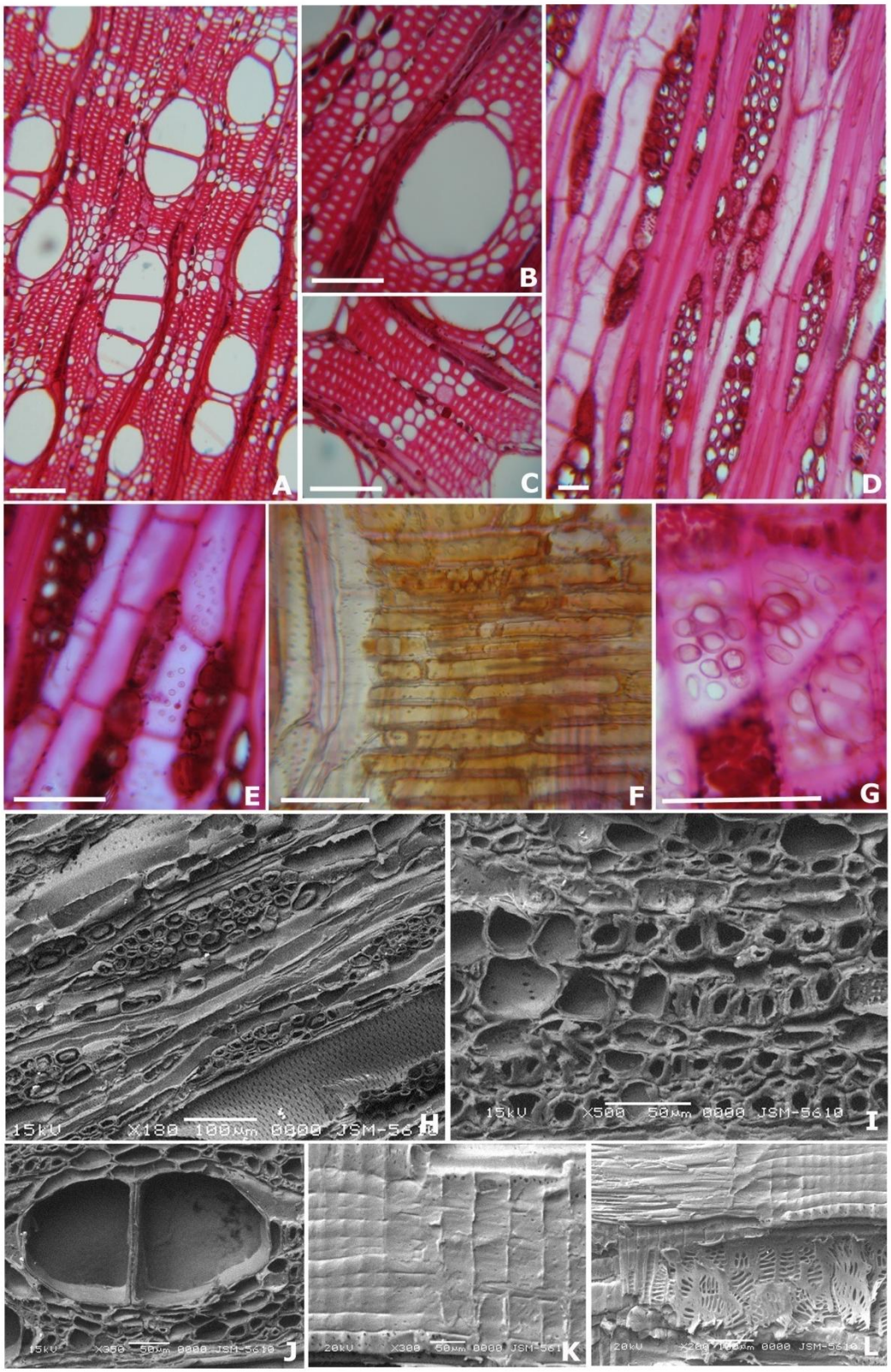

A
B
C
D
E
F
G
15kV X180 100µm 0000 JSM-5610
H
15kV X500 50µm 0000 JSM-5610
I
J
K
L

PLATE 1

Plate 2: Pattern of decay in *S. cuminii* wood infected with the *D. confragosa*

Magnification bar: A to E, H to R - 10 µm; F, G - 5 µm

Transverse view (A to I), Longitudinal view (J to L), Redial view (M to R)

A: Bore holes in axial parenchyma cells (arrow)

B: Medullary rays with bore holes and hyphae (arrow)

C: Buckling of axial Parenchyma cells (arrow)

D: Separation of wall between adjacent vessel element (arrow)

E: Degradation channel formed due to dissolution of middle lamella in the fiber region (arrow)

F: Numerous fine cracks observed in secondary wall of fiber

G: Cracks extend up to other cell wall layers

H: Middle lamella between ray parenchyma cells dissolved forming zig-zag channel (arrow) and some of them showing bore holes (arrow head)

I: Ray parenchyma cell with bore holes

J: Hyphae penetrating vessel element through pits

K: Bore holes in the ray parenchyma cells fused with each other forming large cavity

L: Fibers cells with bore holes

M: Middle lamella degraded separating the cells (arrow). Bore holes in the ray parenchyma cells

N: Hyphae penetrating from ray cell into the vessel element through pits

O: Crack in the cell wall of ray parenchyma cell

P: Fungal hyphae completely colonizing the ray parenchyma cell

Q: Hyphae traversing through pits and colonizing in vessel element

R: Large bore holes in the Fiber cells

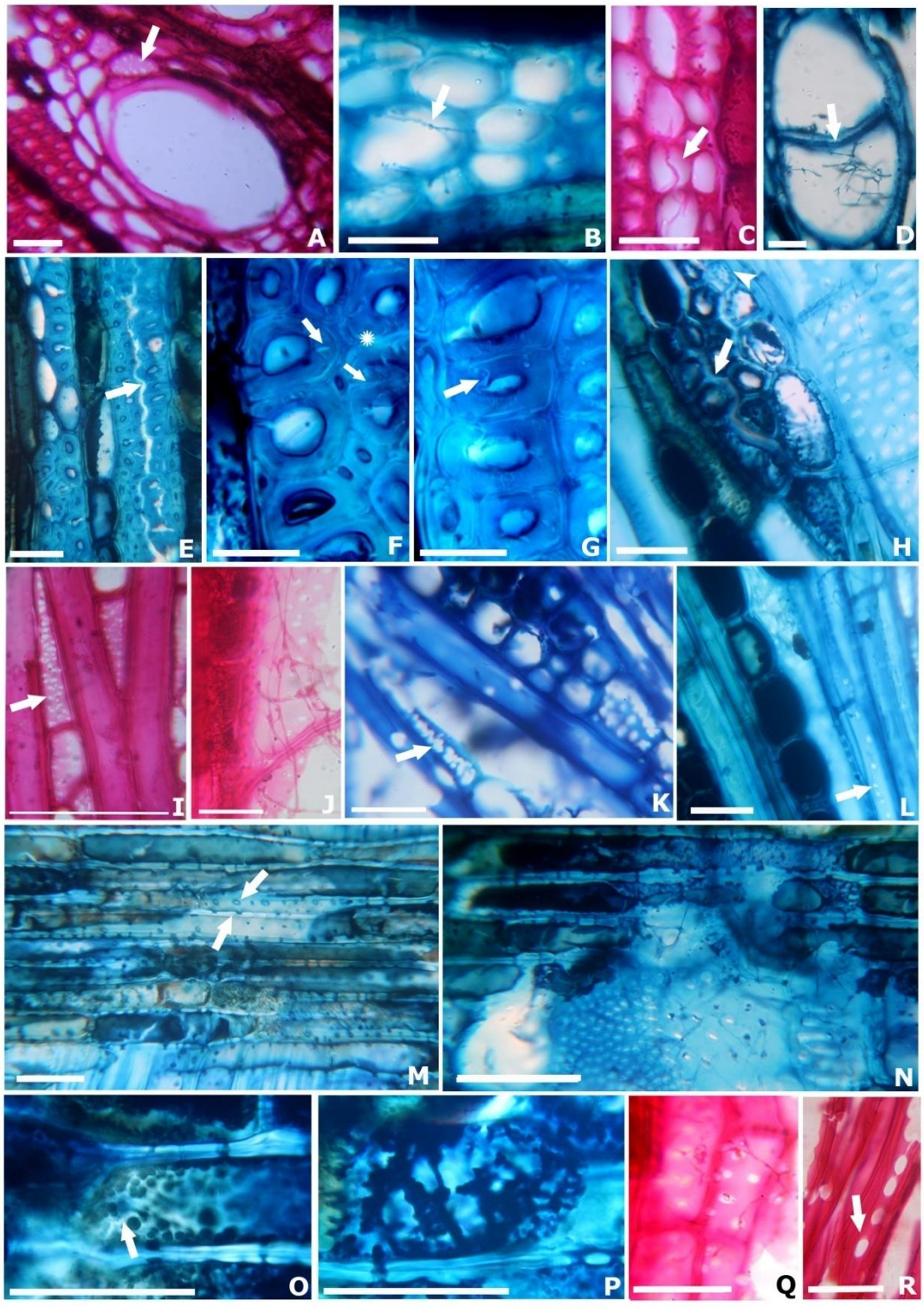


PLATE 2

Plate 3: Pattern of decay in *S. cuminii* wood infected with the *Daedaleopsis confragosa* (Scanning electron microsopy)

Transverse view (A to F), Longitudinal view (G to K), Redial view (L to N)

A: Bore holes in medullary rays (arrow)

B: Bore holes in axial parenchyma cells (arrow)

C: Buckling of parenchyma cell wall

D: Colonization of fungal hyphae is seen in the vessel element. Wall in between two adjacent vessel element is separated (arrow)

E: Degradation of S_2 layer of cell wall vessel element showing separation in the cell wall layers

F: Round to oval cavities in secondary wall of fiber (arrow)

G: Middle lamella between ray parenchyma cells dissolved and hyphae colonizing in the ray parenchyma cell. Arrow points to degraded wall of parenchyma cells

H: Fibers cells with bore holes (arrow head). Ray parenchyma cells completely degraded leaving behind only the outline

I: Separation in the cell wall of ray parenchyma cells due to preferential degradation of S_2 layer of the cell wall along with the middle lamella

J: Vessel element with colonization of hyphae

K: Hyphae penetrating in vessel element from the adjacent ray parenchyma cells

L: Ray parenchyma cells with bore holes in the cell wall

M: Coalescence of bore holes in the cell wall of the ray parenchyma cellss completely degradeding it

N: Colonization of hyphae in vessel element

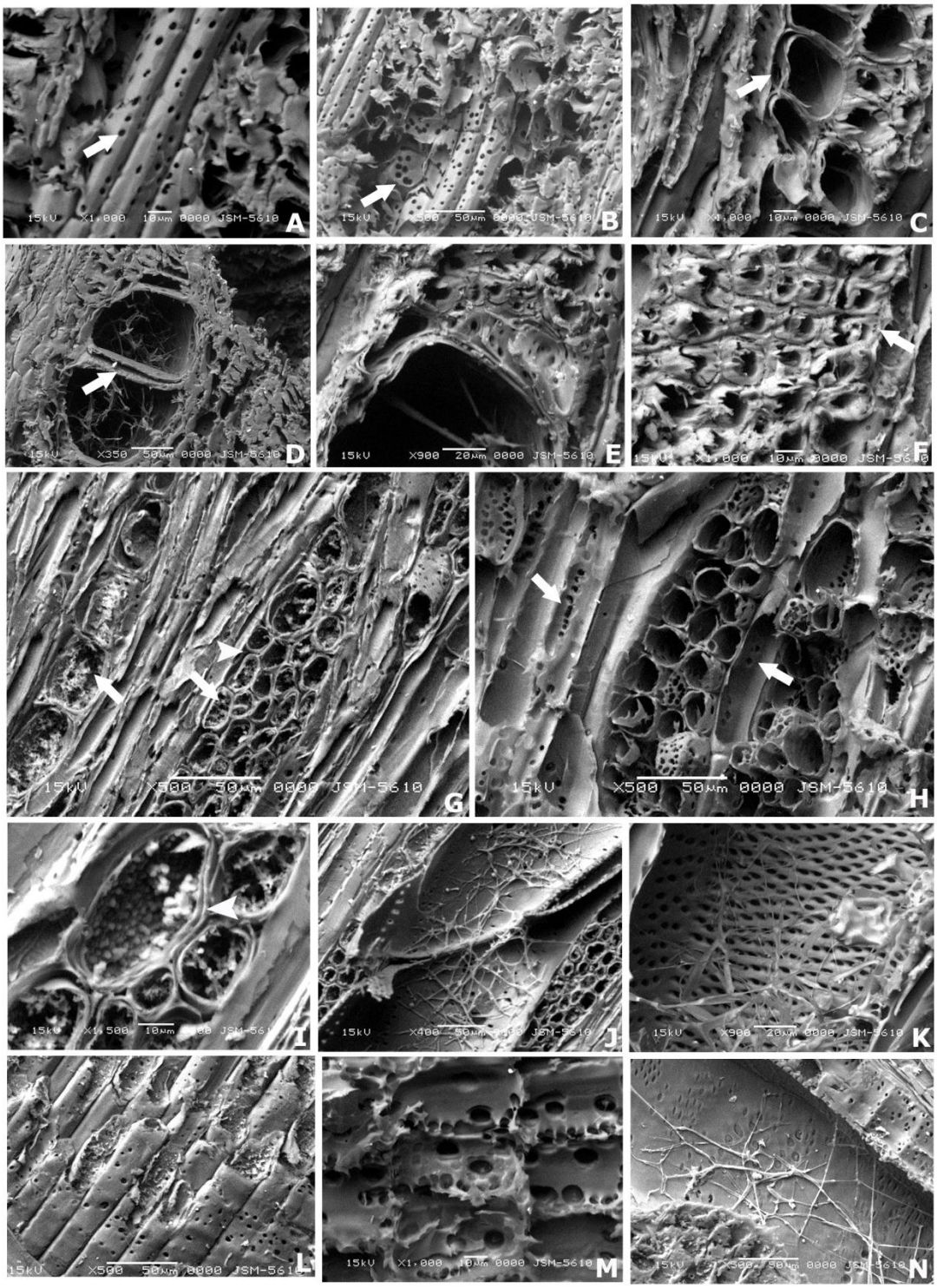


PLATE 3

Plate 4: Pattern of decay in *S. cuminii* wood infected with the *Phellinus pectinatus*

Magnification bar: A to E, G to L - 10 µm; F - 5 µm

Transverse view (A to F), Longitudinal view (G to J), Redial view (K to L)

A: Bore holes in the medullary rays and axial parenchyma cell (arrow)

B: Separation of vessel wall from the adjacent vessel wall by middle lamella dissolution (arrow)

C: Fiber cells shows crack in the S_2 layer of the cell wall (arrow)

D, E: S_2 layer of fiber cell wall degraded (arrow)

F: Buckling of fiber cell wall (arrow)

G: Bore holes in ray parenchyma cells

H: Ray parenchyma cells separated from each other forming zig-zag channel (arrow)

I: Hyphae penetrating the vessel element through pits

J: Middle lamella between the ray parenchyma cells dissolved

K: Bore holes are observed in ray parenchyma cells

L: Ray parenchyma cells colonized with fungal hyphae

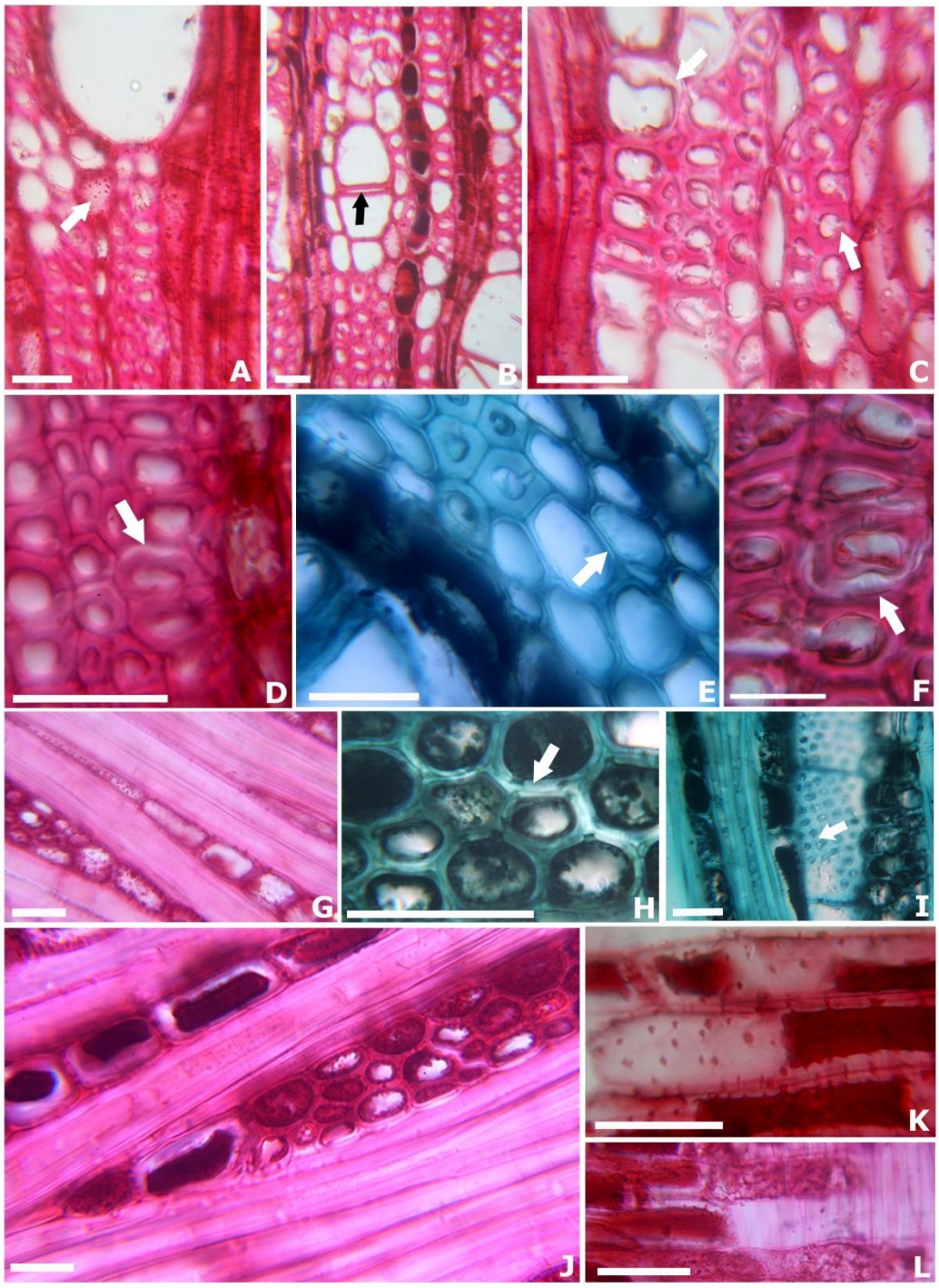


PLATE 4

Plate 5: Pattern of decay in *S. cuminii* wood infected with the *Phellinus pectinatus* (Scanning electron microscopy)

Transverse view (A to D), Longitudinal view (E to H), Redial view (I to L)

A: Wall between two adjacent vessel element separated

B: Buckling of vessel element due to degradation of the S_2 layer

C: Vessel element showing separation of cell wall layer

D: Fiber cell with middle lamella is intact and degradedS2layer

E, F: Middle lamella dissolved between the ray parenchyma cells

G: Colonization of hyphae in vessel element and penetration of hyphae through pits

H: Fiber cell remain intact

I: Bore holes in ray parenchyma cells

J: Ray parenchyma cells filled with fungal hyphae

K: Hyphae penetrating through pits

L: Thin fungal hyphae are clearly seen running in the lumen of fibers cells Perpendicularly (arrow)

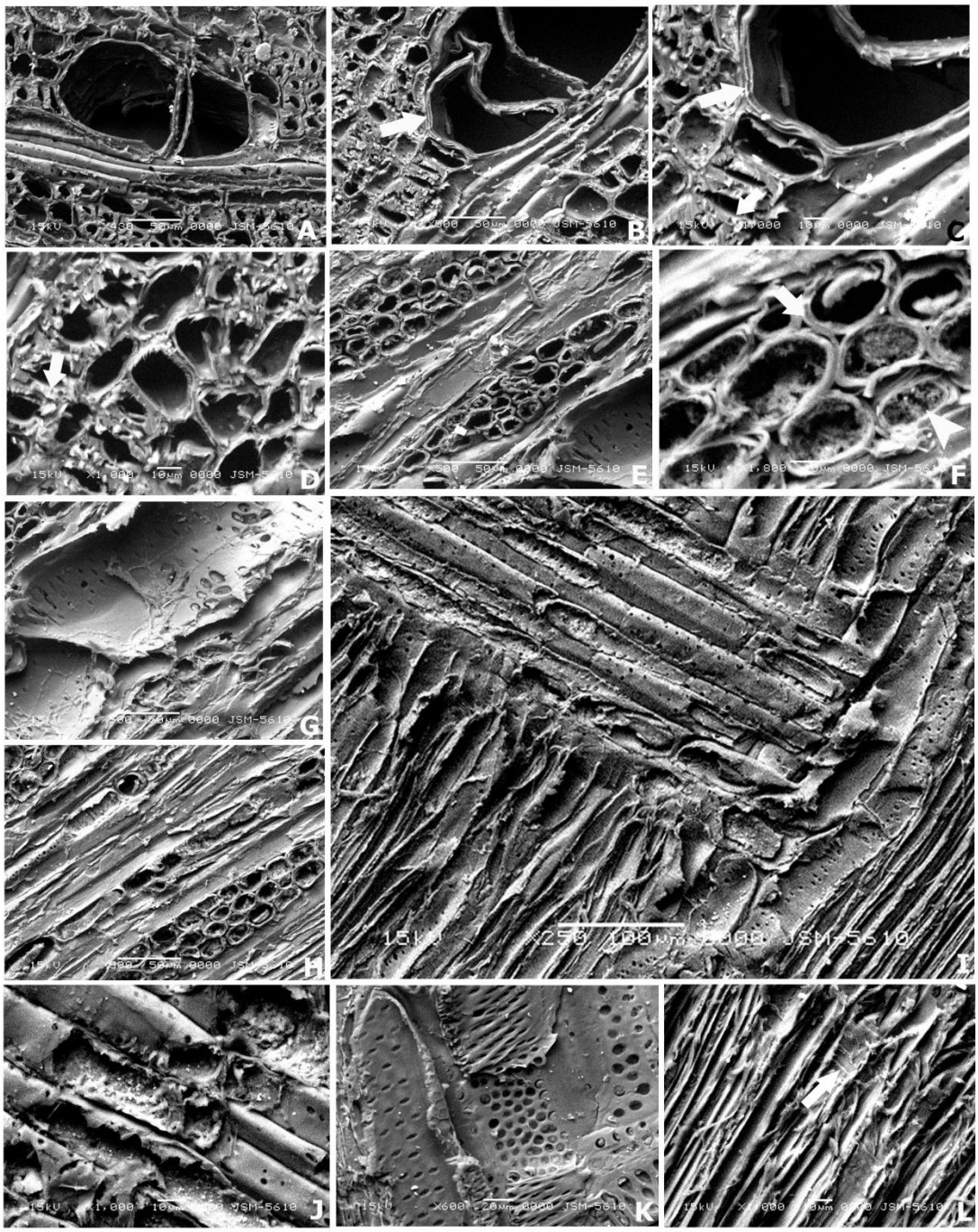


PLATE 5

Normal wood structure of *Bombax ceiba*

Heartwood is basically yellow or shades of yellow. Growth ring boundaries indistinct or absent. Wood is diffuse-porous. Vessel Solitary and outline is circular to oval. Apotracheal parenchyma is arranged in diffuse aggregates (Plate 6 A). Paratracheal axial parenchyma is scanty (Plate 6 D). Intervessel pits alternate (Plate 6 B, C). Septate and non-septate fibers present. Fibers thin to thick walled. Ray cells were multiseriate (Plate 6 E). Prismatic crystals are present in the parenchyma cells. All ray cells are procumbent (Plate 6 F).

Plate 6 G, H and I shows the SEM study of the normal wood structure of wood which shows the same characters as light microscopic study.

Pattern of decay in *Bombax ceiba* wood infected with the *Daedaleopsis confragosa*

Wood blocks of *B. ceiba* was found to be highly susceptible to the *D. confragosa* degradation. Weight loss was found to be maximum within a period of 12 months compared to all the other studied woods infected by *D. confragosa.* Wood of *B. ceiba* is very soft because it contains a large amount of parenchyma tissue which is mainly targeted by the *D. confragosa* and due to which it degrades faster. Plate 7, 8 and 9 shows different stages of degradation by *D. confragosa* in the wood of *B. ceiba.*

In the initial stage of decay *D. confragosa* mainly targets the parenchymatous tissue. In one months incubation period fungal colonization was pronounced in the axial parenchyma, medullary ray cells and also the vessel elements. The hyphae colonize in the vessel element (Plate 7 D) and penetration of hyphae is through the pits (Plate 7 M, N). Under the scanning electron microscopy it is clear that the hyphae penetrates

through pits and due to extensive colonization in very early stage of degradation, whole vessel element is completely filled with fungal hyphae (Plate 8 B; 9 A). Fiber cells remain intact without any alterations (Plate 9 B). By the end of one month bore holes begin to appear in all the wood elements including the fiber cells (Plate 7 A, P; 8 A). Longitudinal erosion cannels were observed in fiber region of the wood (Plate 7 F). Hyphae penetrated in the fiber cell, branched within the lumen and made bore holes in the cell wall (Plate 7 O; 9 D). SEM study of radial view also showed few bore holes (Plate 9 H, I).

Middle lamella begins to dissolve loosening cells (Plate 8 A arrow, F). In radial view hyphae passing through bore holes, completely colonizing it, were observed in the ray parenchyma cells (Plate 7 Q, R, S; 9 E, F, G). Along with the dissolving of middle lamella, secondary wall of the parenchyma cells also appeared to be separated concentrically or slit into cubical fragments at certain sites (Plate 9 B, arrowhead). Extensive delignification occurs in the secondary layer, leading to the accentuation of radial structures. Individual hyphae are recognizable (arrow), oriented in the direction of cellulose microfibrils. After penetrating into the cell wall, the hyphae orient their growth in the direction of cellulosic microfibrils separating it in a concentric manner (Plate 9 B arrowhead). Medullary rays also showed bore holes at this stage (Plate 7 C; 8 A) with some sites coalescence of these holes being prominent. Within 3 months the middle lamella was dissolved and axial, medullary and vessel elements walls showed buckling (Plate 7 E; 8 C). At the later stage of degradation the bore holes in ray parenchyma cells fused with each other forming large cavity and thining out of cell wall (Plate 7 B, L; 8 G, H). Bore holes appeared to coalesce forming large holes on the wall

of all the elements. Middle lamella between the parenchyma cells were degraded and erosion cannels were formed (Plate 7 F). In longitudinal view ray parenchyma cells showed zig-zag shaped erosion channel (Plate 7 J, K). During later stages of incubation period (9-12 months), degradation is pronounced in the parenchyma cells and only ruminants of the ray parenchyma cell walls were observed. The whole cell wall gets completely degraded with unrecognizable cells. Outline of the multiserrate rays could be observed in tangential view (Plate 9 H arrow, I). Middle lamella of the axial parenchyma was dissolved separating the adjacent cells. Along with this also large bore holes appeared in lumen (Plate 9 I).

The whole vessel element wall and surrounding cells were degraded completely (Plate 8 D). Fibers showed cracks in the secondary wall layer starting from the lumen and extending up to secondary wall, but at the same time the middle lamella was intact (Plate 7 G). *D. confragosa* besides degrading middle lamella, lignin from secondary wall is also degraded so the fiber cells become separated (Plate 7 H, I). Degradation of the fiber begins from the S_2 layer of the cell wall by forming small round to oval shaped holes and at the same time middle lamella is observed to be intact (Plate 8 E. F; 9 C).

Pattern of decay in *Bombax ceiba* wood infected with the *Phellinus pectinatus*

Plate 10 and 11 depicts the different stages in degradation of *B. ceiba* wood incubated with the white rot fungi *P. pectinatus*. *P. pectinatus* in wood blocks of *B. ceiba* mainly affected the axial and ray parenchyma. Degradation appeared to be very slow compared to *D. confragosa. P. pectinatus* appeared to degrade *B. ceiba* very slowly. During the initial stages of incubation period (upto one month) no signs of

degradation appear in any of the wood elements. Axial and ray parenchyma, vessel elements and fiber cells appeared to be intact.

At later stages of decay after incubation period of two months decay begins to appear in the form of bore holes and colonization of hyphae within the lumen of axial ray parenchyma cells and vessel element. At this stage the medullary ray cells as well as fiber cells are still intact. In all the three views (TS, TLS, RLS) it is observed that initially the fungus penetrates through axial parenchyma cells surrounding the vessel element by making bore holes and colonizing them (Plate 10 A, G, K; 11F, M). In axial parenchyma cells round and elongated bore holes were formed, and middle lamella between the axial parenchyma cells also get dissolved (Plate 11 A, C arrow (magnified view)). Bore holes in the ray parenchyma were larger in size (0.77 µm) compared to that of the axial parenchyma (0.54 µm). In some of the axial parenchyma cells it appeared to be formed on the lateral side of the cell (Plate 11 N arrow). In some of the ray cells bore holes fuse with each other resulting complete breakdown of ray parenchyma cells (Plate 10 H; 11 G). Leaving behind outline of the separated cells with complete hollow lumen (Plate 11 C arrow head).

Colonization of hyphae in the vessel element starts at this stage (Plate 10 B). Longitudinal and radial view clearly depicts that the penetration of the hyphae in vessel element is through the pits (Plate 10 I; 11 H, I, J). After penetration through the pits the fungal hyphae branch (Plate 11 H, J) and traverse longitudinally parallel to the lumen of the vessel element (Plate 11 I). In the ray parenchyma adjacent bore holes fuse forming erosion channels within the cell (Plate 11 G arrow head; 11 O). With further decay there is a complete fusion of the bore holes forming a large cavity within the lumen leaving

behind the outline (Plate 11 G asterix). Formation of spores/sporulation was observed within some of the ray cells (Plate 11 G arrow) a feature which was not observed in any of the other wood samples incubated by D. *confragosa* and *P. pectinatus* for a period of one year.

This fungus does not affect fibers even at this stage (Plate 10 D). The decay was not uniform, and practically unaltered tissues coexisted with serious damaged ones. At later advanced stages of decay within 9-12 months of incubation period the bore holes within ray parenchyma cells appear to coalesce forming larger holes. Vessel elements wall shows buckling. Towards the end of the degradation and separation of wall between the adjacent vessel element is observed (Plate 10 C; 11 B). Fiber cells show bore holes of very small size at this stage (Plate 11 G arrow). Fiber region showed longitudinal clefts or channels due to which the fiber cells are separated. But wall of the fibers still remained intact and unaffected (Plate 11 D, E). The middle lamella between parenchyma cells were dissolved, but middle lamella within fibers was not degraded (Plate 11 E). Oval shaped bore holes were observed in the S_2 layer of fiber cell wall leaving the middle lamella intact (Plate 11 D). With SEM, holes with irregular rims, and axially elongated troughs, alongside the growing hyphae could be easily observed (Plate 11 G arrowhead). Small bore holes arranged in a row were observed in the fiber cells at a very late stage of decay (Plate 10 J; 11 K). Delignification affected rigidity of the vessel walls and some collapsed vessel elements could be seen under SEM (Plate 11 B). The vessel elements are deformed due to loss of rigidity. In the fibers cells wall remains undegraded (Plate 10 L, M, N; 11 O).

Plate 6: Normal wood structure of *Bombax ceiba*

Magnification bar: A to F - 10 µm

A-F: Light microscopic view of the different elements

G- I: SEM view of different wood elements in different views

A: Solitary vessel and apotracheal parenchyma is diffuse aggregates

B: Alternate pitting in vessel element

C: Radial view shows pits in vessel element

D: Scanty paratracheal axial parenchyma

E: Multiseriate rays

F: Radial view showing the procumbent ray cells

G: Transverse view of apotracheal parenchyma

H: Longitudinal view of multiseriate ray

I: Radial view of ray parenchyma cells

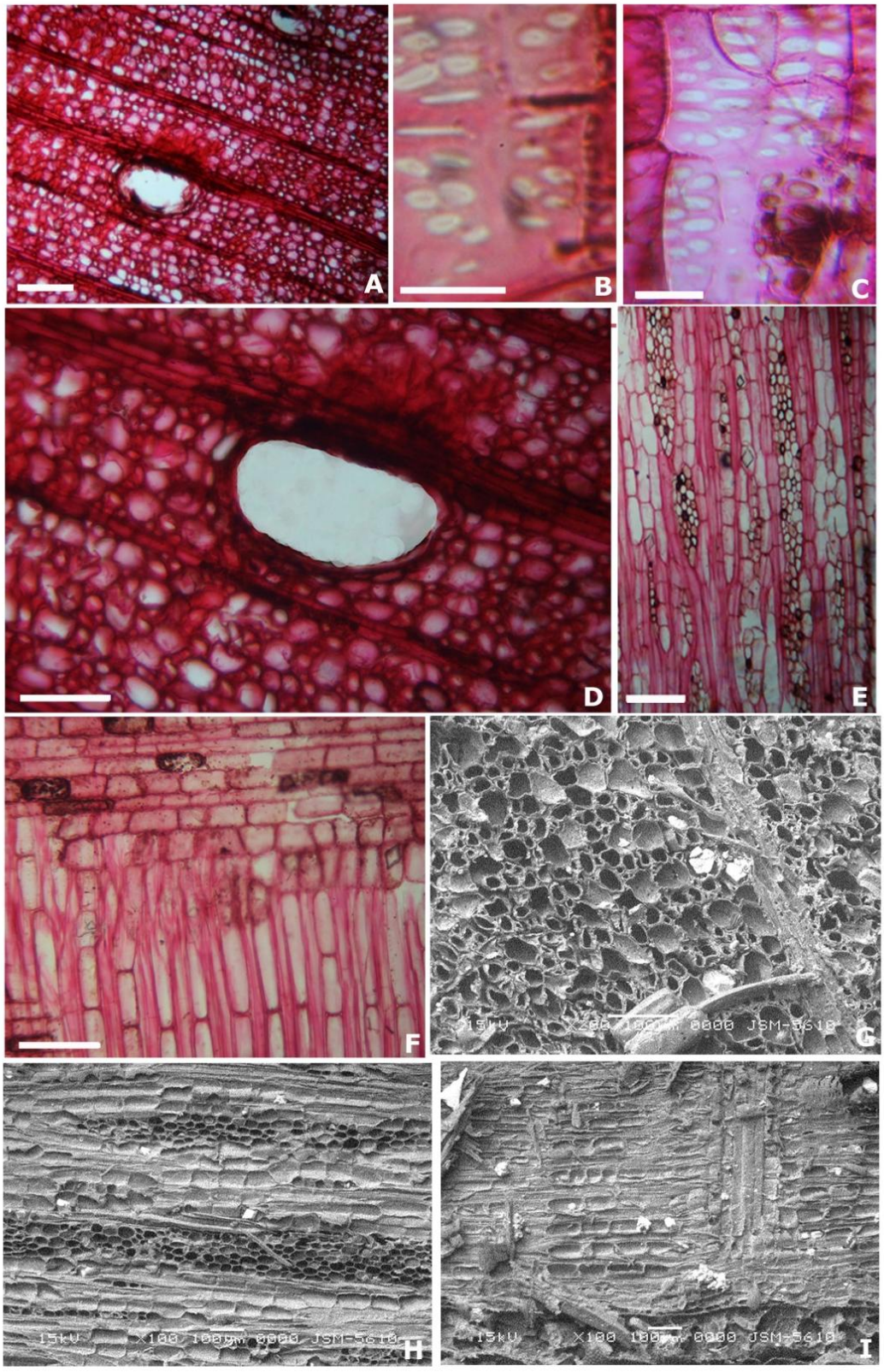


PLATE 6

Plate 7: Pattern of decay in *Bombax ceiba* wood infected with the *D. confragosa*

Magnification bar: A to S - 10 µm

Transverse view (A to I), Longitudinal view (J to O), Radial view (P to S)

A: Bore holes in axial parenchyma cells (arrow)

B: Medullary rays with bore holes

C: Medullary rays with fused bore holes forming large cavity

D: Hyphae penetrating through the vessel element

E: Buckling of vessel element

F: Longitudinal cleft or channel in fiber region

G: Fibers with cracks in the secondary wall layer and intact middle lamella

H, I: Middle lamella and secondary wall degraded separating fiber cells

J, K: Ray parenchyma cell with bore holes and middle lamella dissolved forming zig-zag shaped channels (arrow)

L: Bore holes in ray parenchyma cells fused forming large cavities

M: Hyphae penetrating vessel element through pits

N: Enlarged view of vessel element showing hyphal penetration

O: Hyphae penetrated within a fiber cell lumen

P: Ray parenchyma calls with bore holes and degraded middle lamella

Q: Hyphae traversing through ray parenchyma cells

R: Fungal hyphae penetrating in vessel element through pits

S: Colonization of hyphae in vessel element

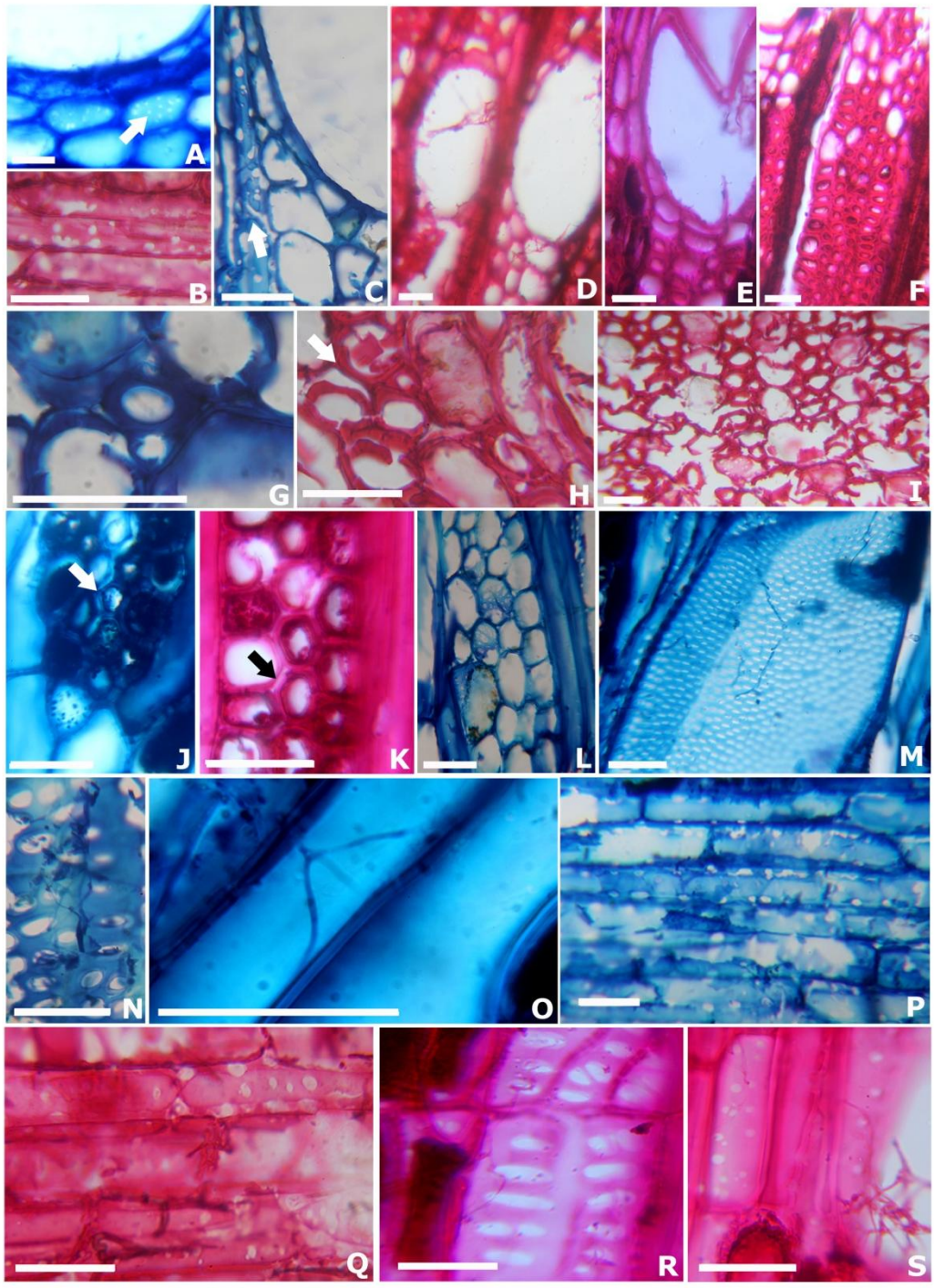


PLATE 7

Plate 8: Pattern of decay in *Bombax ceiba* wood infected with the *Daedaleopsis confragosa* (Scanning electron microscopy)

Transverse view (A to F), Longitudinal view (G, H)

A: Bore holes are seen in axial parenchyma and medullary rays. Arrow points to dissolved middle lamella

B: Vessel element completely filled with fungal hyphae

C: Buckling of vessel element and separation of wall between two adjacent vessel element (arrow)

D: Wood elements completely degraded with unrecognizable cell

E, F: Degradation of the fiber initiated in S_2 layer of the cell wall

G: Bore holes in ray parenchyma cells

H: Bore holes in ray parenchyma cells are fused with each other forming large cavity

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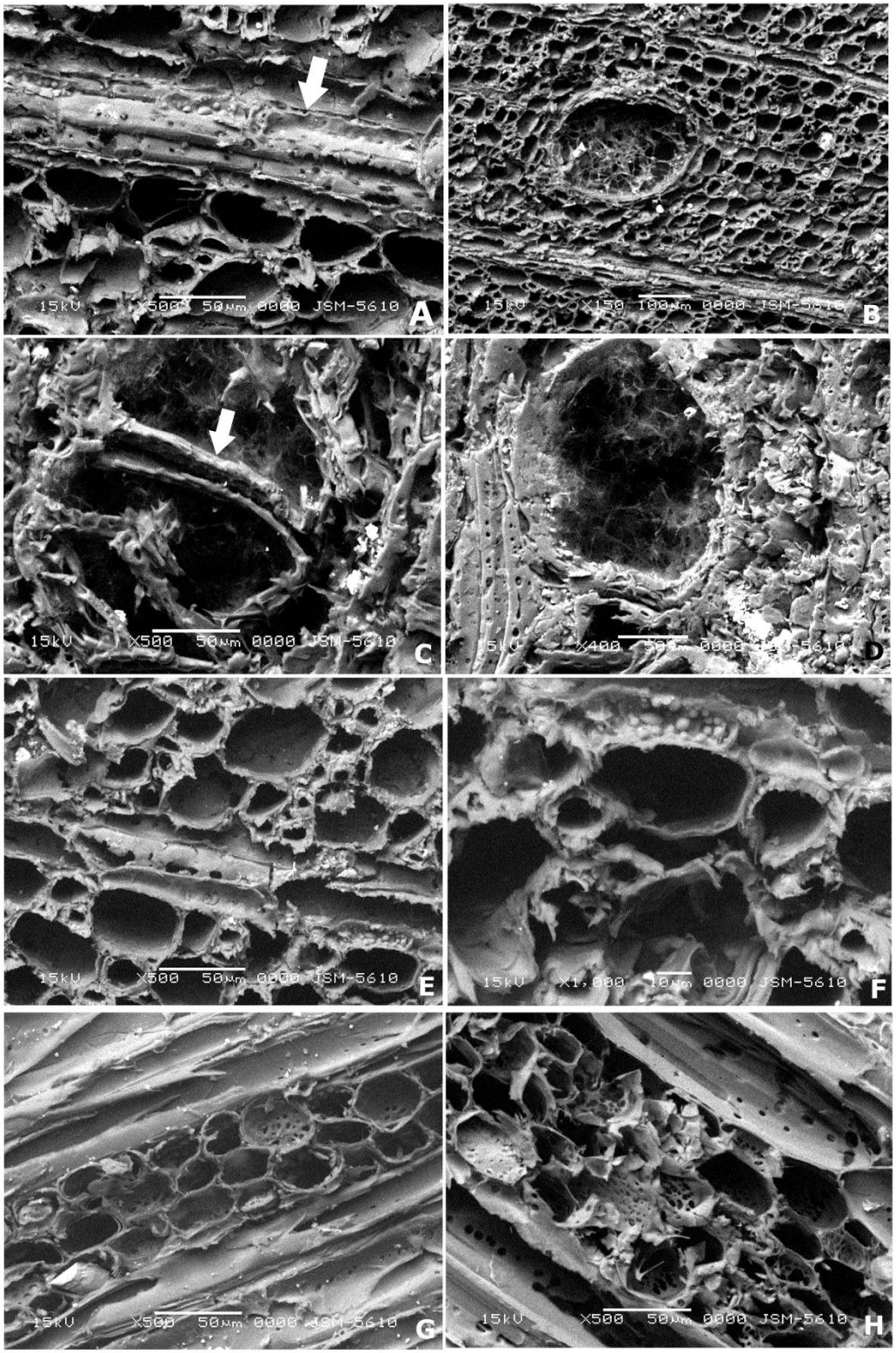
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A
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B
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D
15kV X500 50µm 0000 JSM-5610
E
15kV X1,000 10µm 0000 JSM-5610
F
15kV X500 50µm 0000 JSM-5610
G
15kV X500 50µm 0000 JSM-5610
H

PLATE 8

Plate 9: Pattern of decay in *Bombax ceiba* wood infected with the *Daedaleopsis confragosa* (Scanning electron microscopy)

Longitudinal view (A to D), Radial view (E to I)

A: Vessel element completely filled with thin hyphae

B: Axial parenchyma with bore holes and slits (arrow head)

C: Fiber cell with bore holes (arrow)

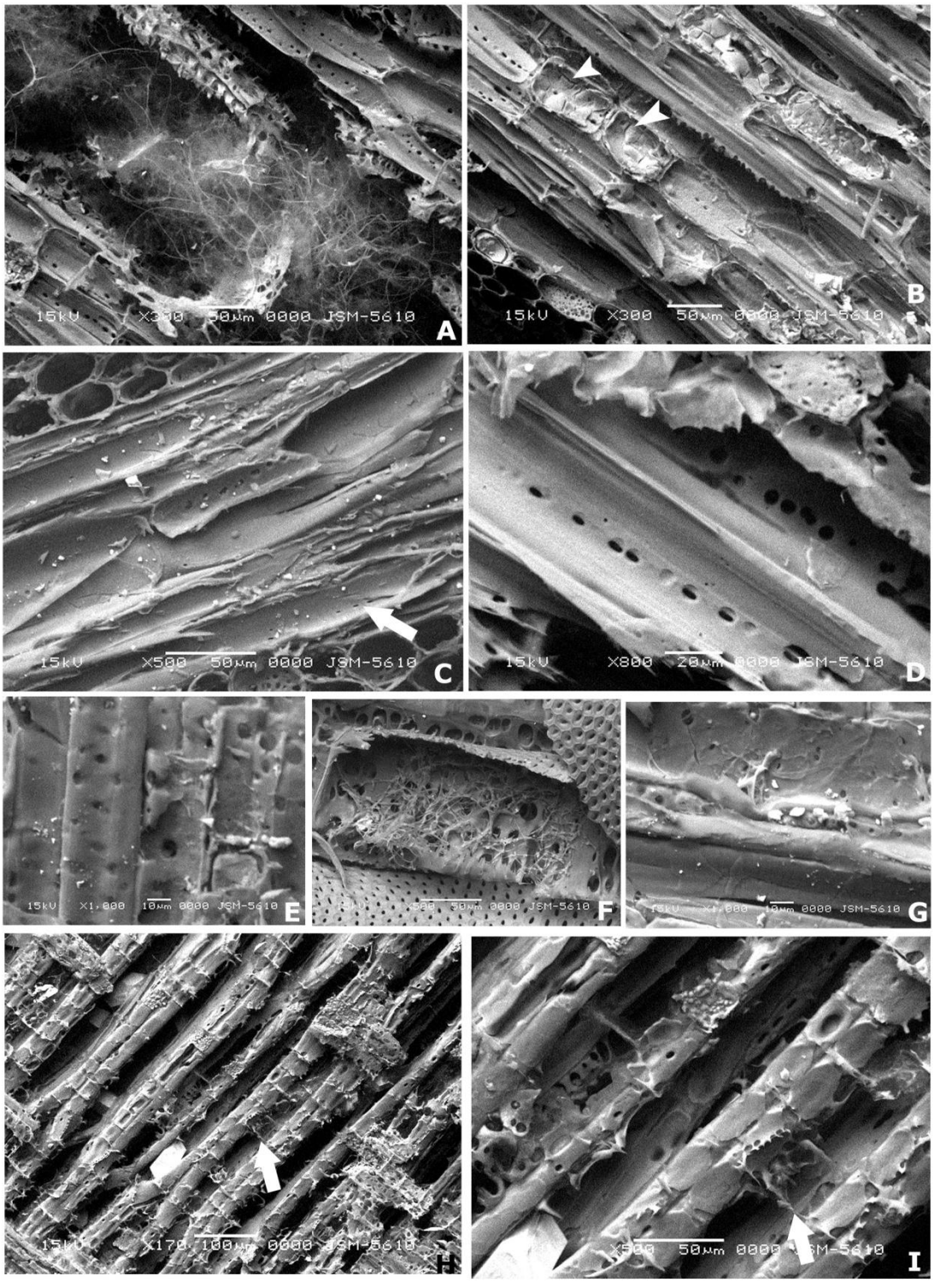
D: Many large bore holes in the fiber

E: Bore holes in ray parenchyma cells

F: Colonization of hyphae in the ray cell

G: Hyphae emerging out from bore hole in a ray parenchyma cell

H, I: Ray parenchyma cell completely degraded with only ruminants of the cell wall (arrow)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C
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D
15kV X1,000 10µm 0000 JSM-5610
E
F
G
H
I

PLATE 9

Plate 10: Pattern of decay in *Bombax ceiba* wood infected with the *Phellinus pectinatus*

Magnification bar: A to N - 10 µm

Transverse view (A to F), Longitudinal view (G to J), Radial view (K to N)

A: Parenchyma cells surrounding the vessel element with bore holes

B: Very thin hyphae colonizing the vessel element

C: Separation of wall between the two adjacent vessel element (arrow)

D: Fibers intact but parenchyma degraded

E, F: Middle lamella in the fiber region degraded forming longitudinal degradation channels

G: Bore holes with fungal hyphae in ray parenchyma cell

H: Fused bore holes leading to complete degradation of ray parenchyma cell

I: Penetration of hyphae through pits in vessel element

J: Small bore holes arranged in a row within the fiber cells

K: Fungal hyphae traversing through the ray parenchyma cells (arrow) and bore holes in the cell wall

L, M, N: Fiber cells undegraded till a long period of incubation

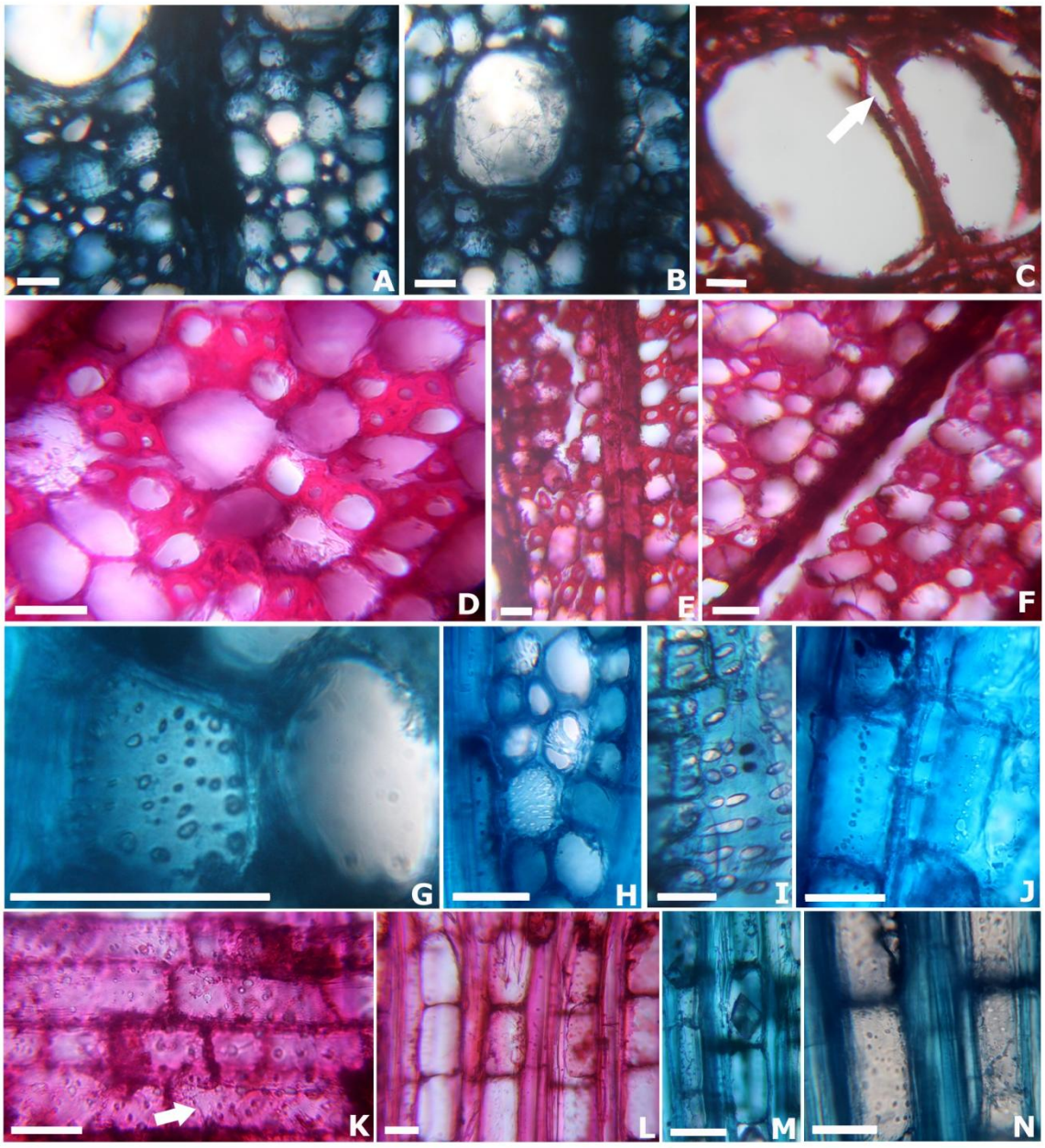


PLATE 10

Plate 11: Pattern of decay in *Bombax ceiba* wood infected with the *Phellinus pectinatus* (Scanning electron microscopy)

Transverse view (A to F), Longitudinal view (G to I, K), Radial view (J, L to O)

A, C: Bore holes in axial parenchyma cells and medullary rays (arrow) Middle lamella between the axial parenchyma cells dissolved

B: Two adjacent vessel wall disfigured and showing separation due to dissolution of middle lamella

D: Middle lamella between parenchyma cells dissolved and fibers intact. Large bore holes within parenchyma cells

E: Middle lamella dissolved at corners of the cell (arrow)

F: Ray parenchyma cells with bore holes (arrow)

G: Bore holes in ray parenchyma cells fused (arrow head) forming a large cavity (asterix). Fiber cells shows very small bore holes (arrow)

H, I, J: Hyphae passing through pits in the vessel element

K: Small bore holes in the fiber cells arranged in a row (arrow)

L: Penetration of hyphae in vessel element through pits

M: Ray parenchyma cells filled with fungal hyphae and a large number of bore holes

N: Axial parenchyma with bore holes (arrow)

O: Fiber cells intact (arrow)

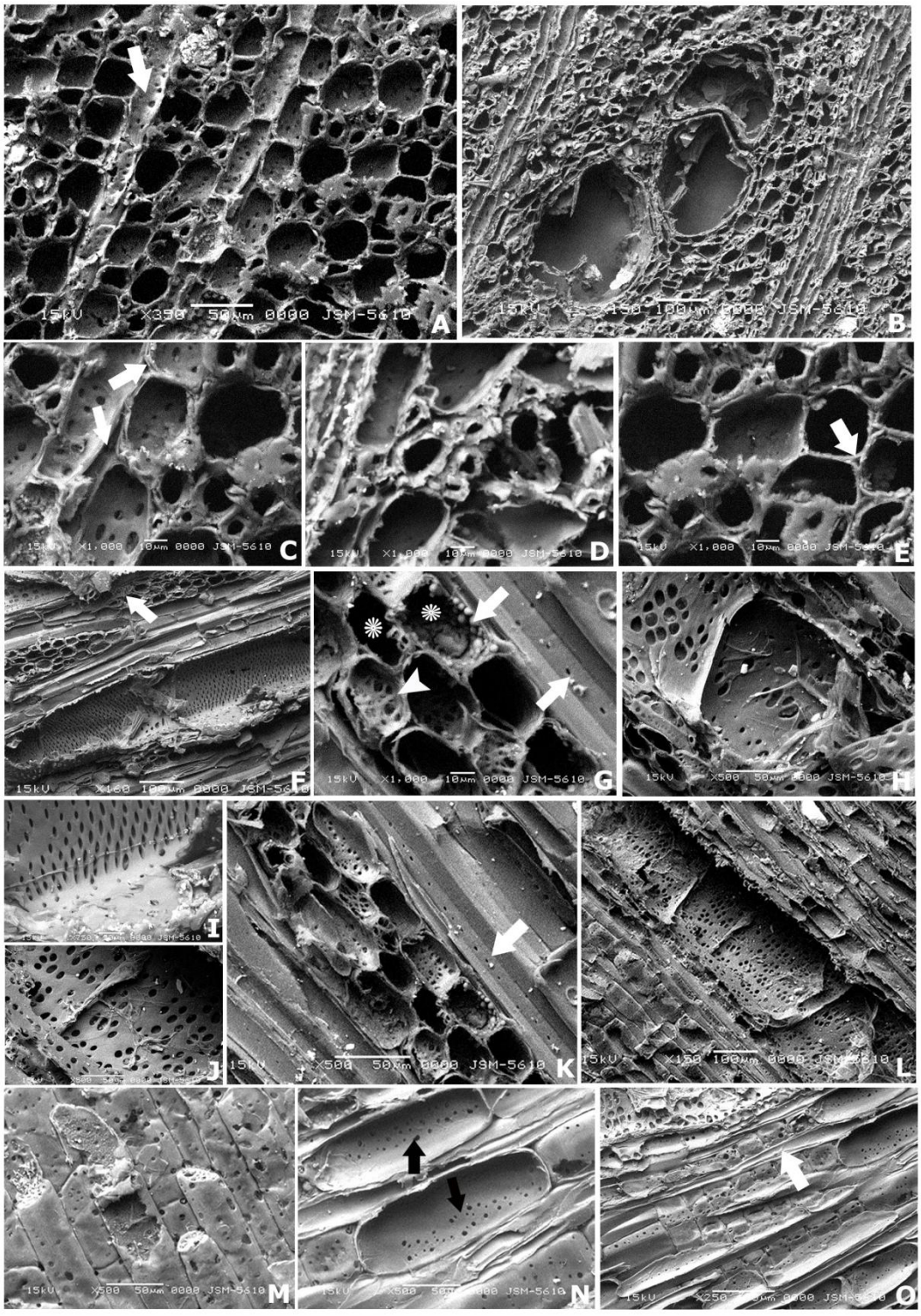


PLATE 11

Normal wood structure of *Mangifera indica*

The wood is yellowish white in colour. Growth ring boundaries is indistinct (Plate 12 A). Vessel elements have more or less the same diameter throughout the wood and hence the wood is diffuse porous. These elements are arranged in radial (Plate 12 B) or diagonal pattern (Plate 12 C).

Axial parenchyma is apotracheal and paratracheal. The apotracheal (axial parenchyma not associated with vessels) axial parenchyma are diffused in aggregates i.e. Parenchyma strands are grouped into short discontinuous strands forming short-discontinuous tangential or oblique lines (Plate 12 A arrow). Paratracheal axial parenchyma is ailiform (parenchyma surrounding with lateral extension which is elongated and narrow) (Plate 12 B arrow). The paratracheal axial parenchyma often coalesces and so is confluent (Plate 12 C arrow). Ray cells are uniseriate or biseriate (Plate 12 D). Cellular composition of rays includes procumbent body ray cells with one row of upright square marginal cells (Plate 12 E).

Fibers are non septate. Prismatic crystals are found present in nonchambered axial parenchyma cells, procumbent and upright ray cells (Plate 12 F, I).

Plate 12 G, H, I, J shows the normal features of the wood under the scanning electron microscopy.

Pattern of decay in *Mangifera indica* wood infected with the *Daedaleopsis confragosa*

M. indica wood is highly susceptible to the fungus *D. confragosa* (Plate 13 and 14). Similar to the observation *B. ceiba* the pattern observed here is that the fungus

mainly affects the ray parenchyma cells, invading and breaking it down completely by making bore holes in them (Plate 13 A; 14 B arrow). Bore holes begin to appear at the very initial stage of 10 days incubation period while in *Bombax* incubated with *D. confragosa* though colonization occurs within first 10 days of incubation period bore holes appear in parenchyma cells only after 30 days.

During initial stages of degradation itself all the parenchyma cells were extensively damaged by bore holes and it completely breaks down (Plate 14 C). Bore holes coalesce to form larger holes (Plate 13 C). *D. confragosa* has primarily affected the medullary rays. Fungal mycelium is clearly observed passing from the medullary ray (Plate 13 A) making bore holes in them (Plate 13 B; 14 A). At final stage of decay the bore hole fuse and degrade the medullary rays (Plate 13 C).

Initially ray parenchyma cells showed bore holes which were round or elongated and oval of 0.5 to 1 µm in size (Plate 13 K; 14 D), which during the later stages of degradation fuses with each other forming large cavities in the cell wall (Plate 13 L). Degradation increase with an incubation period and within 12 months of incubation period only the ruminants of the cell wall remained. In this stage the middle lamella between cells also appeared to be dissolved (Plate 13 M; 14 E arrow).

Radial view confirms that the ray parenchyma cells are the most affected cells of the wood. Both uniseriate and biseriate rays get completely degraded within 12 months of incubation. Initially they form the bore holes, and due to fusion of them in the later stage the whole cell wall was degraded (Plate 13 R; 14 K). With light microscopy and scanning electron microscopy in both tangential view (Plate 13 K; 14 D, E) and radial view (Plate 13 T; 14 H, K) holes with irregular rims and axially elongated troughs

(Plate 13 Q) alongside the growing hyphae could be easily observed. Cracks were easily distinguishable in the ray cells (Plate 13 Q arrow). At a later stages of decay the bore holes increased in size due to the fusion/ coalesce between adjacent bore holes (Plate 13 L, M; 14 K), and with advanced stages of decay the cells had been completely disintegrated leaving behind only remnants of the wall (Plate 14 I, J). Same pattern of degradation was observed in the axial parenchyma which could also be distinctly observed under both light and scanning electron microscopic observations (Plate 13 B, C; 14 I, J).

Vessel element lumen gets colonized by the fungal hyphae in 30 days incubation period. Fiber cells also at this stage shows separation in the middle lamella. Erosion troughs could be identified at certain sites. In the initial stage of degradation fungal hyphae enter the vessel elements through pits on the wall colonize in the vessel and degrade the vessel wall completely (Plate 13 A, N, S; 14 B, F). With the further advancement of degradation due to the dissolution of the middle lamella all the adjacent vessel elements were separated from each other completely, losing its rigidity and leading to buckling of the vessel element (Plate 13 D arrow; 14 B arrow). During pronounced stages of degradation, vessel wall is separated and it showed cleft separating it from the adjacent cells (Plate 13 E arrow). Plate 14 C shows a very high degradation of vessels element with only the crystals remaining intact. The wood block at this stage of decay was very soft. The compound middle lamella is not initially affected by the decay. Few degradation channels were formed due to the degradation of adjacent cell wall. In some regions canal like bore holes 0.33 µm in cross section appears, which connects the neighboring cells together (Plate 13 F; 14 I).

In the initial stage of decay fibers remains intact (Plate 13 O). Individual tracheids become separated from one another by preferential degradation of compound middle lamella (Plate 13 H arrow). Degradation of the middle lamella and degradation of secondary wall layers occurs simultaneously (Plate 13 J), due to which transverse and longitudinal decay channels were formed (Plate 13 G, I). Hyphae are seldom observed in the lumen of the fiber tracheids. Individual round to oval cavities occour in the secondary wall (Plate 13 H arrow head). The cell wall degradation occurs in the immediate surroundings of the hyphae from the lumen towards outwards. At some other sites, areas of compound middle lamella are broken down (Plate 13 G arrow head) along with changes in the secondary wall layer. Degradation of the compound middle lamella results in the tracheids becoming separated from their matrix and individual tracheids occur completely isolated from one another. This also leads to the buckling of cell wall (Plate 13 G arrow). Besides degradation of middle lamella, lignin is also broken down in the secondary wall, which thus appears to be discolored (Plate 13 G).

Longitudinal and radial view of wood fibers showed bore holes towards advanced stages of 12 months degradation (Plate 13 P arrow; 14 K). Scanning electron microscopic study showed all the parenchyma cells removed from the wood, fibers became separated and prominent unaltered crystals were observed (Plate 13 T; 14 G, H).

As the fungus *D. confragosa* has targeted the parenchymatous tissue of the wood the longitudinal view showed the ray parenchyma cells degraded completely.

Pattern of decay in *Mangifera indica* wood infected with the *Phellinus pectinatus*

P. pectinatus infected wood blocks of *M. indica* showed no signs of decay during the initial 20 days of incubation period. Plate 15 and 16 shows the micro photographs of the wood of *M. indica* degraded by the *P. pectinatus.* Decay begins with bore holes in the ray and medullary ray parenchyma cells. Followed by this the vessel elements were found to be affected in two months incubation period. Bore holes in the fibers were observed during the advanced stages of decay. Compared to *D. confragosa* the initiation and advance of decay is comparatively slow with *P. pectinatus*.

In the initial stages of degradation only ray parenchyma cells and medullary rays are degraded. Parenchyma cells present in the wood surrounding the vessel showed fungal hyphae invading though bore holes (Plate 15 A, G, K; 16 A, D) and degradation of middle lamella (Plate 15 L; 16 H arrow) is also observed. Medullary rays also showed bore holes (Plate 15 B; 16 A) but were fewer in number. The fungal hyphae in the medullary ray cells showed erosion channels (Plate 16 A arrow head) along the probably branched hyphal invaded pathway. Some of the ray parenchyma wall was completely degraded leaving behind only their outline (Plate 16 D; 15 J). At this stage the fiber walls are still intact (Plate 15 J arrow). With further decay fungal hyphae in the medullary ray cells showed erosion channels (Plate 16 A, G, I arrow) along the probably branched hyphal invaded pathway. In tangential view middle lamella between the individual ray parenchyma cells completely dissolved (Plate 15 H; 16 D, E).

Vessel element remains unaffected in the initial stage. Very few thin hyphae are observed to be colonizing in the vessel element within two months of incubation period through the pits (Plate 15 C, I, M; 16 F, I). Region of the wall adjacent to the hyphae

degrade along the pathway of the hyphae adjacent to pits forming cracks in the vessel element (Plate 16 F arrow). At the advanced stages of degradation vessel element due to degradation showed separation in the middle lamella (Plate 15 D; 16 B) and under higher magnification it is observed that the compound middle lamella between the vessel is degraded leading to buckling of the walls (Plate 16 B). Adjacent vessel elements were also distinctly separated (Plate 16 B arrow).

Fibers remains unaffected even after an incubation period of 6 months. Long decay channels in the fiber region were observed due to the degradation of middle lamella (Plate 15 E; 16 C arrow). Magnified view showed that the secondary cell wall of fiber cell is degraded and middle lamella is unaffected (Plate 15 F). At advanced stage of decay complete degradation of middle lamella between fiber cells were observed (Plate 16 C) and all the cells were separated from each other.

In the tangential and radial view after the 6 months of incubation period few hyphae passing though them were observed. After penetrating into the lumen hyphae traversed parallelly to the longitudinal axis of fiber cell (Plate 16 J arrow), which form bore holes after 12 months of incubation period (Plate 15 J, N). This observation could be confirmed under the scanning electron microscopic studies and it was also observed that the size of the bore holes was very small (Plate 16 G, K).

Plate 12: Normal wood structure of *Mangifera indica*

Magnification bar: A to F - 10 µm

A-F: Light microscopic view of the different elements

G- J: SEM view of different wood elements in different views

A: Indistinct growth boundaries

B: Vessels elements arranged radially showing ailiform parenchyma (arrow)

C: Vessel elements arranged diagonally showing confluent parenchyma (arrow)

D: TLS showing uni and biseriate rays

E: RLS showing a row of upright square marginal cells (arrow)

F: Prismatic crystal in axial parenchyma cells

G, H: Transverse view of wood elements

I: T. L. S. of wood elements. Prismatic crystals clearly observed

J: R. L. S. of wood elements

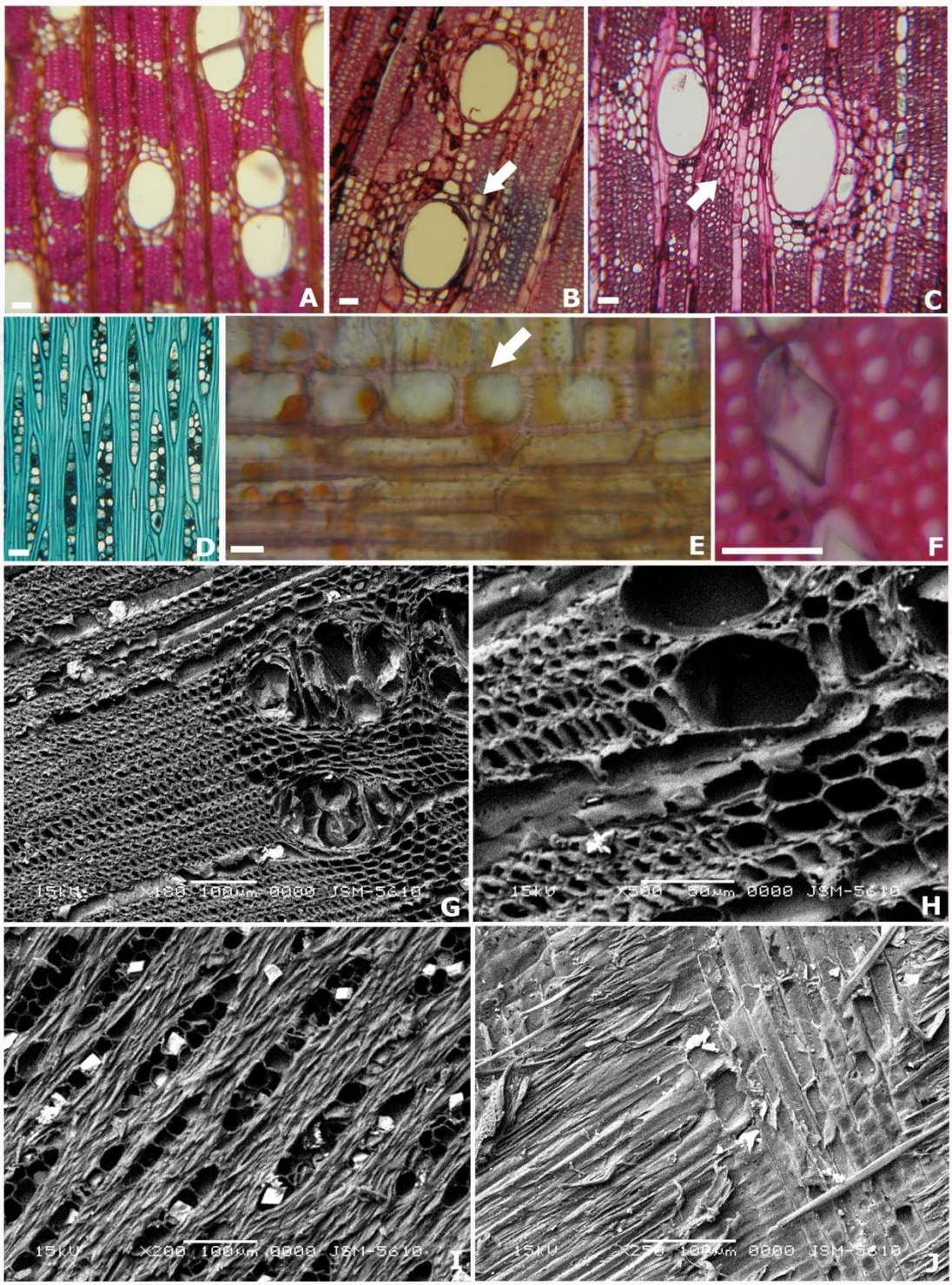


PLATE 12

Plate 13: Pattern of decay in *Mangifera indica* wood infected with the *D confragosa*

Magnification bar: A to E, G, H, J to O, Q to T - 10 µm; F, I, P – 5 µm

Transverse view (A to J), Longitudinal view (K to P), Radial view (Q to T)

A: Bore holes in ray parenchyma cells (arrow)

B: Bore holes in medullary ray cells (arrow)

C: Bore hole of medullary ray cells fused with each other to form a large cavities (arrow)

D: Buckling of vessel element

E: Cleft formed separating vessel wall from the adjacent cells (arrow)

F: Degradation channels formed by the degradation of adjacent cell walls

G: Degradation of middle lamella and secondary wall layers simultaneously. Degradation channel is formed due to dissolution of middle lamella (arrow head). Buckling of fiber cells (arrow)

H: Fiber cells with clefts formed due to degradation of S_2 layer of cell wall (arrow). round to oval cavities occur in the secondary wall (arrow head)

I: Magnified view of the degradation channel (arrow)

J: Simultaneous degradation of middle lamella and secondary wall layers

K: Initially observed bore holes in ray parenchyma cells

L: Bore holes in ray parenchyma fused with each other forming large cavities

M: Degradation of ray parenchyma cells with only the ruminant of the cell wall

N: Hyphal colonization within the vessel element

O: Very small bore holes in fiber cells

P: Fiber cells with large bore holes (arrow)

Q: Hyphae traversing through ray parenchyma and forming cracks in the cell wall (arrow)

R: Bore holes in the ray parenchyma cells.

S: Vessel element with colonization of the hyphae and penetration of hyphae through pits

T: Large number of bore holes in the fibers cells

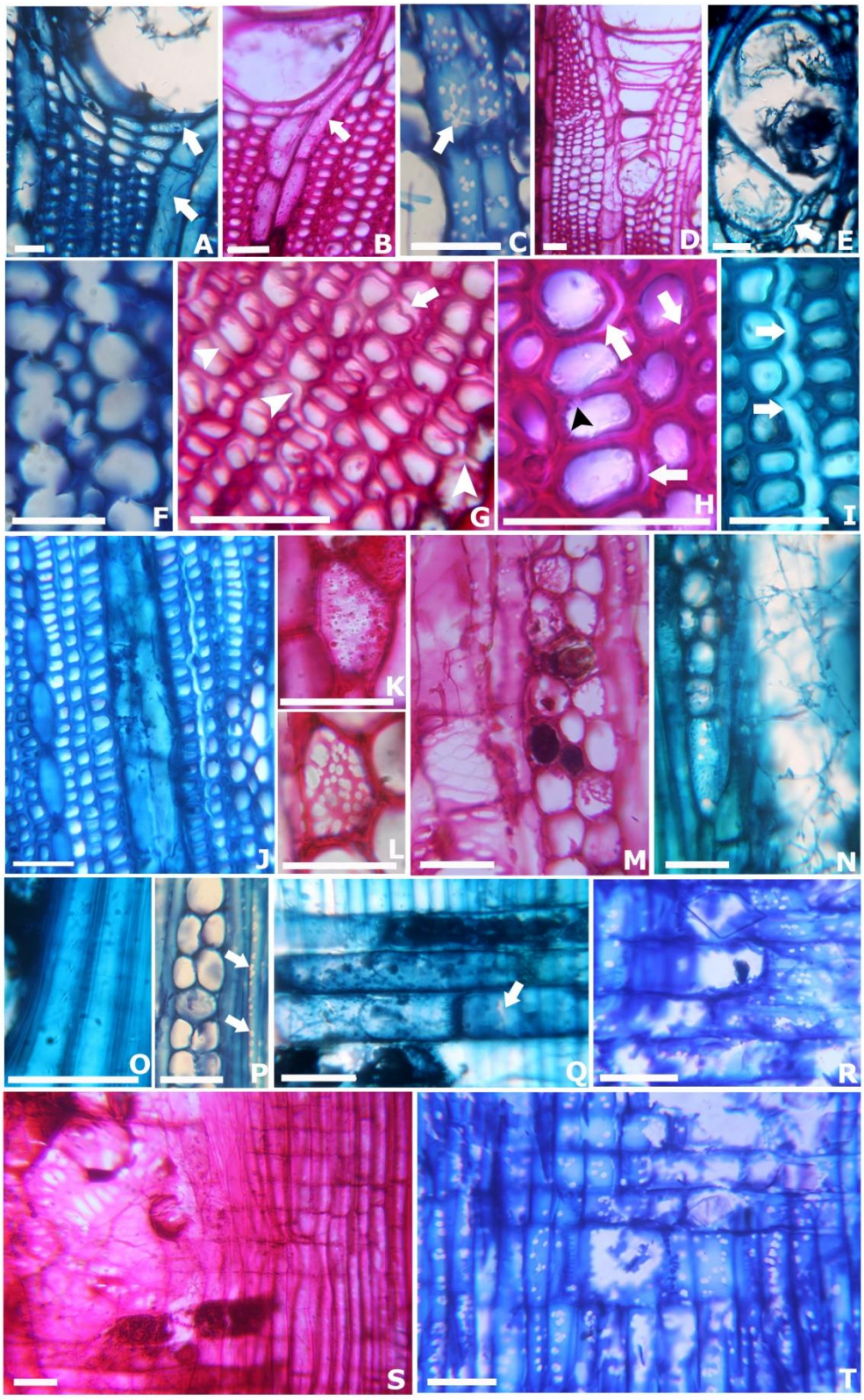


PLATE 13

Plate 14: Pattern of decay in *Mangifera indica* wood infected with the *Daedaleopsis confragosa* (Scanning electron microscopy)

Transvers view (A to C), Longitudinal view (D to G), Radial view (H to K)

A: Bore holes in medullary rays

B: Fungal hyphae colonized in the vessel element

C: Complete degradation of all wood elements

D: Bore holes in ray parenchyma

E: Remnants of the degraded cell wall and middle lamella between ray parenchyma dissolved

F: Hyphae penetrating in the vessel through pits and colonize them

G: Completely degraded wood elements

H: Bore holes in the ray parenchyma cells (arrow)

I: Completely degraded wood elements. Note the presence of large coalesced bore holes

J: Adjacent bore holes fused forming irregular rimed cavities

K: Ray cells and fiber cells completely degraded with large fused bore holes

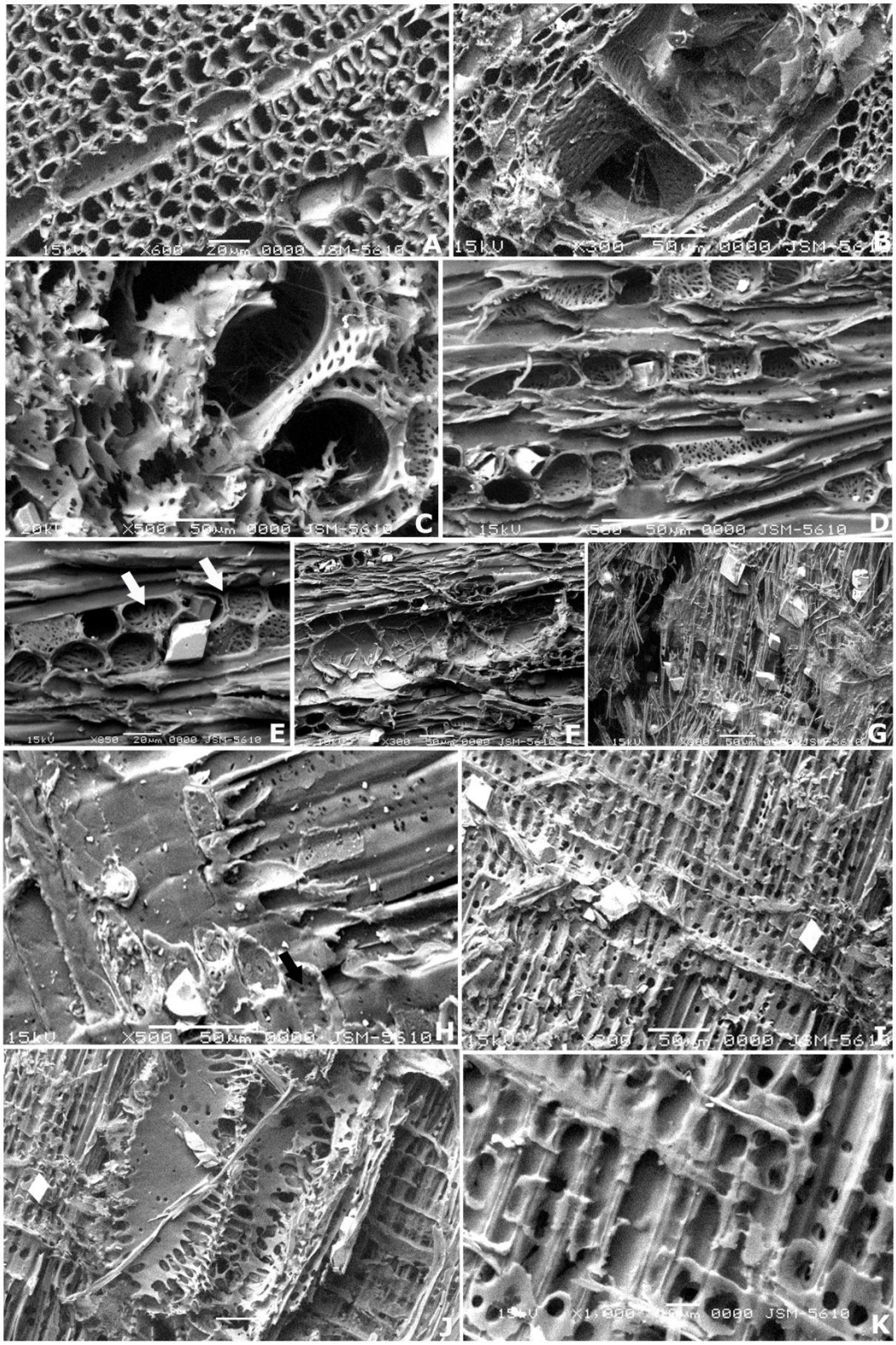


PLATE 14

Plate 15: Pattern of decay in *Mangifera indica* wood infected with the *Phellinus pectinatus*

Magnification bar: B to H, J to N – 10 µm; A, I – 5 µm

Transverse view (A to F) Longitudinal view (G to J), Radial view (K to N)

A: Bore holes in parenchyma cells

B: Meddullary rays with bore holes

C: Colonization of hyphae in vessel element

D: Vessel element wall separation due to degradation of secondary wall layers (arrow)

E: Degradation cannels formed in fiber region due to the separation of middle lamella

F: Magnified view of the degradation cannels

G: Ray parenchyma cells with small sized bore holes with fungal hyphae

H: Middle lamella between ray parenchyma cells dissolved forming zig-zag channel (arrow)

I: Hyphae penetrating the vessel element through pits

J: Fibers cells with penetration of hyphae in the lumen (arrow)

K: Ray parenchyma cell with bore holes

L: Middle lamella degraded separating ray parenchyma cells

M: Colonization of hyphae in the vessel element

N: Bore holes with hyphae in the wall of the fiber cells

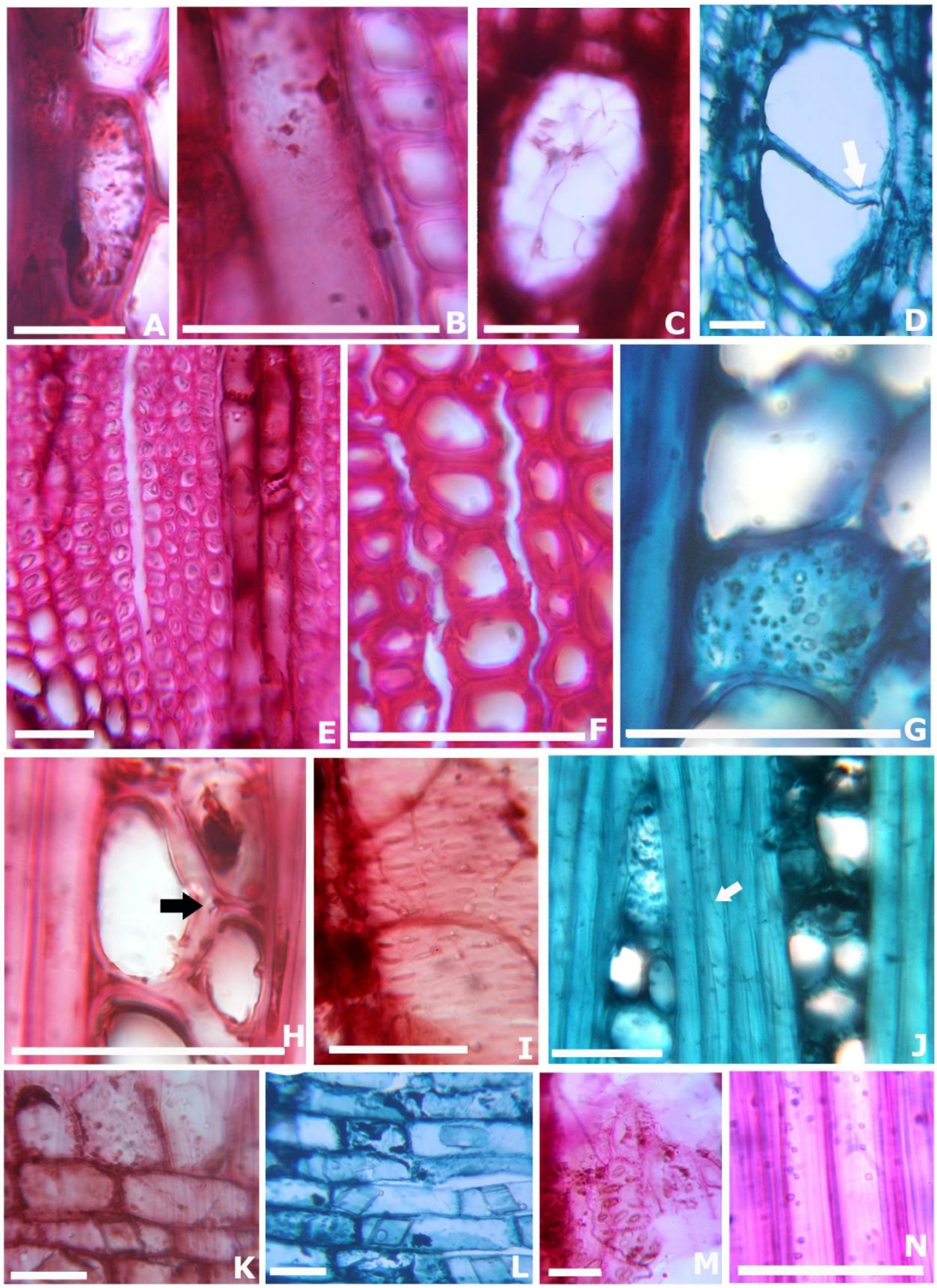


PLATE 15

Plate 16: Pattern of decay in *Mangifera indica* wood infected with the *Phellinus pectinatus*

(Scanning electron microscopy)

Transverse view (A to C), Longitudinal view (D to G), Radial view (H to K)

A: Bore holes are seen in parenchyma cells and medullary rays (arrow). Arrowhead points to cracks formed breaking wall into small cubical units (arrow head)

B: Buckling of vessel element wall (arrow)

C: Degradation channel in the fiber region (arrow)

D: Bore holes are fused and thinning of cell wall (arrow)

E: Ray parenchyma cell wall separated with completely degraded lumen

F: Colonization of hyphae (arrow) in vessel element

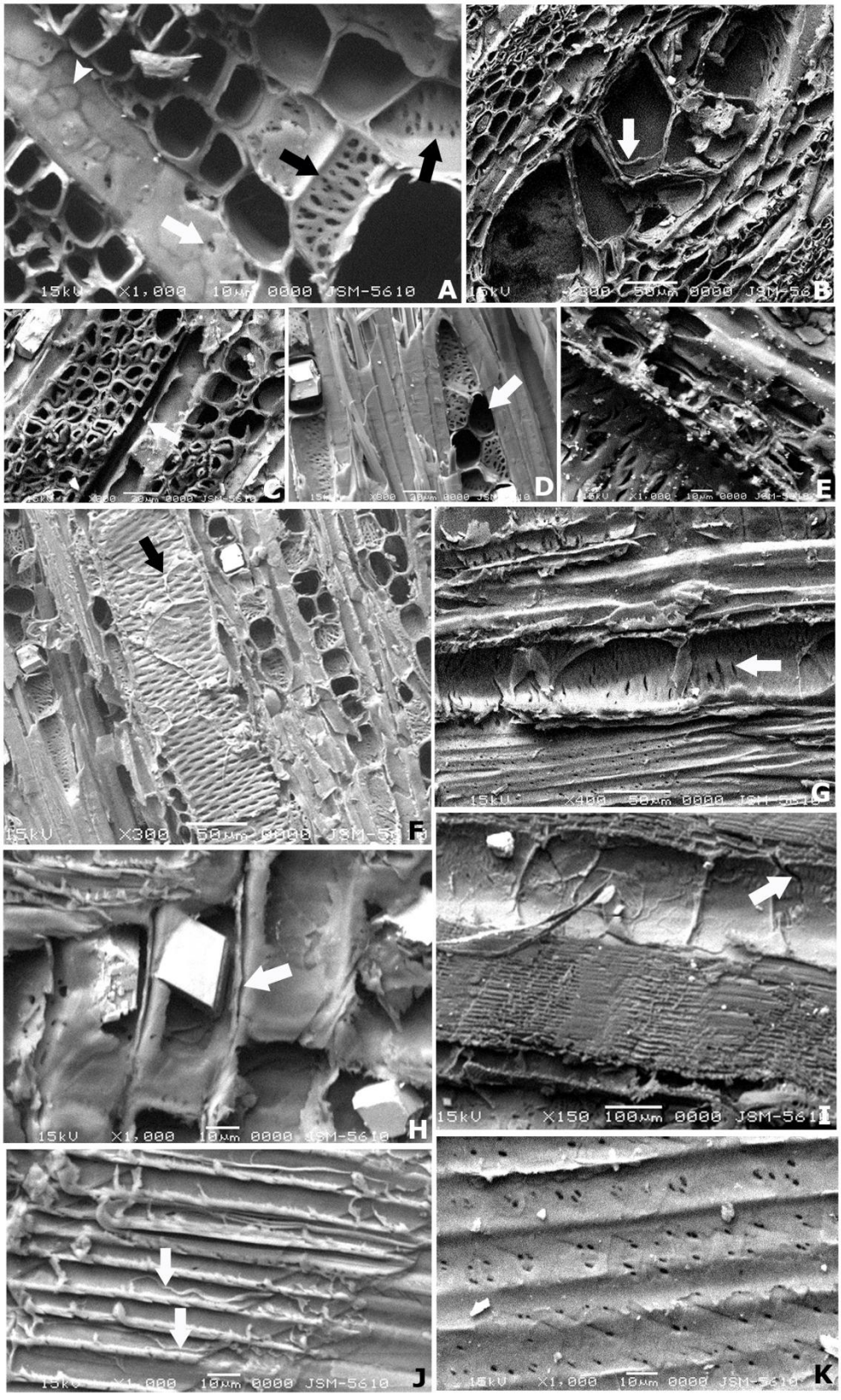
G: Small sized bore holes in the fibers and vessel element wall cracked (arrow)

H: Complete degradation of middle lamella (arrow) and bore holes in ray parenchyma cells

I: Vessel element colonized by fungal hyphae and vessel wall broken (arrow)

J: Hyphae passing parallel through the lumen of the fibers

K: Oval and elongated bore holes in the fiber cells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B
C
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E
15kV X300 50µm 0000 JSM-5 F
G
15kV X1,000 10µm 0000 JSM-5610 H
15kV X150 100µm 0000 JSM-561 I
J
15kV X1,000 10µm 0000 JSM-5610 K

PLATE 16

Normal wood structure of *Pithecellobium dulce*

Heartwood was red or shades of red in colour. Heartwood colour was found to be darker than sapwood colour. Growth ring boundaries indistinct or absent. Wood diffuse-porous (vessel elements have more or less same diameter throughout the wood). Vessel Solitary or in radial multiples of 2 common. Solitary vessel outline is circular to oval (Plate 17 A). Simple perforation plates are present.

Apotracheal and paratracheal axial parenchyma present Axial vasicentric, (Plate 17 A) around the vessels). Intervessel pits are alternate and polygonal in shape (Plate 17 D). Ray cells are uni - biseriate (Plate 17 B, C). They show bordered pitting. All ray cells procumbent (Plate 7 E, F).

Plate 17 G, H, I, shows the normal structure of wood under Scanning electron microscope.

Pattern of decay in *Pithecellobium dulce* wood infected with the *Daedaleopsis confragosa*

Unlike in *B. ceiba* and *M. indica D. confragosa* in *P. dulce* showed resistance to decay during the first month of incubation period. All the wood elements appeared to be intact. Only after one month of incubation period the decay is initiated by formation of bore holes in axial parenchyma cells. After a incubation period of 2 months vessel elements, fiber cells and medullary rays were still intact. Pattern of decay by *D. confragosa* in the wood of *P. dulce* is represented in Plate 18 and 19.

Under all the three different views (TS, TLS, RLS) similar features were observed. Decay started from the axial parenchyma cells in the form of bore holes

(Plate 18 A, N, S, P; 19 A, B, M) and middle lamella is dissolved at the corners of parenchyma cell (Plate 18 D arrow; 19 J, K arrow). Medullary rays also showed the bore holes (Plate 18 B). Hyphae passing through bore holes of the medullary ray was very clearly observed in transverse view (Plate 19 A). Indicating that the hyphae penetrated through parenchyma cells in the wood. At some sites in the parenchyma cells the secondary layer gets dissolved continuing into the adjacent cell forming erosion channels (Plate 18 A, E). Centrifugal direction of delignification with remnants of lignin in cell corners and portions of middle lamella and erosion troughs were observed at some sites (Plate 18 E, F). Cells get separated, lose its rigidity and also get deformed (Plate 19 H). Cell wall of the adjacent cells get completely dissolved and due to that a long degradation channel is formed (Plate 18 E, F). As the degradation increase the ray parenchyma cells showed complete degradation (Plate 19 L).

At later stages of decay (3 months incubation period) bore holes appear in the medullary ray cells also. Vessel elements get colonized with hyphae. Penetration of the hyphae and further its colonization take place through pits (Plate 18 C; 19 C, D), which at the end of the degradation shows separation of vessel wall (Plate 19 D, E). At this stage in both longitudinal and radial view the vessel elements show colonization of the fungal hyphae (Plate 18 J, R; 19 H, N). During severe stages of decay bore holes are larger in parenchyma (axial and medullary) cells. Oblique cracks appear in fiber cells which are perpendicular to the longitudinal axis of fiber cells (Plate 18 K; 19 H). With further stage of degradation larger cracks are noticed on the vessel wall (Plate 18 K, L, M; 19 H) along with bore holes which are large at some sites (Plate 19 I, N).

Fibers are affected only during the later stages of degradation (9 and 12 months). In transverse sections, delignification could be easily observed in fibers. Many of them showed a concentric delignification starting from the lumen surface (Plate 18 G, H, I). S_2 layer of these fiber cells get degraded while the middle lamella is still intact. Within the S_2 layer the enzymatic activity of the hyphae seems to advance preferentially between the lamellae following the directions of the fibrils, the cell wall collapsing into submicroscopic layers. This degradation further leads to individual lamellae of the inner secondary wall dissolving inwards to the lumen (Plate 18 H arrow head).

Samples exposed to fungi for 6 -12 months showed advanced delignification in the fiber cells and vessel elements. In the vessel elements erosion channels were observed (Plate 19 H, N arrow head). Fiber cells in longitudinal view showed obliquely arranged erosion troughs run parallel with the cellulose microfibrils (Plate 19 K). These erosion troughs were of varying diameter. Scanning electron micrographs showed these slits very prominently extending from the lumen outwards (Plate 19 F, G). Extensive delignification occurring in the S_2 layer leading to the accentuation of radial structures. It initiates with clefts in the S_2 layer. These radial structures /clefts increase in size with advanced decay and appear perpendicular to the middle lamella (Plate 19 G) at a later stage completely breaking down the S_2 layer (Plate 19 F arrow). In some of the fiber cells although hyphae are present within the lumen of fibers it shows inherent resistance to degradation.

In a tangential view fibers showed elongated radial slits arranged at a distance and parallel to one another following the direction of microfibrils (Plate 18 O arrow; 19

K). And it shows some probably secondary exudates filled in the rays as well as in fiber cells (Plate 18 Q). In radial view fiber shows very few bore holes (Plate 19 O).

Pattern of decay in *Pithecellobium dulce* wood infected with the *Phellinus pectinatus*

The pattern of decay followed by *P. pectinatus* on *P. dulce* wood blocks appear to be the same as that of *D. confragosa*, Plate 20, 21 and 22 shows the pattern of decay. Anatomical cell wall alterations were very well depicted. *P. pectinatus* showed initiation of wall degradation within the initial incubation period of 30 days unlike *D. confragosa* in which initial signs of degradation was noticed only after an incubation period of 2 months.

Initiation of the degradation begins by bore holes in both axial and medullary ray parenchyma cells (Plate 20 I, B). Within a period of 2 months incubation time erosion channels starts appearing in between the parenchyma cells extending in to the fiber tracheids (Plate 20 A, D, E; 22 A). Middle lamella between the ray parenchyma cells get dissolved at the corners initially (Plate 20 C arrow), followed by which the degradation progresses separating the adjacent cells (Plate 22 E). At some instances secondary wall layer appears to get separated rather than the middle lamella (Plate 21 B). In longitudinal and radial view bore holes in the ray parenchyma cells were observed (Plate 20 H, I; 21 C; 22 G, H). Due to enhancement of the degradation in the final stages, the whole cell wall of parenchyma cell is degraded (Plate 21 D).

At the later stages of degradation i.e. incubation period of 9 to 12 months cell wall alterations appeared to be very prominent. Vessel elements showed great resistance

against the fungal invasion and so very scarce colonization appeared in the lumen of vessel element, except they were separated from each other due to the crumbling of middle lamella (Plate 20 F; 21 H, I). Signs of erosion channels were observed in few vessel elements breaking its walls into small cubical fragments or units (Plate 22 C, I; 20 J, 21 E).

Fiber cells showed the most prominent alteration in the S_2 layer. Large concentric clefts appear in the S_2 layer distinctly separating it from the outer layer (Plate 20 G, H; 22 B). Longitudinal tangential and radial view of the fiber cells at the advanced stage of decay shows clear obliquely arranged troughs running parallel with the cellulose microfibrils (Plate 21 A, B, G; 22 F, J, K).

Transverse view of the fiber cells shows the separation of microfibrils into submicroscopic microfibrils (Plate 20 H). Bore holes arranged in a row is observed in radial view (Plate 21 F). Radial slits and presence of fungal hyphae within the lumen was also noticed (Plate 20 H arrow head).

Plate 17: Normal wood structure of *Pithecellobium dulce*

Magnification bar: A to F - 10 µm

A-F: Light microscopic view of the different elements

G- I: SEM view of different wood elements in different views

A: Growth boundary indistinct and vasicentric axial parenchyma cells

B: Biseriate rays

C: Uniseriate rays

D: Intervessel pits alternate and polygonal in shape

E, F: Ray cells procumbent

G: Transverse view shows vessel element

H: Longitudinal view of normal wood

I: Radial view of normal wood shows ray cells

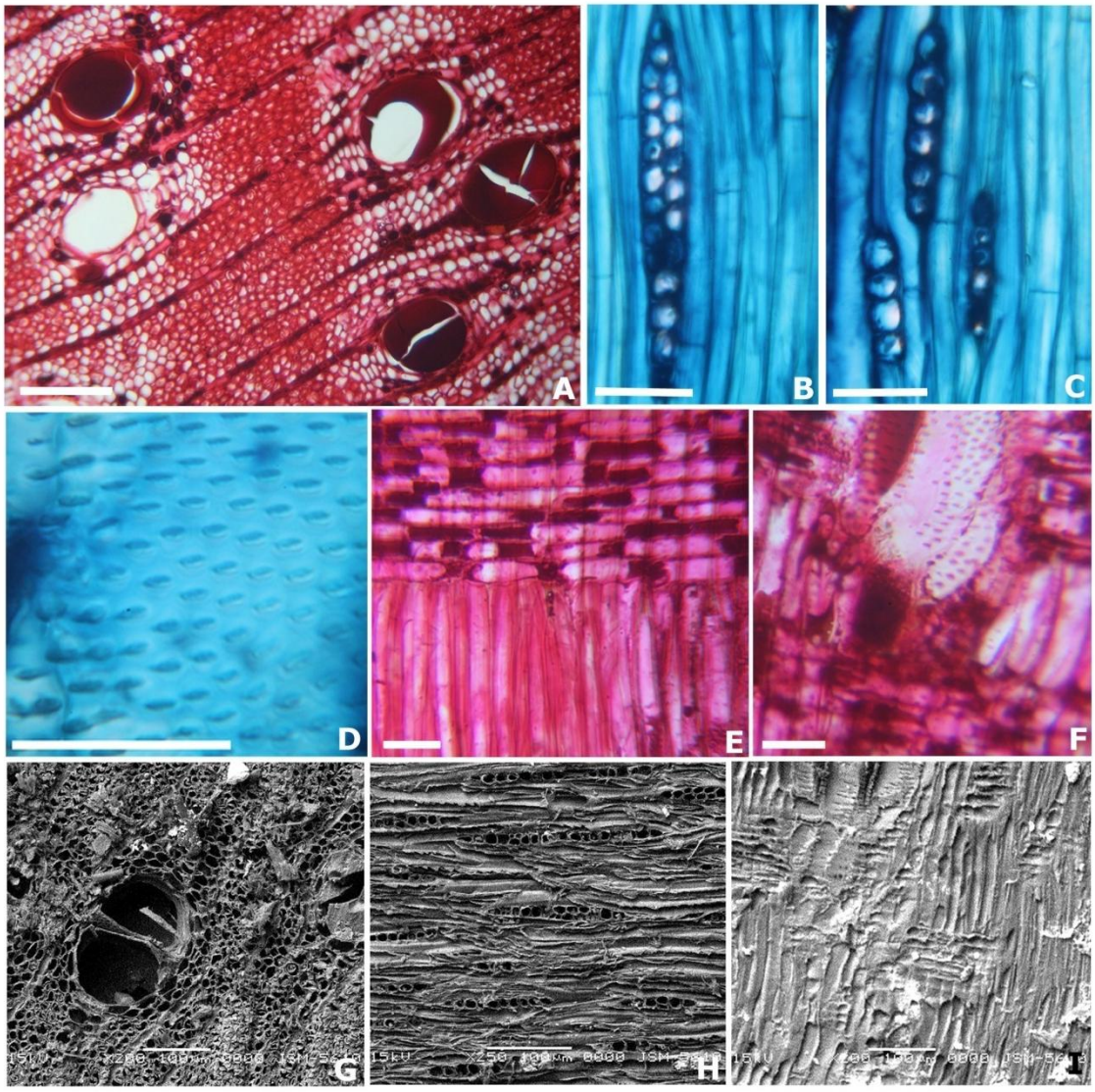


PLATE 17

Plate 18: Pattern of decay in *P. dulce* wood infected with the *D. confragosa*

Magnification bar: B to J, L to T - 10 µm; A, J – 5 µm

Transverse view (A to I), Longitudinal view (J to O, Q), Radial view (P, R to T)

A: Axial parenchyma cells surrounding the vessel element showing bore holes

B: Medullary rays with bore holes

C: Hyphae colonized in the vessel element and separation between the adjacent vessel wall (arrow)

D: Middle lamella at the corners between the parenchyma cells dissolved (arrow)

E, F: Long degradation channel formed in parenchyma region by degrading entire wall (arrow)

G, H: Secondary wall of the fibers with clefts (arrow) loosening the inner wall layer. Splits starting from the lumen extending up to S_2 layer of the cell wall (arrow head)

I: Fiber cells showing loosening of the cell wall

J: Fungal hyphae colonized in the vessel element

K: Hyphae penetrated through pits in the vessel element and cracks are observed in the vessel element wall

L, M: Vessel wall degradation increased with large cracks (arrow)

N: Ray parenchyma cells with bore holes

O: Fiber cells with elongated radial slits (arrow)

P: Ray parenchyma cells with bore holes

Q: Exudates filled in the ray cells and fiber cells

R: Hyphae penetrated in the vessel through pits

S: Ray parenchyma cells with cracks

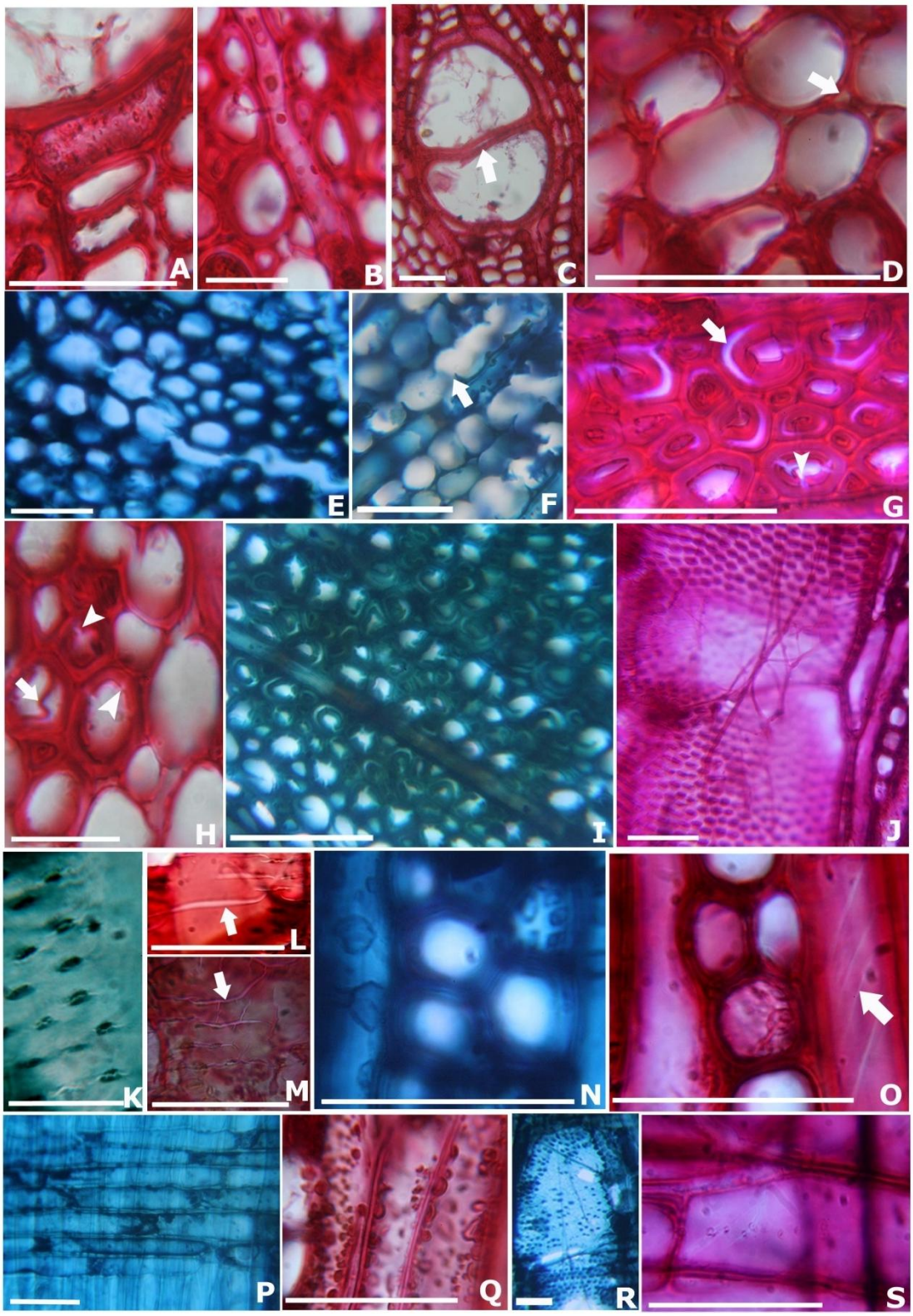


PLATE 18

Plate 19: Pattern of decay in *Pithecellobium dulce* wood infected with the *Daedaleopsis confragosa* (Scanning electron microscopy)

Transverse view (A to G), Longitudinal view (H to K), Radial view (L to O)

A: Degradation channel formed between parenchyma cells

B: Middle lamella in between the ray parenchyma cell dissolved

C: Hyphae colonized in the vessel element

D, E: Separation of adjacent vessel wall (arrow)

F: Clefts formed on fiber wall (arrow)

G: Secondary wall of the fibers with numerous clefts and splits starting from the lumen extending up to S_2 layer. Fibers walls at some site completely broken down forming a large cavity (arrow)

H: Vessel element with cracks and colonized by fungal hyphae

I: Big holes formed in the vessel element wall

J: Middle lamella in between the ray parenchyma cell dissolved

K: Adjacent ray parenchyma cells separated (arrow) due to dissolution of Middle lamella. Fiber cells with rradial slits

L: Complete degradation of ray parenchyma cells

M: Bore holes in ray parenchyma cells and their separation (arrow)

N: Vessel wall degraded with large cavity (arrow) and with cracks perpendicular to its longitudinal axis (arrow head)

O: Bore holes in fibers cells

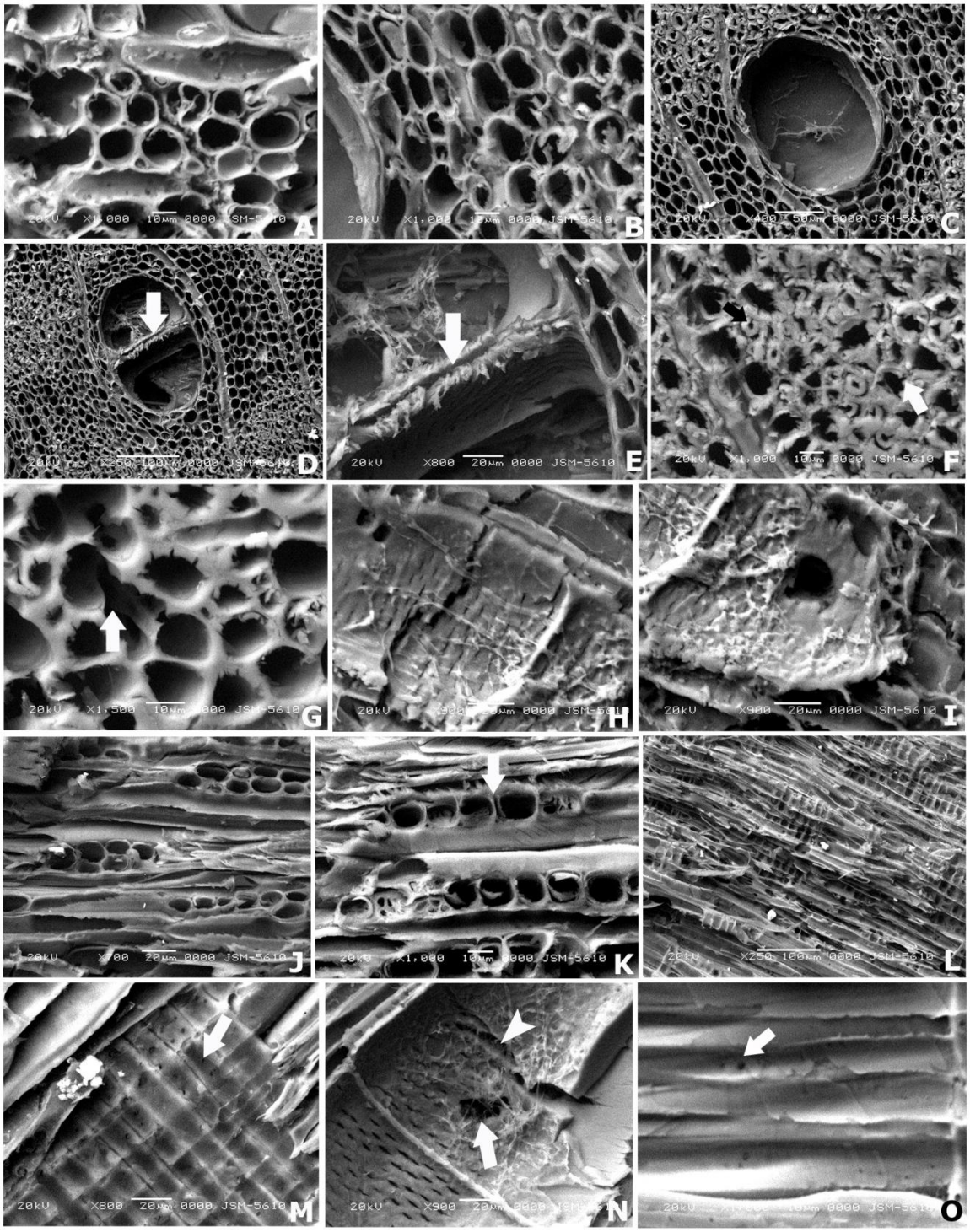


PLATE 19

Plate 20: Pattern of decay in *Pithecellobium dulce* wood infected with the *Phellinus pectinatus*

Magnification bar: A to J - 10 µm

Transverse view (A to H), Longitudinal view (I to J)

A: Decay channels formed due to degradation of middle lamella between them

B: Bore holes with fungal hyphae in medullary rays (arrow)

C: Middle lamella between the parenchyma cells dissolved (arrow)

D: Degradation channels separating the cells but with intact cell wall

E: Secondary wall layer of axial parenchyma cells separated in a concentric manner

F: Wall between two adjacent vessel element separated (arrow)

G: Bore holes in secondary wall layer of the fiber cell (arrow)

H: Cracks in fiber cells (arrowhead) extending from lumen towards outer wall. Also concentric clefts separating S_2 layer from outer layer observed (asterix)

I: Bore holes in the ray parenchyma cells

J: Hyphae penetrated in to the vessel element through pits and forms crack in vessel wall starting from one pit to another

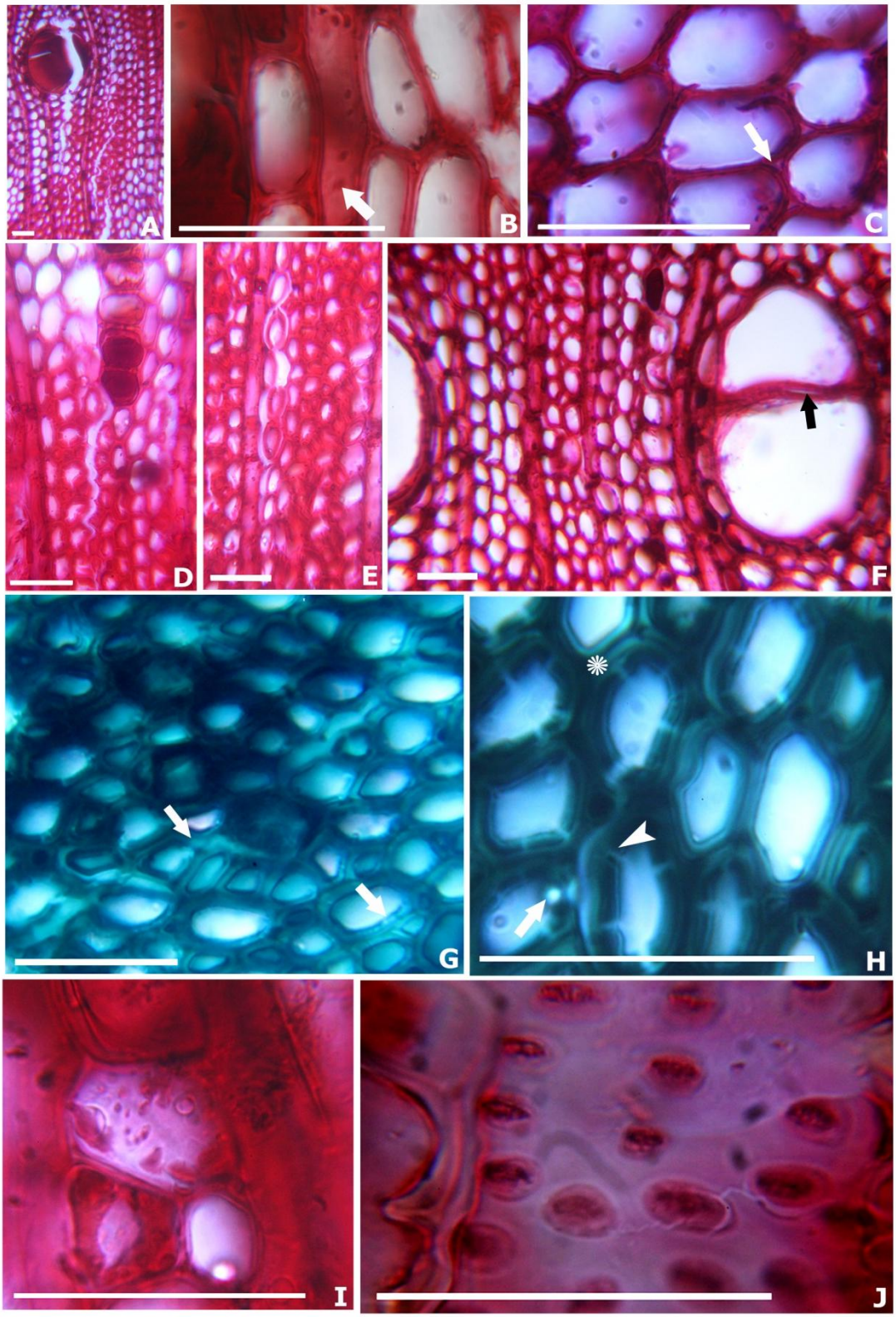


PLATE 20

Plate 21: Pattern of decay in *Pithecellobium dulce* wood infected with the *Phellinus pectinatus*

Magnification bar: A to G - 10 µm

Longitudinal view (A, B), Radial view (C to G), Scanning Electron Microscopy - Transverse view (H, I)

A: Fibers with long slit like cavities in the direction of microfibrils (arrow)

B: Fibers with long slit like cavities (arrow) and ray parenchyma cells showed loosening (arrow head)

C: Bore holes in ray parenchyma cell (arrow)

D: Complete cell wall of ray parenchyma cell degraded (arrow)

E: Vessel wall with cracks in between the pits (arrow)

F: Bore holes in fiber cells (arrow)

G: Radial slit in fiber cells (arrow)

H, I: Separation of adjacent vessel element wall (arrow)

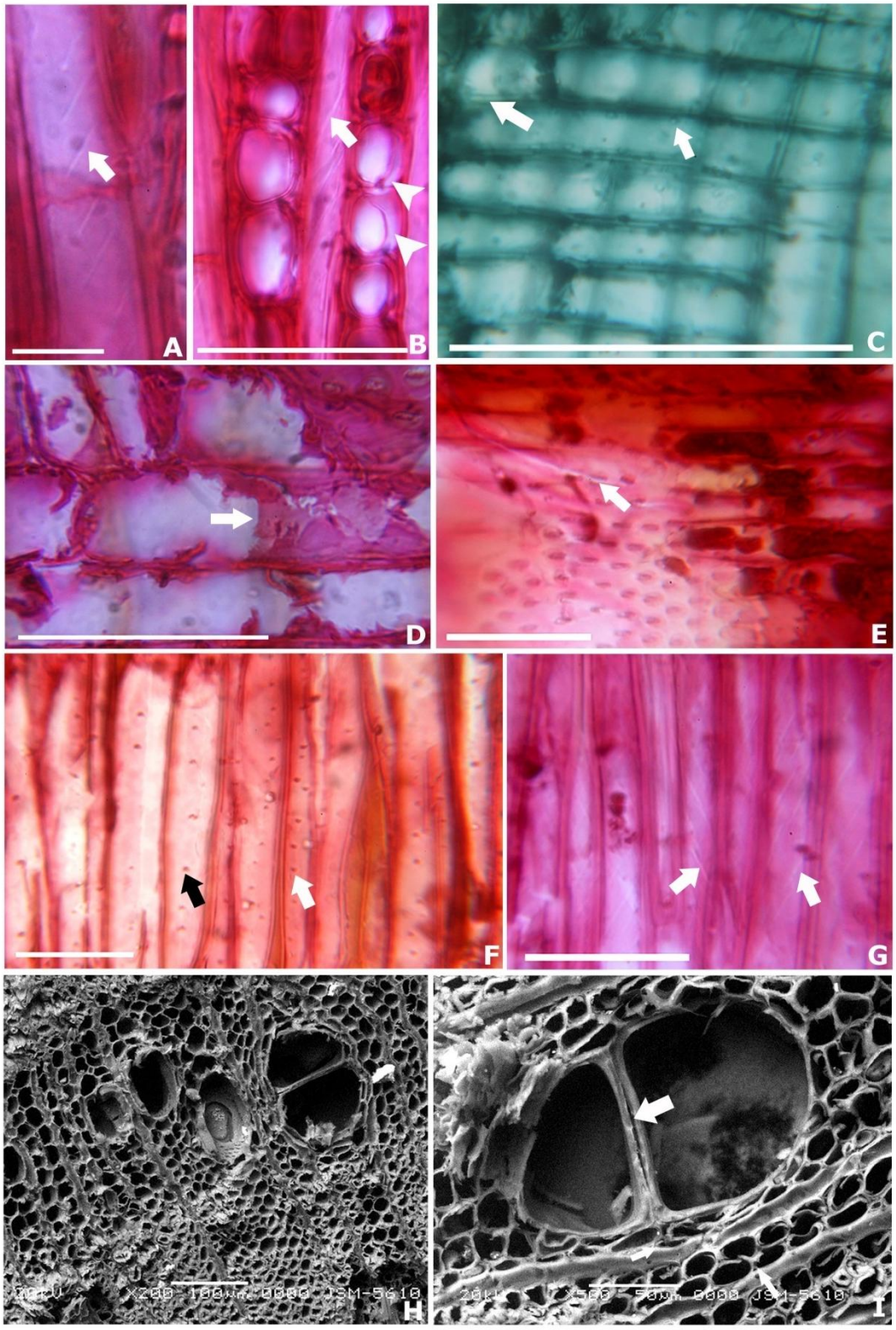


PLATE 21

Plate 22: Pattern of decay in *Pithecellobium dulce* wood infected with the *Phellinus pectinatus* (Scanning electron microscopy)

Transverse view (A, B), Longitudinal view (C to F), Radial view (G to K)

A: Bore holes in medullary rays (arrow)

B: Redial slit from the lumen towards the S_2 layer in fiber cells (arrow). S_2 layer of fiber cells separated from outer wall layer (arrow head). Middle lamella intact

C: Vessel wall with large number of cracks breaking into small cubical units

D: Bore holes in the ray parenchyma cells (arrow)

E: Middle lamella between the ray parenchyma cells dissolved (arrow)

F: Radial slits in fiber cells

G, H: Bore holes in ray parenchyma cells

I: Vessel wall with cracks in between the pits

J, K: Large number of radial slit in the fibers cells

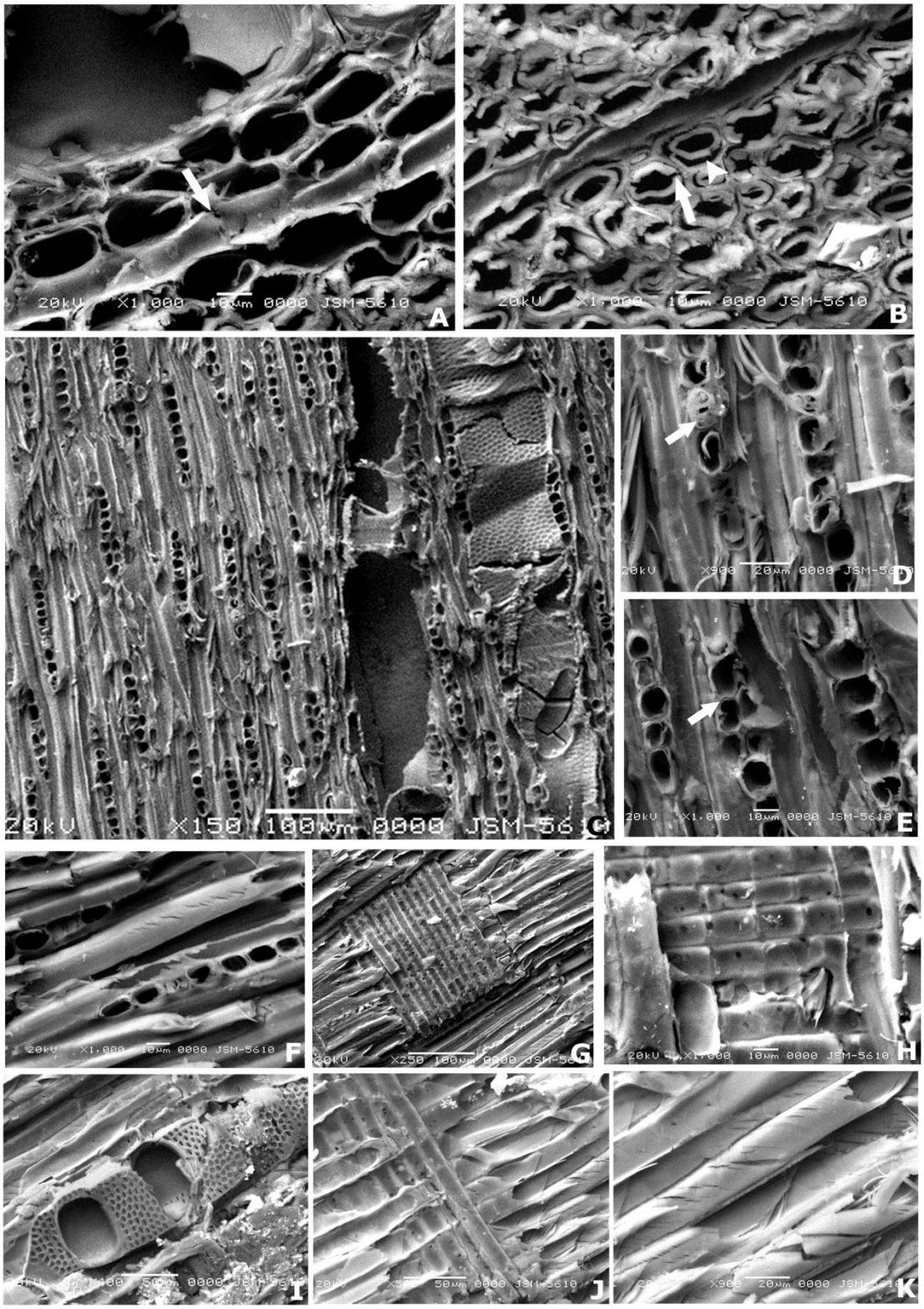


PLATE 22

Normal wood structrure *of Eucalyptus globulus*

Wood is basically brown or shades of brown. Heart wood colour is darker than sap wood. The wood is of commercial importance, it is used in construction and traditionally widely used in paper making. Growth ring boundaries indistinct or absent. Wood is diffuse-porous (vessel elements have more or less same diameter throughout the wood). Vessels exclusively solitary (Plate 23 A). Shape of vessel is circular–oval.

Apotracheal and paratracheal axial parenchyma present (Plate 23 A). The apotracheal (axial parenchyma not associated with vessels) axial parenchyma are diffuse in aggregates ie. Parenchyma strands grouped into short discontinuous strands grounded into short-discontinuous tangential or oblique lines (Plate 23 A). Paratracheal axial parenchyma vasicentric (parenchyma cells forming complete circular to oval sheath around a solitary / multiple vessel).

Rays exclusively uniseriate (Plate 23 B). Vessel-ray pits with much reduced borders to apparently simple: pits rounded or angular (Plate 23 C, E). Rays with procumbent, square and upright cells mixed throughout the ray (Plate 23 D). Fibres are Non-septate and thin- to thick-walled.

Plate 23 F, G, H is shows SEM micrographs of normal wood features in transverse longitudinal and radial view respectively.

Pattern of decay in *Eucalyptus globulus* wood infected with the *Daedaleopsis confragosa*

Wood of *Eucalyptus globulus* decayed by *D. confragosa* showed all the features of degradation shown by the other selected woods. *E. globulus* wood is traditionally

used for making paper by the industries, so it is very important to study the decay pattern of this wood infected by the two potential white rot fungi *D. confragosa* and *P. pectinatus*. *E. globulus* incubated with *D. confragosa* shows initiation of decay within 10 days of incubation period. Fiber cells were found to be resistant to the decay for about 3 months incubation period.

In the initial stages all the three views (TS, TLS, RLS) of wood clearly showed that the decay begins through the parenchyma cells surrounding the vessel element which showed bore holes (Plate 24 A, H, N; 25 A, J), and medullary rays which also showed the bore holes (Plate 24 B; 25 A, C). In a magnified view the hyphae was very clearly observed to be penetrating into the cell by bore hole (Plate 25 K). With an advancement of degradation middle lamella between the axial parenchyma cells were dissolved and so the cells separated (Plate 25 A, F; 24 I, O). During the last stage of degradation within 6 months incubation period due to fusion of the adjacent bore holes the wall gets degraded and, only the ruminants of the ray parenchyma cell wall are observed (Plate 25 L). Within 12 months of incubation period the wood elements i.e. vessel element and parenchyma were completely degraded and was replaced with fungal hyphae. Fiber cells were not much affected like the vessel element and parenchyma (Plate 25 E).

Vessel element showed colonization in the lumen by penetrating through pits (Plate 24 D, J, K, P, Q; 25 B, M, G) within 10 days of incubation period. In the transverse view degradation of the S_2 layer of the cell wall was observed (Plate 24 E). At the later stages of degradation the entire wall of vessel element was separated (Plate 24 F) from the adjacent cells.

Fibers showed penetration of hyphae (Plate 24 M) within 3 months of incubation period. Horizontal clefts or channels were formed due to degradation of middle lamella and simultaneously S_2 layer of the cell wall was also degraded separating the cells from each other (Plate 24 G, 25 D). Sometimes cell wall between the fibers dissolved forming large cavities (Plate 24 C). At the advanced stage of the degradation in longitudinal and radial view of the wood, fiber cells showed large bore holes (Plate 24 L arrow; 25 H, I, J). Bore holes in the fibers are of very large conspicuous size in comparison to the ray parenchyma cells (Plate 25 N).

Pattern of decay in *Eucalyptus globulus* wood infected with the *Phellinus pectinatus*

Eucalyptus globulus decayed by *P. pectinatus* also showed all the features of degradation showed by the other selected woods. Axial and ray parenchyma cells were mainly affected. For a long period of incubation (6 months) the vessel elements and fibers remained unaffected. *E. globulus* incubated with *Phellinus pectinatus* showed signs of decay during the initial 10 days itself only the fiber cells remained intact without alteration upto 3 months incubation period.

Medullary rays and ray parenchyma during the first ten days of incubation period showed presence of bore holes (Plate 26 A arrow; 27 A). Middle lamella between the axial parenchyma cells dissolves and so the cells become separated. (Plate 27 C arrow, D; 26 I).

The procumbent ray cells show its lumen completely filled with fungal hyphae (Plate6 26 M). With further advancement in incubation period the middle lamella between the ray cells appears to be dissolved (Plate 26 M). Cell wall of the ray

parenchyma cell is also degraded (Plate 26 N). Within the axial parenchyma in a longitudinal view branching of hyphae within secondary wall was clearly visible (Plate 26 K). With highly advanced stage of degradation the uniseriate ray cells were completely degraded leaving behind only the outline of the cells (Plate 27 E, F), which was clearly visible (Plate 27 G, H) in radial view.

Hyphae penetrated and colonized the vessel element through pits and also showed branching within the lumen of the vessel element (Plate 26 B, J; 27 H, I). Cracks were observed in the vessel wall (Plate 26 L, O). With an advance in degradation it was observed that the S_2 layer of the cell wall is degraded in a concentric manner separating the microfibrillar lamella (Plate 26 C arrow, F; 27 A, C arrow).

In all the other studied wood species initiation of the decay begins by formation of bore holes in parenchyma cells (ray and medullary ray). In *E. globulus* infected with *Phellinus* initial stages of decay are observed in the form of cracks/ splits on the wall of the ray parenchyma cells (Plate 26 A arrow).

Within an incubation period of 3 months the fiber cells begin to show separation. Degradation of middle lamella in the fiber region leading to horizontal clefts or channels were observed (Plate 26 F; 27 B). In this stages few of the fiber tracheids are decayed largely while the ray cells showed no structural changes (Plate 26 D). The cell wall degradation occurs in the immediate surroundings of the hyphae from the lumen outwards and further forming a complete channel of decay. Fiber cells showed concentric degradation of S_2 layer separating it completely from the S_1 layer (Plate 26 H; 27 B arrow).

Middle lamella was hardly degraded even at the latter stages of decay. Though large clefts were observed (Plate 26 G; 27 B, C arrow) in S_2 layer (Plate 26 E) the middle lamella still appeared to be intact. Fiber cells showed the hyphae traversing through the cell (Plate 27 J), and at a very late stage of decay (12 Months) small bore holes of about1.85 µm size also appeared in the fiber cells with middle lamella still intact (Plate 27 E, F, K; 26 P).

Plate 23: Normal wood structure *of Eucalyptus globules*

Magnification bar: A to E - 10 µm

A-E: Light microscopic view of the different elements

F- H: SEM view of different wood elements in different views

A: Solitary vessels with circular to oval shape and paratracheal axial parenchyma vasicentric.

B: Rays exclusively uniseriate.

C: Vessel ray pits rounded to angular in longitudinal view

D: Radial view shows the procumbent ray cells.

E: Vessel ray pits round to angular in radial view

F: Transverse view of wood shows solitary vessel

G: Uniseriate ray cells

H: Procumbent ray cells in the radial view

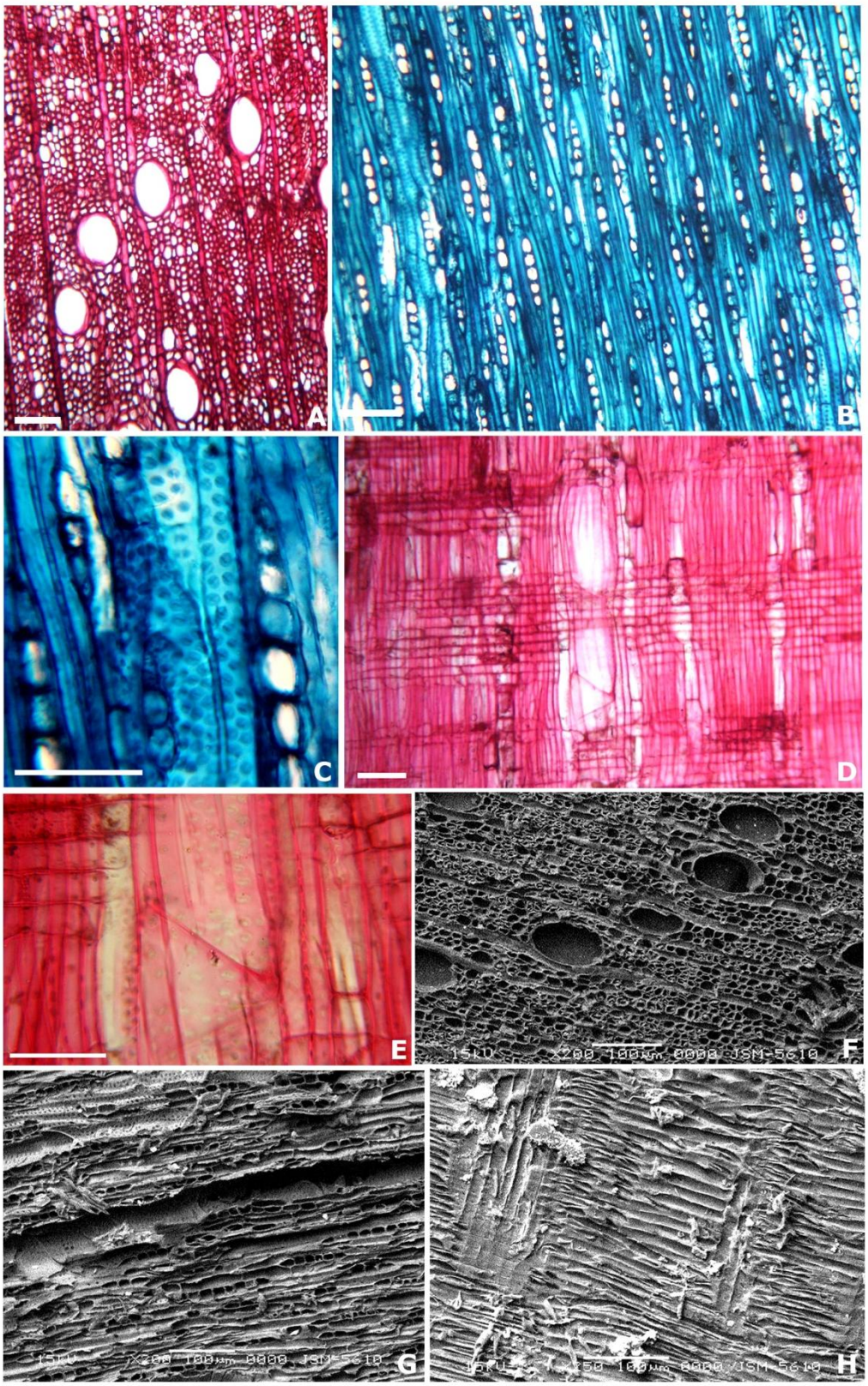


PLATE 23

Plate 24: Pattern of decay in *Eucalyptus globulus* wood infected with the *Daedaleopsis confragosa*

Magnification bar: A to Q - 10 µm

Transverse view (A-G), Longitudinal view (H to M) Radial view (N to Q)

A: Ray parenchyma cells surrounding the vessel element with bore holes

B: Bore holes in medullary rays

C: Cell wall between the fibers dissolved forming large cavities

D: Hyphae colonized in the vessel element

E: S_2 layer of the Vessel cell wall forming channel and adjacent parenchyma delignified

F: Buckling of vessel element wall

G: S_2 layer of fiber wall degraded separating fiber cells

H: Bore holes in ray parenchyma cells

I: Middle lamella between the ray parenchyma cells dissolved

J: Hyphae penetrated in the vessel element through pits

K: Hyphae colonized in the vessel element

L: Fiber cells with large bore holes (arrow) and ray parenchyma cell disfigured

M: Hyphae traversing in the lumen of fiber cells parallel to its longitudinal axis

N: Parenchyma cells with large bore holes merged with each other forming a pathway between adjacent cells

O: Middle lamella dissolved in between ray parenchyma cells

P: Colonization of hyphae in the vessel element

Q: Hyphae penetrating through pits into the vessel element

PLATE 24

Plate 25: Pattern of decay in *Eucalyptus globulus* wood infected with the *Daedaleopsis confragosa* (Scanning electron microscopy)

Transverse view (A to E), Longitudinal view (F to I), Radial view (J to N)

A: Medullary rays and parenchyma cells with bore holes and middle lamella between the axial parenchyma cells dissolved separating them

B: Hyphae colonized within lumen of vessel element

C: Medulary ray with bore holes (arrow)

D: Middle lamella between fiber cells is degraded

E: Wood elements completely degraded and replaced with fungal hyphae

F: Middle lamella between the ray parenchyma cell dissolved

G: Hyphal colonization in the vessel element

H, I: Large bore holes in the fiber cells

J: Bore holes in ray parenchyma and fiber cells

K: – Hyphae penetrating in the ray parenchyma cell through bore hole (arrow)

L: Ray parenchyma cells completely degraded and only remnants of cell wall left

M: Hyphae penetrated and colonized in the vessel element

N: Large number of large bore holes in fiber cells

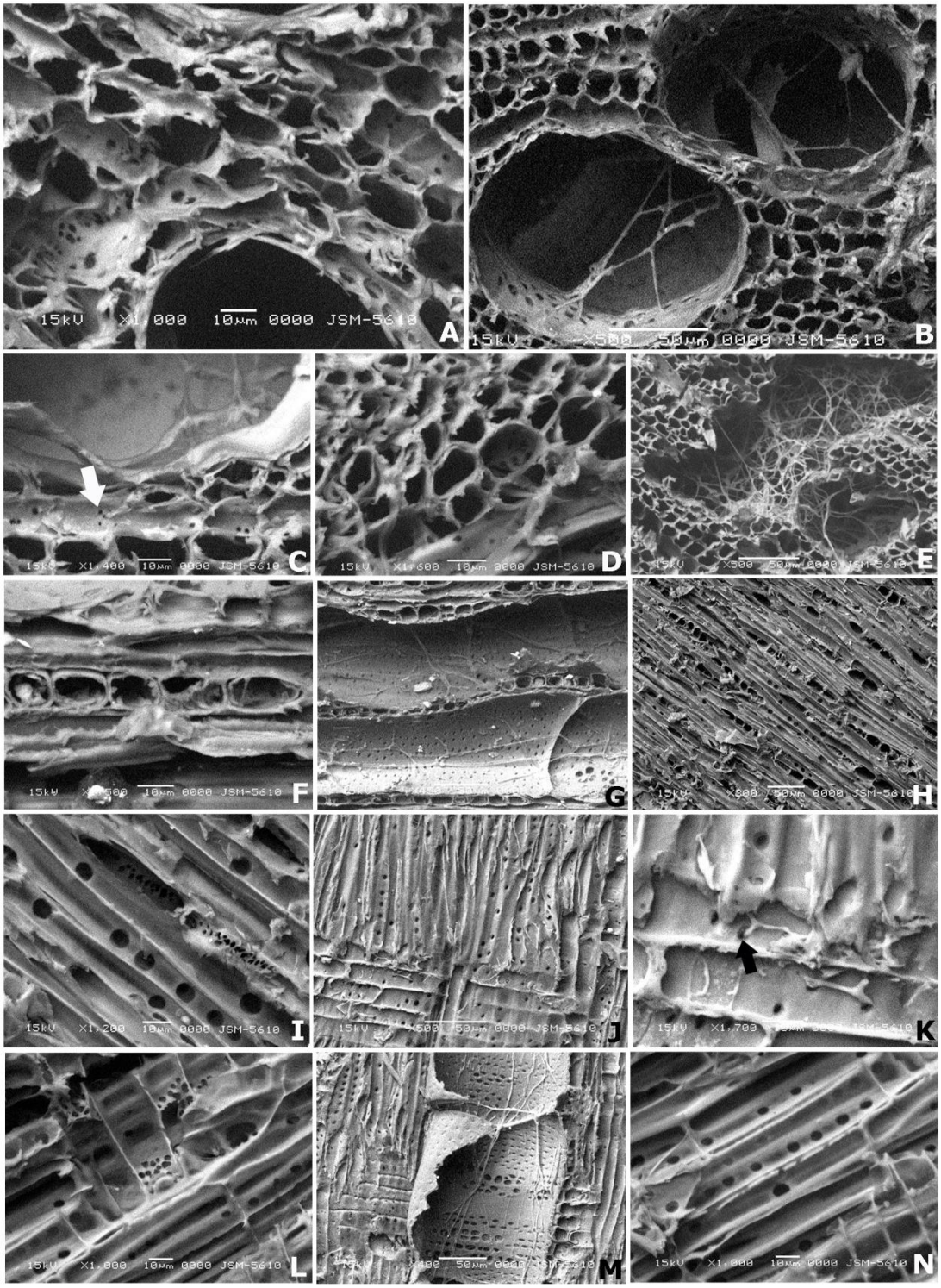


PLATE 25

Plate 26: Pattern of decay in *Eucalyptus globulus* wood infected with the *Phellinus pectinatus*

Magnification bar: B to K, M to P - 10 µm; A, L – 5 µm

Transverse view (A to G), Longitudinal view (I to L) Radial view (M to P)

A: Cracks in axial parenchyma cells (arrow)

B: Hyphae colonized in the vessel element

C: S_2 layer of the vessel cell wall degraded separating microfibrils (arrow)

D: Degradation channels/pathways formed passing through the completely degraded wall region (arrow)

E: Middle lamella degraded between the fibers forming longitudinal channel

F: Medulary ray cells separated from adjacent cells due to decay channels

G, H: Long horizontal clefts or channels formed in between the fiber cells (arrow)

I: Middle lamella between the ray parenchyma cells dissolved

J: Hyphae penetrated in to the vessel through pits

K: Cell wall of axial parenchyma with cracks

L: Cell wall of vessel element with cracks between the adjacent pits

M: Middle lamella between the ray parenchyma cells degraded

N: Ray parenchyma cells wall degraded

O: Ray parenchyma cells with bore holes

P: Bore holes in fiber cell

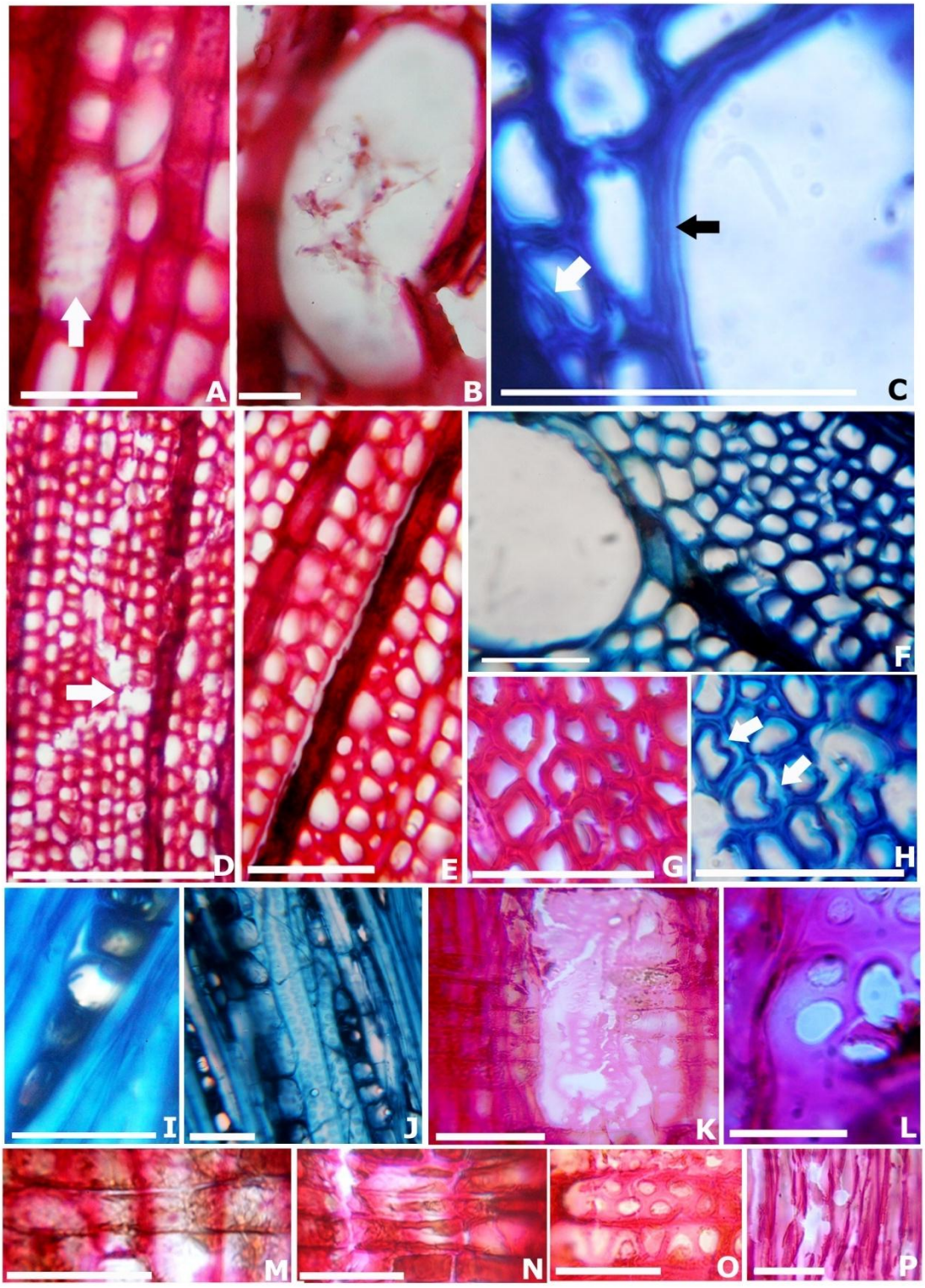


PLATE 26

Plate 27: Pattern of decay in *Eucalyptus globulus* wood infected with the *Phellinus pectinatus*

(Scanning electron microscopy)

Transverse view (A to C), Longitudinal view (D to F), Radial view (G to K)

A: Bore holes in medullary rays and fiber cells with horizontal clefts or channels (arrow)

B: Middle lamella between the axial parenchyma cells dissolved leading to its buckling (arrow)

C: S_2 layer of the vessel cell wall degraded separating other cell wall layers (arrow)

D: Middle lamella between the ray parenchyma cells dissolved (arrow)

E, F: Bore holes in fiber cells and vessel element (arrow)

G: Middle lamella in between the ray parenchyma cells dissolved and with bore holes

H: Hyphae penetrated in the vessel element

I: Penetration of the hyphae in vessel element through pits

J: Fiber cells with fungal hyphae running through the lumen

K: Bore holes in cell wall of the fibers (arrow)

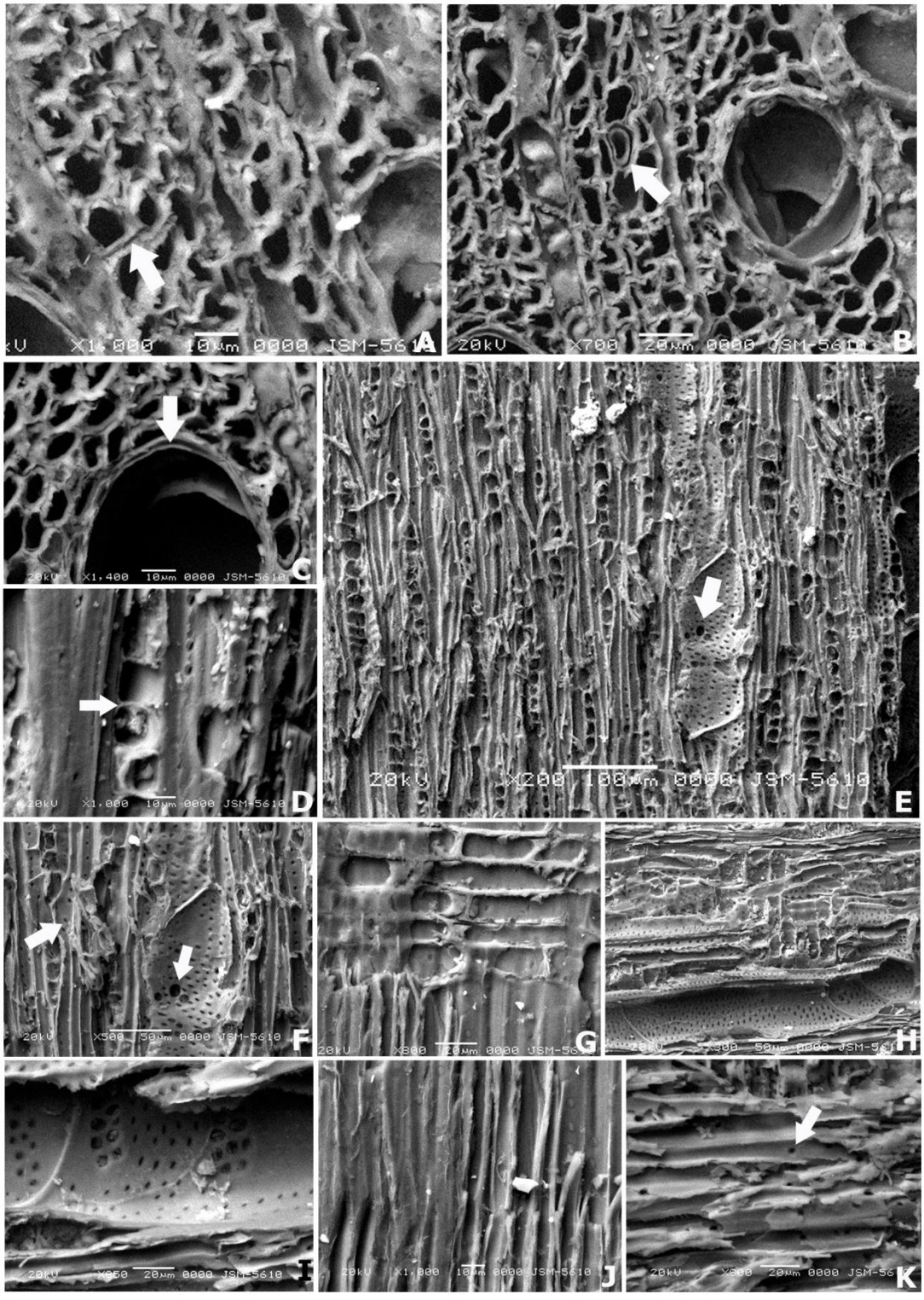


PLATE 27

II) DISCUSSION

Research in wood decay began in Europe with discoveries by Robert Harting before the turn of the 20th century. Harting in 1878 separated the white rots from the brown rots by the colour of decayed wood.

All the fungi included in the Basidiomycetes group causes white rot. There are some of the fungi under the group Ascomycetes, and family xylleariaceae also known to cause white rot (Sutherland and Crawford 1981).

Commonly the term 'white rot' is used to describe forms of wood decay in which the wood assumes a bleached appearance and where lignin as well as cellulose and hemicelluloses is broken down. Amount of degradation of lignin and cellulose and other cell-wall constituents depends on the species of fungus and conditions existing in the wood. White rot degradation are generally familiar in two forms, selective delignification and simultaneous rot on the basis of the diversity of the wood decay pattern (Blanchette 1984 ; Adaskaveg and Gilbertson 1986; Raymer and Boddy 1988).

Falck (1926) was the first to show the basic chemical changes that accompanied white rots and brown rots. White rot fungi removed lignin and cellulose, where as brown rot fungi removed only cellulose. Bjorkman *et al.* (1949) separated the white rot group into 2 categories. Some white rot fungi were found to remove all cell wall components, where as others were found to remove lignin and hemicelluloses, first leaving the cellulose. He identified the categories as white rot and corrosive rot respectively. Thus biochemically in the selective delignification at the early stage of decay lignin is broken down more than cellulose.

Decay is a natural process, it may be observed on living trees as well as dead and decaying logs of trees. Many white rot fungi can degrade all structural component

of wooden cell wall (Adaskaveg *et al.* 1990; Kirk and Highley 1973; Kirk and Moore 1972; Blanchette and Reid 1986) and some white rot fungi selectively degrades lignin over other wood polymers (Adaskaveg *et al.* 1990; Luna *et al.* 2004).

Worral *et al.* (1997) has studied the comparison of wood decay among divers lignicolous fungi. They have selected 78 different fungal species responsible for white, brown and soft rot, among them Aphyllophorale showed the highest weight loss and fungi in other groups of Basidiomycetes also showed high weight loss.

In the present study degradation of wood blocks were found to be higher with *D. confragosa* compared to that *P. pectinatus* (Table 16, 17 and Figure 22 A and B).

Weight loss in all wood species was also observed to be maximum with fungus *D. confragosa* in comparison to *P. pectinatus* in all the selected wood species within an incubation period of 12 months.

S. cuminii and *P. dulce* wood did not show much weight loss when treated with both the fungi. *M. indica* and *E. globulus* wood showed moderate amount of weight loss. *B. ceiba* wood with *D. confragosa* showed the highest weight loss, but with *P. pecinatus* it showed lesser weight loss.

D. confragosa also showed a high amount of loss in the lignin content in comparison with *P. pectinatus* with all selected wood species at all different the incubation periods (Table 18, 19 and Figure 23 A, B).

Among all the selected wood species the highest lignin loss was observed in *B. ceiba* wood, with both the fungi and lowest lignin loss was observed in *S. cuminii* treated with *D. confragosa* and *P. dulce* with *P. pectinatus*.

Percentage loss in lignin content also indicated that the *S. cuminii* and *P. dulce* wood was quite resistant to the infection and *B. ceiba* wood was most susceptible to both the fungi.

Less amount of loss in the cellulose content is always favorable for biopulping industry. Here percentage cellulose loss was slightly more in all the wood blocks infected with the *P. pectinatus* in comparison with the *D. confragosa*, except in *P. dulce* wood (Table 20 and 21).

Figure 24 A and B clearly indicated that the highest amount of cellulose loss was observed in the wood of *B. ceiba* with both the white rot fungi, but it is more with the *P. pectinatus*. In wood of *M. indica* also the loss of cellulose is at higher rate in comparison to other three wood species.

Rio *et al.* 2002 studied weight loss and lignin loss during *Eucalyptus* wood decay by *Bjerkandera adusta* FIB 538, *Peniophora lycii* FIB 507, *Peniophora cinerea* FIB 508, *Peniophora* sp. FIB 509 and *Phanerochaete crassa* FIB 511 and FIB 539 for 8 weeks of incubation. Here the large variation is observed in the weight loss (4 to 20 %) and lignin loss (11 to 45 %) with the different fungal species. In the present study also the both the fungi showed variation in weight loss, lignin loss and cellulose loss. All the studied parameters showed greater loss with *D. confragosa* in comparison to *P. pectinatus*. They observed the highest weight loss and lignin loss in the degradation by *P. crassa* FIB 511, which was 20 % and 45 % respectively. In present study all the wood species infected with the *D. confragosa* and *P. pectinatus* showed lower weight loss and lignin loss, but in case *B. ceiba* infected with *D. confragosa* showed higher weight loss but loss of lignin was less. While *Eucalyptus* wood infected with *P. lycii*

FIB 507 17 % weight loss after 8 weeks which resembles to the results in the present study on *Eucalyptus* wood degraded by *D. confragosa* for 2 months but it was greater than that of *Eucalyptus* wood degraded by *P. pectinatus*.

A study on *E. globulus* wood degraded by six white and brown rot fungi and their incubation period varying from 30 days to 1 year showed weight loss ranging from 1.7% to 42% (Ferraz *et al.* 2000). This results is in accordance with the weight loss obtained in *E. globulus* wood infected by the *P. pectinatus* which was in range of 2.02 to 40.21 %, and in *D. confragosa* infected the weight loss obtained was much higher (3.08 to 66.51 %).

Another study on *E. globulus* wood degraded by *Grammothele subargentea* for 30 days 8.2 % wood mass loss and 13.5 % lignin loss was obtained (Saparrat *et al.* 2008). Which is exactly the same amount of weight loss obtained in *E. globulus* wood infected with the *D. confragosa* (8.37 %), but the lignin loss was 24.8 %, which was approximately double. While in case of *E. globulus* wood infected with *P. pectinatus* lower weight loss and lignin loss could be obtained.

Eucalyptus siderxylone infected with *Polyporus versiocolar* and *Poria monticola* showed 18 % and 27 % weight loss respectively after 8 weeks of incubation (Hart and Hillis 1974). But both fungi *D. confragosa* and *P. pectinatus* showed lesser amount of weight loss in *E. globulus*.

Bombax buonopozense infected with *Botryodiplodia theobromae* showed 30.5 % weight loss, 85.8 % cellulose loss and 99.5 % lignin loss within 56 days of incubation period (Umezurike 1968). This study completely disagreed with results of

the present study. Such a high lignin and cellulose loss was not obtained when infected with both the fungi *D. confragosa* and *P. pectinatus*.

Blocks prepared from sap wood of *Bombax ceiba* (Semal) were used for providing nutrients to the actively growing test fungus in culture bottles (Pant and Tripathi 2010).

Different species of white rot fungi *Ganoderma* were evaluated for the weight loss, in oak wood. *G. meredithiae, G. zonatum, G. oregonense* and *Ganoderma* sp. showed weight loss in the range of 38 to 52 %, except *G. colossum* within the 20 weeks of incubation and White fir wood with *Ganoderma* sp. showed weight loss ranging from 21 to 26 % (Adaskaveg *et al.* 1990). But in present study there was a weight loss of 20.84 to 29.5 % after a period of 6 months for *D. confragosa* and 9.68 to 27.36 % with *P. pectinatus*. Results of weight loss by *D. confragosa* agrees with results of white fir wood with *Ganoderma* sp.

In the present study from an overview of the results of the chemical analysis, in all the combinations both fungi showed degradation of the lignin faster than that of cellulose, indicating the selectively delignifying nature of the selected fungi *D. confragosa* and *P. pectinatus.* Wood of *B. ceiba* infected with *D. confragosa* showed highest degradation, while the *P. dulce* wood infected with the *P. pectinatus* showed the lowest degradation in an incubation period of 12 months.

Weight loss experiment supplemented with *invitro* decay experiments brought about greater insight into the pattern and intensity of decay.

Macroscopically difference in the selective delignification was recognized by light patches, as preferential lignin degradation remaining pure cellulose giving light

appearance. Morphologically the wood decayed by the selective delignifying white rot fungi is fibers due to dissolution of lignin rich middle lamella and the separation of individual cell elements from their matrix. Thus if the decayed wood has the fibrous consistency we can assume selective delignification macroscopically (Schwarze *et al.* 2000).

Anatomical studies have revealed that a lot changes take place in the wood microstructure during progressive stages of fungal decay (Luna *et al.* 2004). Anatomical description of decay caused by brown rot, white rot and soft rot is given by many authors using light microscopy and scanning electron microscopy (Cowling 1961; Corbert 1965; Wilcox 1968; Wilcox 1993; Liese 1970; Erickson *et al.* 1990; Blanchette 1991).

Taking into account the incubation period and the alterations in the wood elements brought about by the two fungi *D. confragosa* and *P. pectinatus* it can be seen that the pattern of decay was found to be the same with some variations which were more prominent in some of the wood samples. Formation of bore holes in parenchyma tissue, colonization of vessels elements, dissolution of middle lamella and breakdown of cellulose into submicroscopic layers are some of the most common characteristic features noted. The initiation and intensity of decay varied in the different wood species with the different incubation period.

In *S. cuminii* wood blocks infected with *D. confragosa* ray parenchyma and medullary rays showed bore holes within 30 days of incubation period simultaneously with, vessel element showing colonization of hyphae, while with *P. pectinatus* all this features were observed after 2 months incubation period. With *D. confragosa* fusion of

bore holes in the ray parenchyma at the later stage of decay were observed. Fiber cell wall showed degradation of S_2 layer after 6 months, a feature which was observed only after 12 months incubation with *P. pectinatus*. Even 12 months of incubation with *D. confragosa*, showed very few bore holes in fiber cells.

In *B. ceiba* wood infected with *D. confragosa* after 10 days of incubation colonization of hyphae in axial and medullary parenchyma cells were observed and after 30 days of incubation bore holes begin to appear, but with *P. pectinatus* colonization and bore holes occurred in ray parenchyma cells after 2 months. Medullary rays were not affected with *P. pectinatus*. With the white rot fungus *D. confragosa* vessel element showed colonization during initial 10 days and buckling due to loss of rigidity was observed after 3 months while with *P. pectinatus* buckling was observed only after 6 months. Fiber cells showed bore holes at 2 months of incubation period which increased upto 12 months with of *D. confragosa* while with *P. pectinatus* bore holes were observed only after 12 months.

In *M. indica* wood infected with *D. confragosa* axial and ray parenchyma cells showed signs of degradation science the beginning (10 days), while with *P. pectinatus* this character was observed only after 30 days of incubation period. After 3 months of incubation period wood infected with *D. confragosa* bore holes started fusing with each other and with advanced stage of degradation (12 months) complete cell wall was degraded. While till 12 months of incubation with *P. pectinatus* fusion of bore holes was not observed. Wood when incubated upto 30 days with *D. confragosa* showed colonization of hyphae in vessel element, while in *P. pectinatus* very scarce amount of colonization in vessel element was observed even at 2 months of incubation period.

Buckling of vessel element wall was observed after 6 months and at 12 months of incubation complete vessel wall was degraded with *D. confragosa*, while in *P. pectinatus* after 9 months buckling was observed. Fibers showed boreholes after 2 months of incubation in case *D. confragosa*, while *P. pectinatus* showed penetration of hyphae is observed after 6 months and at 9 months of incubation bore holes were prominently observed.

In the wood of *P. dulce* axial parenchyma and medullary ray cells showed bore holes after 2 months of incubation period with both the fungi. With *D. confragosa* colonization of hyphae was observed in vessel element wall after 3 months but in *P. pectinatus*, within 6 months of degradation vessel wall showed separation. Fiber cell wall showed cracks in the direction of microfibrils with both the fungi.

In the wood of *E. globules* both the fungi showed almost the same pattern of degradation in case of axial and ray parenchyma and vessel element. In case of fibers *D. confragosa* showed bore holes after 9 months of incubation while *P. pectinatus* showed the bore holes only after 12 months of incubation.

During the initial stage of degradation parenchyma tissue of wood is observed in all the wood species infected with the *D. confragosa* except wood of *P. dulce*. But with *P. pectinatus* the degradation of the parenchymatous tissue of wood is a very slow, only wooden blocks of *E. globulus* showed degradation of parenchymatous tissue from the initial stage. Bore holes of the axial and ray parenchyma cells were fused in all the wood infected with *D. confragosa*. With the fungi *P. pectinatus* bore holes of axial and ray parenchyma did not showed fusion, but in the wood of the *B. ceiba* fusion is observed.

Vessel element of the woods infected with *D. confragosa* showed colonization of hyphae within 20 to 30 days of incubation, only except in the wood of *P. dulce* it showed very rare colonization (that is after 3 months). With *P. pectinatus* very scarce colonization of hyphae is observed after 2 months in the wood of *M. indica, S. cuminii and B. ceiba*, while in *P. dulce* colonization was not observed and in *E. globulus* colonization was observed from 10 days of incubation itself.

In the wood of *S. cuminii* and *E. globulus* fibers showed bore holes only at 12 months of incubation, *B. ceiba* and *M. indica* wood showed bore holes after 2 months of incubation with the fungus *D. confragosa*. While with using the fungus *P. pectinatus* wood of *E. globulus, M. indica* and *B. ceiba* showed bore holes at the 9 months of incubation period. *S. cuminii* wood infected with the *P. pectinatus* did not show bore holes. Fibers of *P. dulce* wood infected with both the fungi showed radial slits in the direction of microfibrils at the 6 months of incubation period.

For all white rots, ray parenchyma of softwoods and hardwood were attacked (Anagnost 1998). Present study as discussed earlier in this section agrees with this statement as in all the five wooden species selected (*Mangifera indica, Syzygium cuminii, Bombax ceiba, Eucalyptus globulus, Pithecellobium dulce*) infected with both the basidiomycetes fungi (*D. confragosa* and *P. pectinatus*) showed that the degradation begins with the ray parenchyma or the axial parenchyma cells. Incubation period for the initiation of the decay varied with the different wood species. D. confragosa treated wood blocks of *M. indica* and *E. globulus* showed initiation of decay within first 10 days of incubation period, evidenced by presence of bore holes in the axial and ray

parenchyma cells. These cells in the wood blocks of *S. cuminii* and *P. dulce* appeared to be resistant upto an incubation period of 20 and 30 days respectively.

Except for *E. globulus* wood samples in all the other four wood species initiation of decay by *P. pectinatus* was prominent only within 30 days incubation period. In *E. globulus* bore holes appeared within first 10 days of incubation period.

Degradation of ray parenchyma and axial parenchyma cells by the *D. confragosa*, showed the bore holes initially which merge with each other completely degrading the cell wall. All the wood infected with *P. pectinatus* also showed the bore holes in the ray parenchyma and axial parenchyma, but they did not show fusion with each other. The bore holes formed with *P. pectinatus* is very small compared to that of the *D. confragosa.*

Degradation of middle lamella occurs in conjunction with extensive lignin degradation in the secondary wall. At the late stage, individual cells become separated from their matrix (Harting 1878; Blanchete 1984). Mechanism behind this kind of degradation is explained by Schwarze *et al.* (2000). Hyphae penetrated in the lumen of the cell produce enzymes which in early stage of decay diffuse into secondary wall, after that diffusion extends to middle lamella and so preferential lignin degradation leads to the separation of individual cells. In present study the wood of *E. globulus, S. cuminii, P. dulce* infected with both (*D. confragosa* and *P. pectinatus*) selected fungus showed the same results.

Cell separation is best indicator of the selective delignification (Angnost 1998) which agrees with the present studies. In *B. ceiba* and *M. indica* wood infected with

both the fungi showed the degradation of middle lamella forming longitudinal degradation channels.

Lamellar collapse of the secondary wall in to submicroscopic layers is also possible (Harting 1878 and Peek *et al.* 1972). Further extensive delignification may also occur in S_2 layer leading to the accentuation of the radial structures (Schwarze and Engels 1998). Here fine cleft appears in the S_2 layer of the degrading cell wall due to local degradation of cellulose and hemicellulose which enlarge the cleft and forms cavity, which are separated from one another by radial structures running perpendicular to the middle lamella.

In present study accentuation of the radial structures in secondary wall layers is observed in the wood of *P. dulce* and *S. cuminii* infected by both the white rot fungi (*D. confragosa* and *P. pectinatus*). Wood of *B. ceiba* infected with *D. confragosa* only showed the formation of cleft in the S_2 layer of the cell wall, but the wood degraded with *P. pectinatus* did not show the formation of the radial structures in secondary wall layers. Such a character is not observed in wood of *M. indica* and *E. globulus* infected with both the white rot fungi (*D. confragosa* and *P. pectinatus*).

Another obvious character of the selective delignifying white rot fungi is the buckling of the different cells. The buckling in of the cellwalls is based on the fact that after their separation, the cellulose rich secondary walls can still be heavily stressed under tensile loading but easily fail under compression load (Schwarze 1995). In the present study the cells which shows buckling differs with the wood species as well as fungal species. *S. cuminii* wood infected with *D. confragosa* showed buckling of axial parenchyma cells. Vessel element showed buckling in the case of *S. cuminii* wood

infected with both the fungi (*D. confragosa* and *P. pectinatus*), and *M. indica*, *E. globulus* woods degraded by the *D. confragosa.*

When fiber buckling occurs in wood, it has the consequence that the cellulose microfibrils, which run in a shallow spiral to the cell wall axis, straighten out and thus can be stressed up to 20 % more under tensile loading (Gordon 1976). Fiber buckling is observed in almost all the studied cases of the degradation, only except *B. ceiba* wood with both the fungi and *S. cuminii* wood with *P. pectinatus* did not show buckling of the fibers, which is one of the important character of the selective delignification. Wood infected with selective delignification causing fungus showed zone with partially buckled fibers due to which wood becomes softer and behaves mechanically like wood that has lost some load-bearing strength (Matthech and Breloer 1994).

Here it is important to mention that when extensive selective delignification does occur in a wood, in the later stages of the decay, cellulose degradation take place. As a result of this besides the initial change in the stiffness and compression strength, latter on the tensile strength of the wood is also very severely modified (Pratt 1979; Schwarze 1995). For the paper and pulp industry loss of cellulose is not lucrative at all. All the selected five wood species (*Mangifera indica, Syzygium cuminii, Bombax ceiba, Eucalyptus globulus, Pithecellobium dulce*) infected with both the basidiomycetes fungi (*D. confragosa* and *P. pectinatus*) showed dual pattern of degradation i.e. selective delignification in the initial stage (till the 6 months of incubation) followed by simultaneous rot during advanced stage (9 and 12 months of incubation) of decay. Initially the separation of the cells due to dissolution of middle lamella, accentuation of secondary cell wall layer and buckling of the all cells were the characteristic feature of

both strains, but in the advanced stage of decay, formation of erosion channels were observed only in parenchymatous cells and bore holes were in fiber cells were also observed. At the advance stage, formation of bore holes were observed in all the wood species infected with both the selected fungi (*D. confragosa* and *P. pectinatus*) except *P. dulce* wood, which is a character of the simultaneous rot (Anagnost 1998). In the wood of *P. dulce* infected with both the fungi (*D. confragosa* and *P. pectinatus*) fibers in tangential section showed hyphal growth within the S_2 layer, recognized by the tube like or slit like cavities always in the direction of cellulose microfibrils, which resembles the soft rot decay. Such kind of decay is reported in the Maple wood by *Armilllaria mellea* (Schwarze *et al.* 2000). This agrees with Anagnost (1998) who mentioned that selective white rot fungi can produce anatomical features similar to simultaneous white rot fungi. In advanced stage, degradation by *D. confragosa* was so distinct that rays were completely destroyed while many cells including vessels were either deformed or destroyed due to loss of rigidity of their walls. But with the *P. pectinatus* decay is slow, as at the advanced stages of decay also the ray parenchyma and other cells remain intact.

Koyani *et al.* (2011), worked on the wood of *Azadirachta indica* infected with *Chrysosporium asperatum* and *Trichoderma harzianum*. They observed the dual pattern of degradation with both the strains that is selective delignification in the initial stage and simultaneous rot during advanced stages of decay which resembled with the present study of decay with both the fungi (*D. confragosa and P. pectinatus*).

Blanchette (1980), studied the degradation of *Pinus monticola* and *Larix occidentalis* by *Phellinus pini* using scanning electron microscopic study. In both the wood selective degradation of lignin is observed. Other features they have observed

were, decomposition of middle lamella between fibers, secondary wall with separated microfibrillar structures, complete degradation of ray parenchyma cells presence of fungal hyphae and bore holes in some cells. As fungi *P. pini* with these characteristics is suggested to be used in biopulping process and as all the above mentioned characters are also observed in all the selected wood decayed by *D. confragosa and P. pectinatus* in the present study, white rot fungi *D. confragosa and P. pectinatus* can also be suggested for the biopulping.

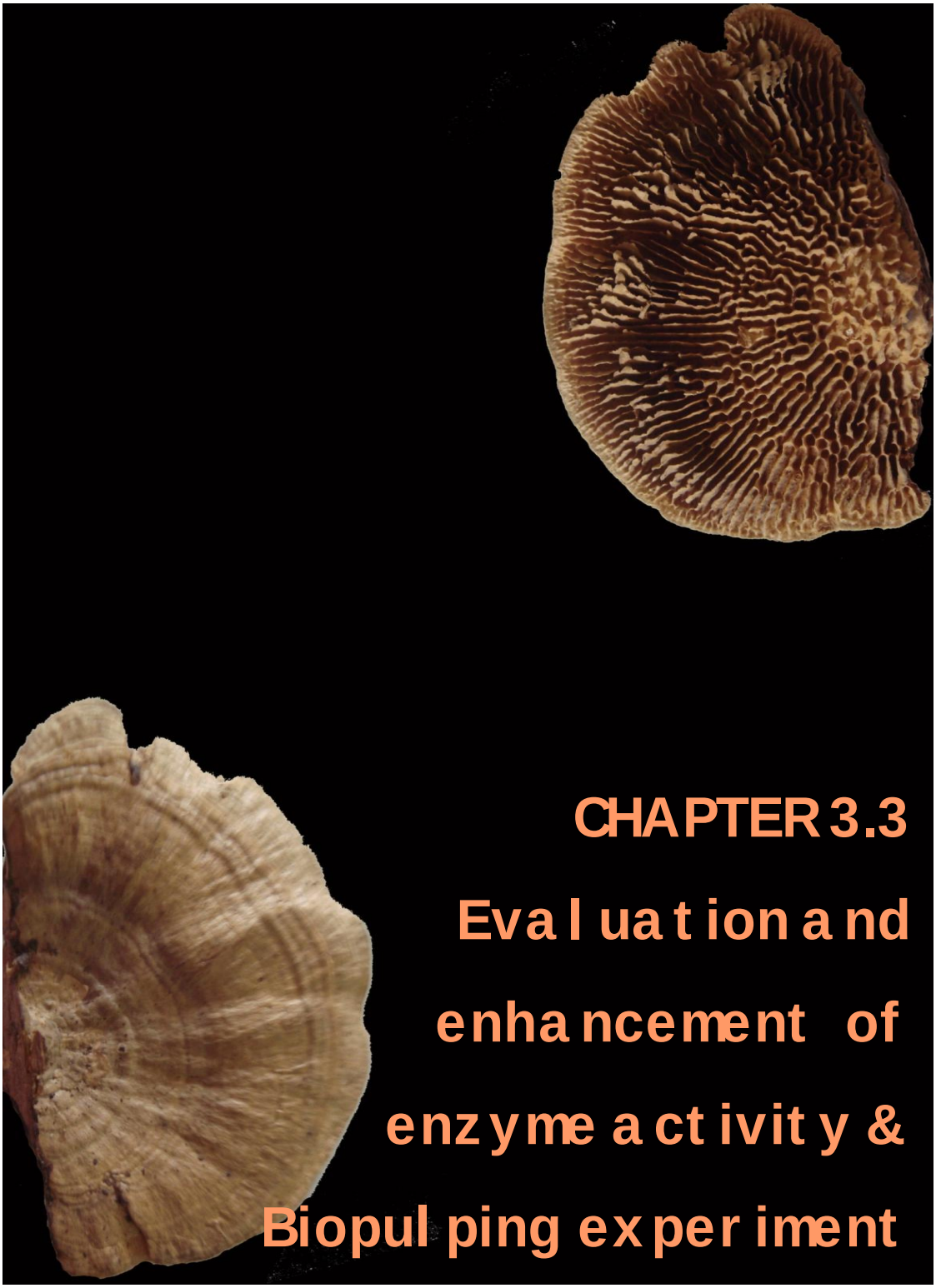
Wood of *Acacia koa* var. *koa* degraded by *Phellinus kawakamii* showed that only the vessel element is resistant to the degradation and all other cells including fibers are completely degraded (Blanchette *et al.* 1988). But in present study results of *P. pectinatus* did not showed such features.

Poplar wood infected with *Ganoderma lucidum* showed few characters of selective delignification along with few characters of simultaneous decay (Luna *et al.* 2004). Aspen and birch wood infected with *Phlebia termellosus* showed selective degradation of lignin (Blanchette and Reid 1986).

Aspen wood was used for degradation under the laboratory condition by three white rot fungi *Ischnoderma resinosum, Poria medulla-pnis,* and *Xylobolus frstulatus,* out of which the *X. frustulatus* was able to form pockets of delignification in the wood (Otjen and Blanchette 1985)

Blanchatte *et al.* in 1994 studied 26 white rot fungi which were able to remove lignin selectively from coniferous and hardwood trees, and he found that the degradation of middle lamella and fibrillar appearance of cell wall layers are the main anatomical features separating selective white rot decay from the others. In this

experiment decay chambers were incubated for 4, 8 and 12 weeks, which means maximum incubation was of 84 days only. But in present study the decay chambers were incubated for the 10 and 20 days, 1, 2, 3, 6, 9 and 12 months which gave more insight into the pattern of degradation of wood. In all cited studies the signs of simultaneous rot was observed after 12 weeks where as in case of *D. confragosa and P. pectinatus* showed the signs of simultaneous rot after 6 months of incubation. On the basis of the present study and the observations it can be considered that both studied fungi (*D. confragosa and P. pectinatus*) are selective delignifying white rot fungi and can be used in the biopulping process.



CHAPTER 3.3

Evaluation and enhancement of enzyme activity & Biopulping experiment

I) RESULT

1) Evaluation of enzyme activity

In paper and pulp industry different enzymes are utilized to dissolve lignin and keep cellulose intact. White rot fungi are known to produce different ligninolytic enzymes like MnP, LiP, AAO and Laccase. In this study screening of some white rot fungi was done to identify the ability of fungi to produce large quantities of ligninolytic enzymes. Fungal biomass after each incubation period was also evaluated of *D. confragosa* and *P. pectinatus*

The activity of lignin degrading enzymes MnP, LiP, Laccase, AAO and fungal biomass was determined after an incubation period of 3-30 days. Biomass of both the fungi *D. confragosa* and *P. pectinatus* showed an increase from the third day of the incubation to 20 days period (0.12± 0.01 to 0.49±0.052) after which there was a reduction in the fungal biomass (Table 22).

Days	Fungal biomass (gm)	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)
3	0.12±0.01	0	0	13.32±0.39	0.63±0.01
5	0.13±0.005	0.062±0.001	0	15.49±0.24	0.74±0.01
10	0.17±0.01	0.39±0.036	0.94±0.17	19.53±0.45	0.87±0.005
15	0.35±0.02	0.77±0.036	1.71±0.072	20.51±0.24	0.91±0.023
20	**0.49±0.052**	0.94±0.06	1.77±0.07	22.41±0.47	0.97±0.01
25	0.4±0.01	**1.7±0.09**	**2.49±0.03**	**24.25±0.17**	**1.07±0.03**
30	0.25±0.02	0.63±0.10	1.8±0.07	23.43±0.23	0.87±0.027

Table 22: Bioassay of lignin degrading enzymes and fungal biomass of *Daedaleopsis confragosa*

Days	Fungal biomass (gm)	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)
3	0.12±0.008	0	0	19.50±0.45	0.69±0.02
5	0.13±0.005	0	0	22.87±0.30	0.86±0.005
10	0.14±0.002	0	0	26.67±0.36	0.92±0.002
15	0.16±0.005	0	0	27.27±0.51	0.93±0.01
20	**0.21±0.01**	0	0	28.30±0.70	1.07±0.02
25	0.16±0.007	0	0	**29.49±0.29**	**1.21±0.008**
30	0.16±0.016	0	0	28.72±0.28	0.93±0.004

Table 23: Bioassay of lignin degrading enzymes and fungal biomass of *P. pectinatus*

The maximum growth of the fungus was observed on the 20th day (Fig. 25 A). Similarly the fungal biomass of *P. pectinatus* gradually increased in the fungal biomass till the 20th day, after that there was a decrease. On the 20th day fungal biomass was the highest (Table 23; Fig. 25 B). In *D. confragrosa* all the lignin degrading enzymes were produced in the maximum amount on 25th day (Table 22). Production of MnP was not initiated on 3rd day, but it slowly initiated by 5th day which increased and reached to a maximum on the 25th day, followed by which there was a decline (Fig. 26 A). LiP production initiated only on the 5th day and then similar to MnP the graph shows the peak activity on the 25th day after which it declines (Fig. 26 B). Enzymes AAO and Laccase were produced from day one with a gradual increase and reaching its peak on the 25th day and further declining the activity by 30th day (Fig. 26 C, D). Table 21 shows the results of liginolytic enzyme activity of *P. pectinatus*. MnP and LiP enzymes were not produced by this fungus (Fig. 27 A, B). Only AAO and Laccase enzymes were produced (Table 23). Similar to *D. confragosa* AAO and Laccase enzyme activity of *P. pectinatus* reached its peak on 25th day after which the activity declined (Fig. 7 C,D).

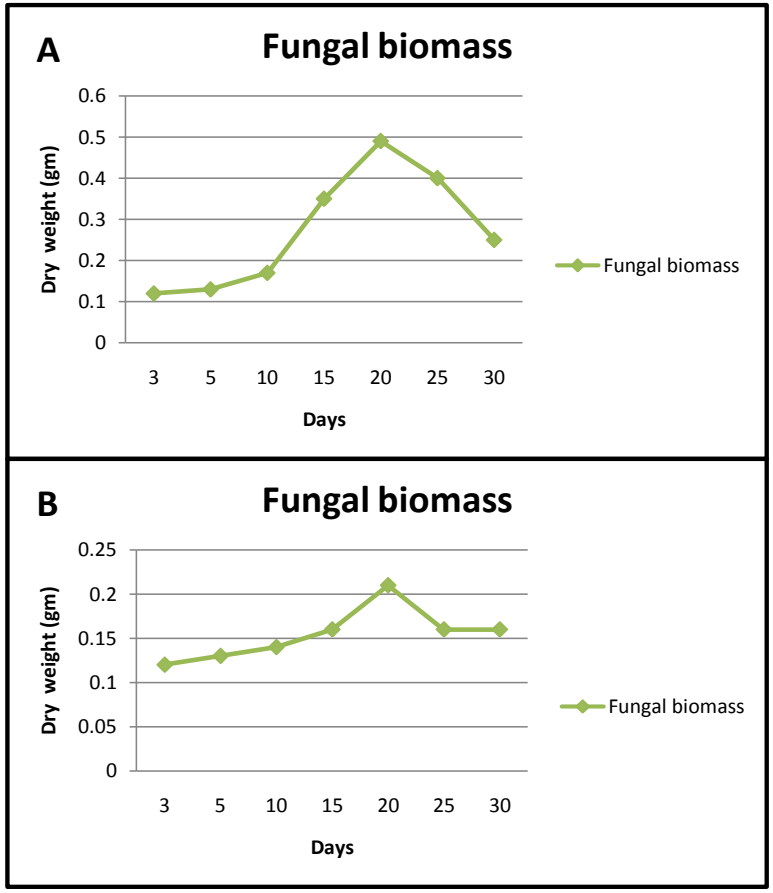


Figure 25 : Variation in fungal biomass with different incubation period
A - *Daedaleopsis confragrosa*
B - *Phellinus pectinatus*

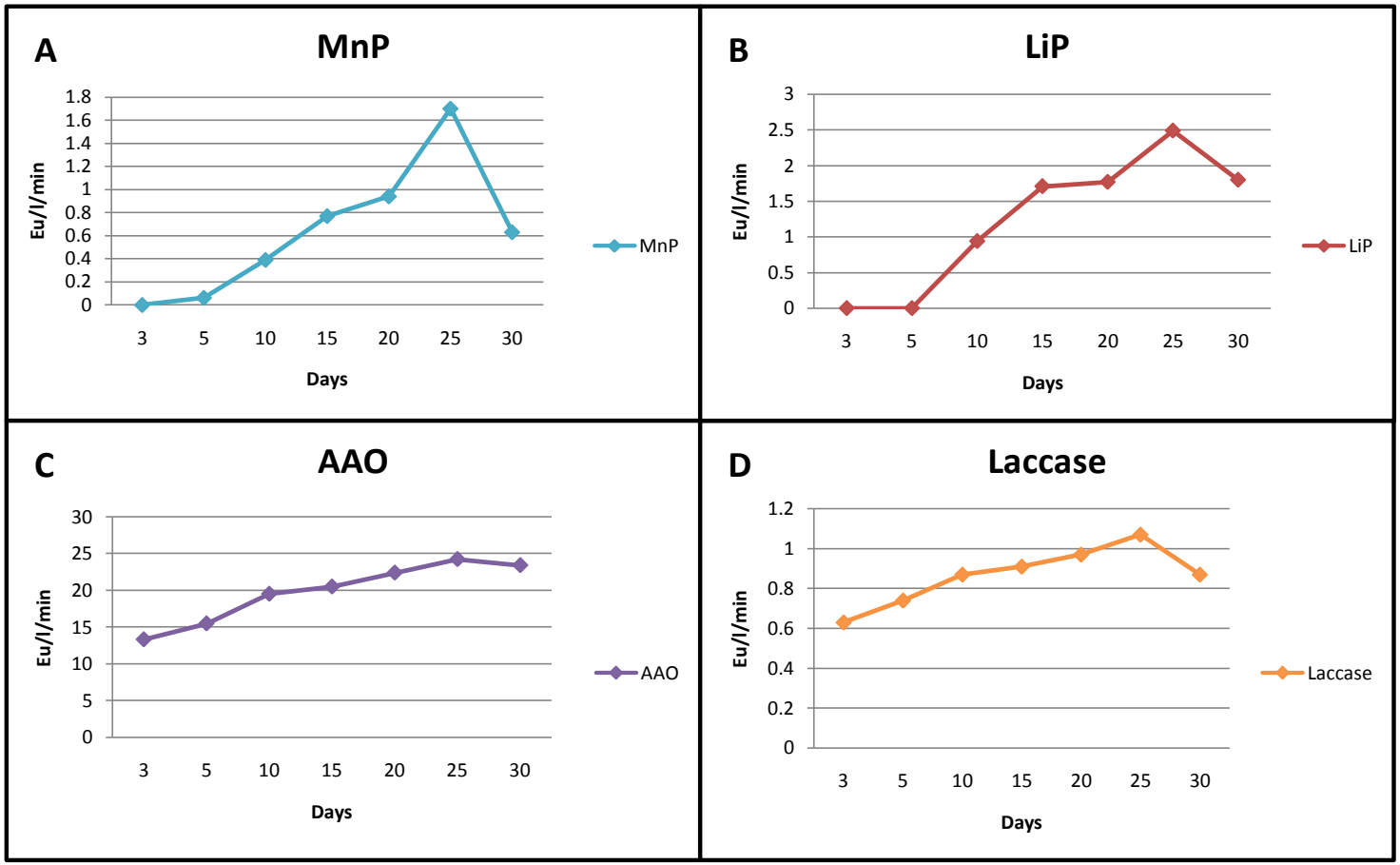


Figure 26 : Ligininolytic enzymes produced by *Daedaleopsis confragosa* during different incubation period

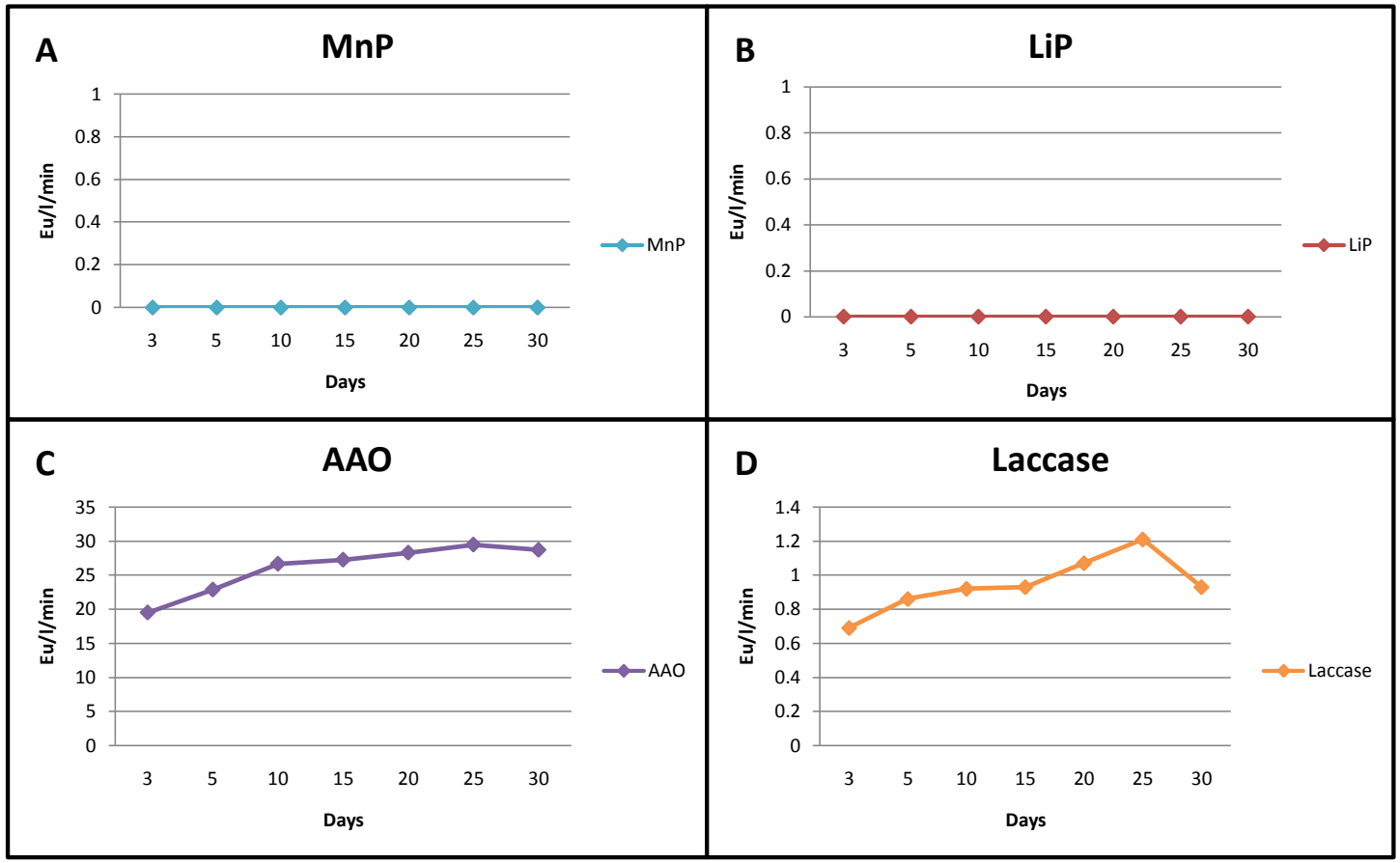


Figure 27 : Ligininolytic enzymes produced by *Phellinus pectinatus* during different incubation period

2) Patameters affecting enzyme activity

a) Effect of pH

It is important to study the pH stability of the different ligninolytic enzymes because in the paper industry the current pulping process takes place under extreme conditions (pH is very highly basic and very high temperature). In the present study pH stability of the different ligninolytic enzymes (MnP, LiP, AAO and Laccase) have been evaluated. In order to study the effect of different pH on the enzyme production, the fungal enzyme extract samples were incubated at different pH and evaluated for the best stability.

pH	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)
1	0.72±0.08	0.56±0.003	14.85±0.43	0.8±0.025
2	0.84±0.03	1.81±0.015	18.07±0.37	0.9±0.016
3	0.98±0.03	2.3±0.063	22.58±0.54	0.97±0.009
4	1.05±0.04	2.37±0.018	23.49±0.21	1.06±0.002
5	1.68±0.018	2.53±0.036	24.37±0.53	1.08±0.006
6	1.7±0.05	2.5±0.062	11.54±0.07	1±0.01
7	1.67±0.02	2.44±0.06	10.92±0.22	0.97±0.02
8	1.56±0.025	2.25±0.04	10.68±0.22	0.92±0.006

Table 24: Effect of pH on ligninolytic enzymes activity of *Daedaleopsis confragosa*

pH	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)
1	0	0	15.98±0.33	1.01±0.012
2	0	0	16.7±0.33	1.03±0.012
3	0	0	22.94±0.14	1.06±0.02
4	0	0	26.69±0.35	1.13±0.01
5	0	0	28.99±0.36	1.2±0.01
6	0	0	28.03±0.37	1.16±0.027
7	0	0	16.57±0.37	1.04±0.06
8	0	0	15.66±0.18	0.7±0.52

Table 25: Effect of pH on ligninolytic enzymes activity of *Phellinus pectinatus*

pH of the solution is very important for the enzyme activity. Table 24 shows pH stability of ligninolytic enzymes produced by *D. confragosa.* MnP is not found stable at very high acidic pH (1 to 4 pH), but it was stable in the pH range of 5-7 (Fig. 28 A). Similarly LiP was also not much stable in the initial pH range (1-3), but it was stable in the pH range of 4 to 7 (Fig. 28 B). As seen in figure 28 C AAO enzyme was stable in the pH range of 3 to 5. It was inactive in the neutral pH. Enzyme laccase showed its stability in the pH range of 4 to 6 (Fig. 28 D). It was observed that all the enzymes showed less stability in basic pH 8 and optimum pH for all the enzymes was 5.

Table 25 shows effect of pH on different ligninolytic enzymes secreted by the *P. Pectinatus.* MnP and LiP enzymes were not produced by this fungus (Fig. 29 A and B). AAO enzyme was stable in the pH range of 5 to 6. In highly acidic, neutral and in basic pH it showed reduction in the enzyme activity (Fig. 29 C). Laccase enzyme was stable in the pH range of 4 to 6 (Fig. 29 D). Similar to *D. confragosa* in *P. pectinatus* the optimum pH for all enzymes could be considered to be pH 5.

For further optimization studies on bioprocess parameters, pH 5 was taken as optimum for maximum ligninolytic enzyme activity.

b) Effect of Temperature

It is important to detect the temperature stability of the different ligninolytic enzymes (MnP, LiP, AAO and Laccase) produced by both the selected fungi. The pH stability of different ligninolytic enzymes secreted by both the fungi were detected by incubating the enzyme containing supernatants at 25, 35, 45, 55, 65, 75 ˚ C in water baths for four hours to allow ample time for denaturation and then carry out the

enzyme assays. Table 26 and 27 shows the effect of temperature on the ligninolytic enzyme activity of the two fungi.

Temperatures (˚C)	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)
5	1.69±0.07	2.65±0.2	24.04±0.18	1.05±0.004
15	1.72±0.042	2.57±0.16	24.73±0.25	1.05±0.004
25	1.73±0.031	2.49±0.036	24.26±0.18	1.08±0.036
35	1.69±0.034	2.34±0.13	24.05±0.2	1.01±0.01
45	1.69±0.007	2.18±0.03	24.61±0.2	1.04±0.005
55	1.109±0.036	2.01±0.063	23.42±0.27	0.93±0.005
65	0	0.96±0.03	18.35±0.33	0.73±0.004
75	0	0	0	0

Table 26: Effect of different temperatures on ligninolytic enzyme activity of *Daedaleopsis confragosa*

Temperatures (˚C)	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)
5	0	0	29.47±0.31	1.18±0.021
15	0	0	29.09±0.35	1.22±0.013
25	0	0	29.49±0.29	1.22±0.008
35	0	0	29.63±0.09	1.21±0.016
45	0	0	28.17±0.33	1.17±0.006
55	0	0	25.34±0.83	1.1±0.02
65	0	0	19.14±0.37	0.86±0.005
75	0	0	0	0

Table 27: Effect of different temperatures on ligninolytic enzyme activity of *Phellinus pectinatus*

Activity of MnP enzyme was stable in the temperature range of 5˚C to 45˚C produced by *D. confragosa*. At 55˚C it showed slight decrease in the activity and at 65˚C it abruptly decreased to zero (Fig. 30 A). LiP enzyme was stable in the range of 5˚C to 45˚C after which the activity gradually declined, and at 75˚C it became zero (Fig. 30 B). AAO activity was observed in the range of 5˚C to 45˚C and, at 55˚C and 65˚C activity reduced becoming zero at 75˚C (Fig. 30 C). For laccase, 5˚C to 45˚C range was

found suitable for its stability (Fig. 30 D) and the activity becomes zero at 75˚C. In this fungus all the enzymes showed the best stability at 25˚C.

Table 27 shows the stability of different ligninolytic enzymes produced by *P. pectinatus* at different temperatures. AAO enzyme production was stable in the range of 5˚C to 45˚C and then it gradually reduced and became zero at 75˚C (Fig. 31 C). Whereas laccase was found to be stable in the range of 5˚C to 55˚C and then reduced to zero at 75˚C (Fig. 31 D). At 25˚C all the enzymes showed maximum activity. For further study 25˚C temperature was taken as optimum for maximum ligninolytic enzyme activity.

3) Study on enhancement of enzyme activity

Different chemicals were used in varying concentration and enhancement in the enzyme activities were evaluated. In order to evaluate the effect of different chemicals on the enzymatic activity, five different chemicals in varying concentrations were used and evaluated. In order to carry out the experiment 3 % MEA medium was prepared and autoclaved and supplemented with different concentration of chemicals (Ethanol, Veratryl alcohol, Xylidine, Yeast Extract, Peptone) and inoculated with a 9 mm disc of 10 day old culture. After the completion of incubation period enzyme assays were carried out.

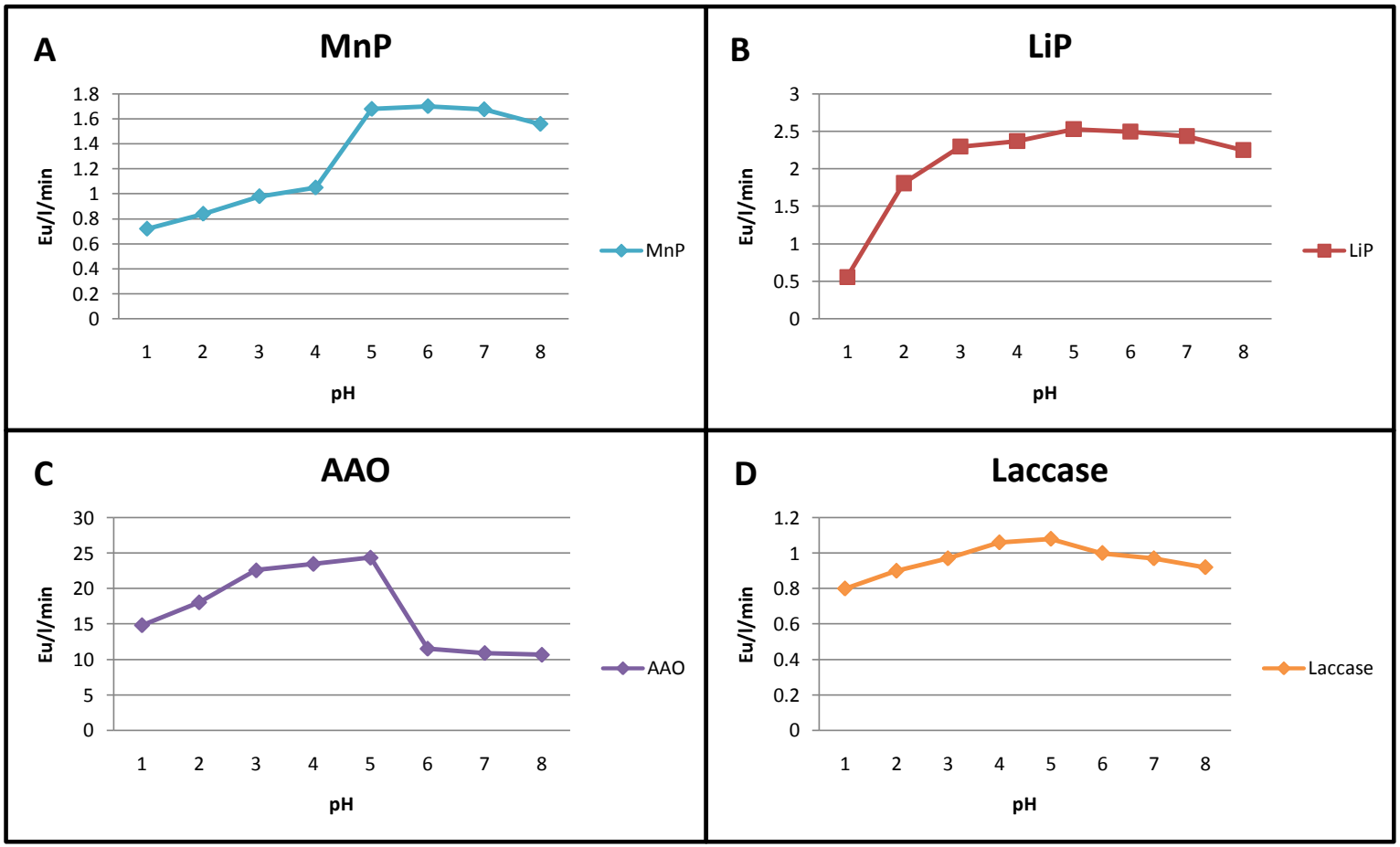


Figure 28: Effect of pH on ligninolytic enzymes activity of *Daedaleopsis confragosa*

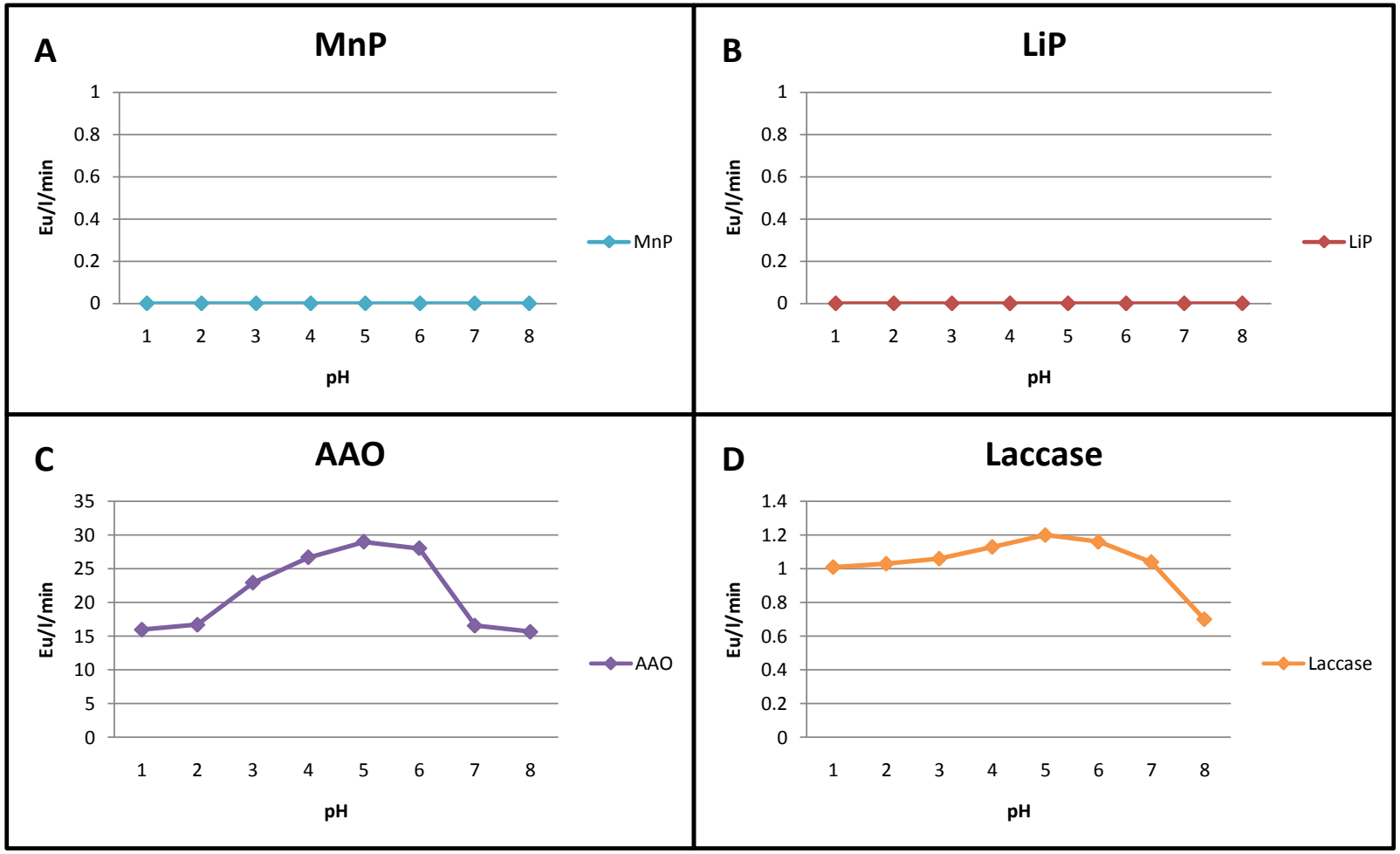


Figure 29: Effect of pH on ligninolytic enzymes activity of *Phellinus pectinatus*

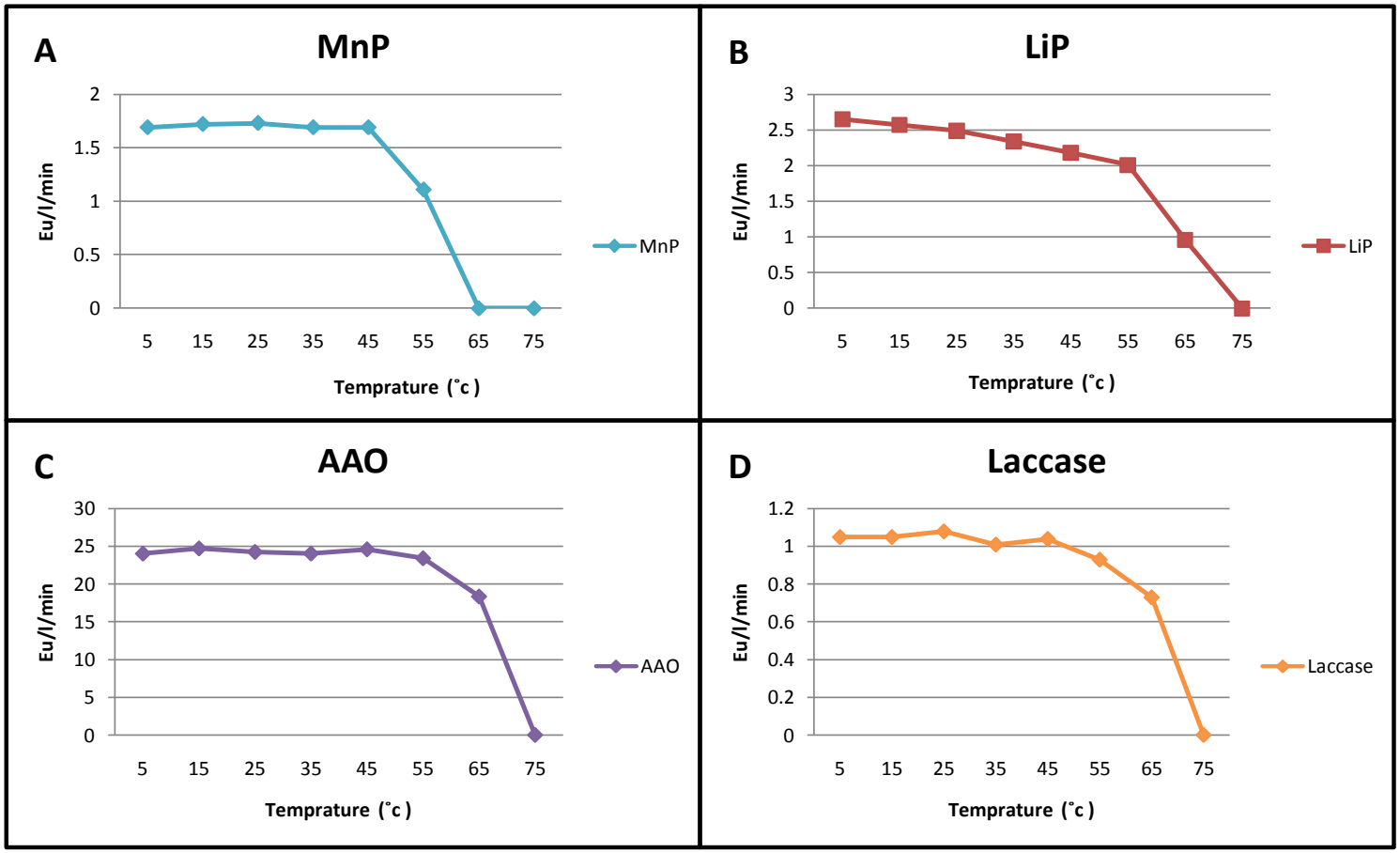


Figure 30: Effect of different temperatures on ligninolytic enzyme activity of *Daedaleopsis confragosa*

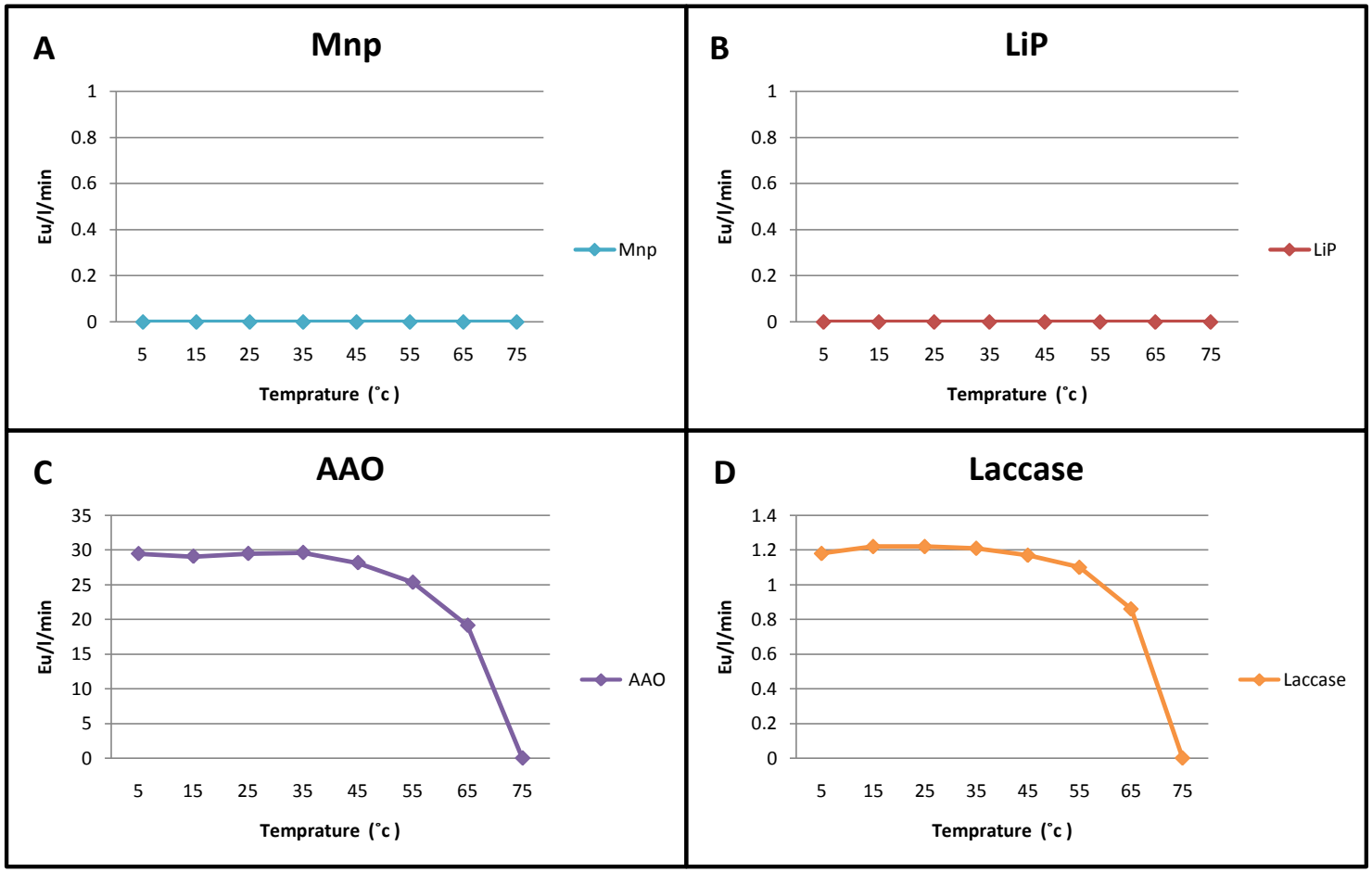


Figure 31: Effect of different temperatures on ligninolytic enzyme activity of *Phellinus pectinatus*

a) Influence of Ethanol (ETH) on enzyme production

The effect of different concentration of ethanol on biomass yield and enzyme production was evaluated in the MEA medium. Ethanol in the concentration of 1%, 2%, 3%, 4%, 5% was added to the media after it was autoclaved (Lee *et al.*, 1999). Control for the experiment was with the absence of ethanol.

Concentration of Ethanol	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)
control	1.73±0.03	2.49±0.04	24.26±0.1	1.07±0.04
1%	2.96±0.08	4.46±0.05	28.2±0.03	0.98±0.01
2%	2.04±0.03	3.49±0.04	25.33±0.15	0.87±0.01
3%	1.91±0.03	3.08±0.03	24.47±0.06	0.86±0.01
4%	1.08±0.03	2.15±0.04	20.56±0.19	0.78±0.01
5%	0.98±0.03	1.78±0.07	16.28±0.12	0.71±0.002

Table 28: Effect of different concentrations of Ethanol on ligninolytic enzyme activity

Effect of different concentration of ethanol on the ligninolytic enzyme activities are shown in Table 26. Addition of 1%, 2% and 3% ethanol resulted in a significant increase in the production of all ligninolytic enzymes except laccase. A higher concentration of ethanol (4% and 5%) decreased the amount of enzyme production compared to the control.

The most significant increase was found with 1% concentration in case of MnP, LiP and AAO. Amongst all the enzymes highest enhancement was observed in Lip (Fig. 32 B) which was 79.11%.

A significant enhancement was also observed in MnP (Fig. 32 A) i.e. 71.09%. Enhancement of AAO was the least as can be very distinctly seen in Figure 32 C (14.38%). Laccase did not show any enhancement with the ethanol (Fig. 32 D).

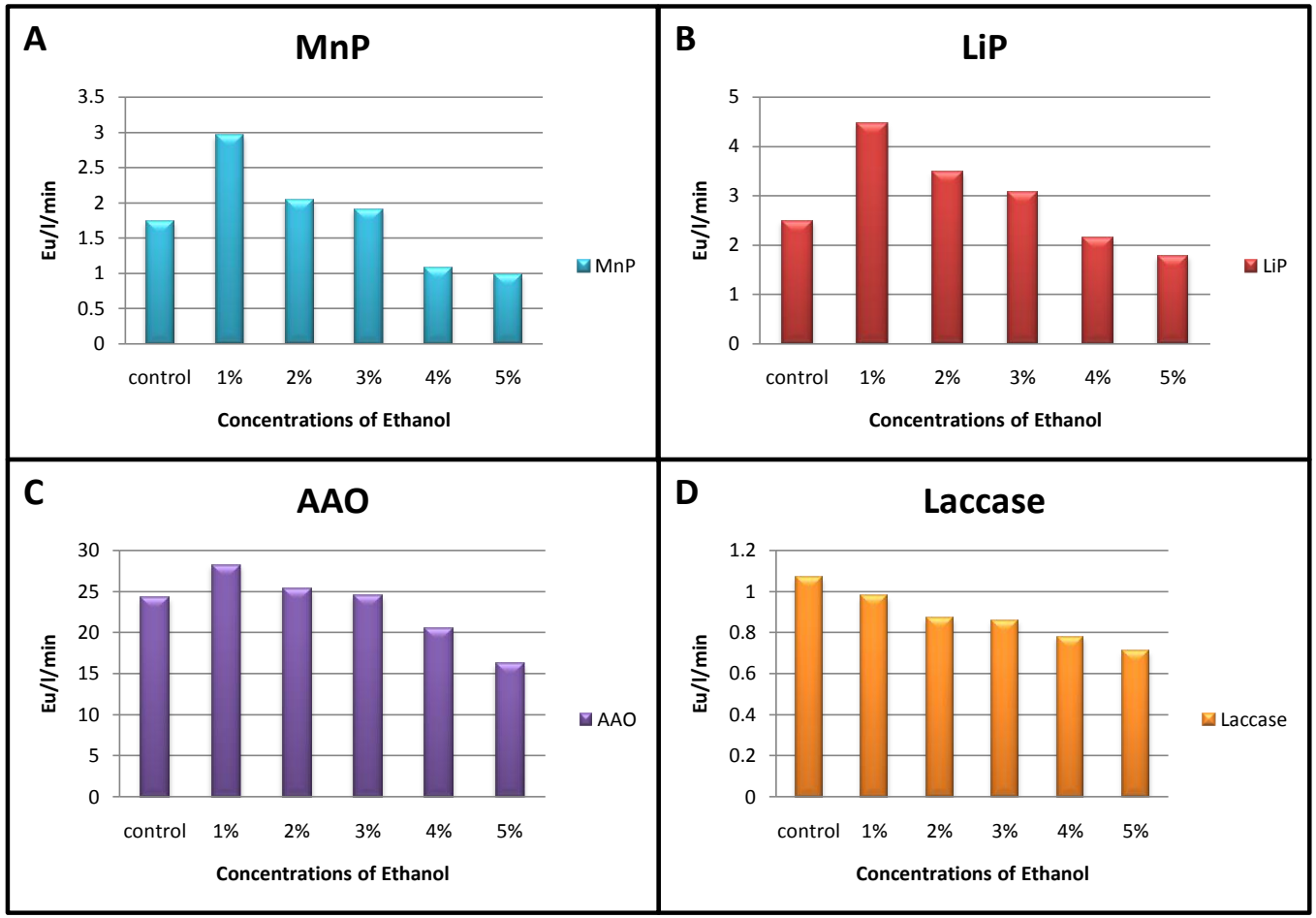


Figure 32: Effect of different concentrations of ethanol on ligninolytic enzyme activity of *Daedaleopsis confragrosa*

b) Influence of Veratryl Alcohol (VA) on enzyme production

In the experiment, to determine the optimal concentration of veratryl alcohol for induction of biomass yield and enzyme production MEA medium was used. Veratryl alcohol was added to MEA media to obtain a final concentration of 4 mM, 8 mM, 12 mM, 16 mM, 24 mM. (Barbosa *et al.,* 1996) A control treatment was included in the experiment that contained no veratryl alcohol.

Concentration of Veratryl Alcohol	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)
control	1.73±0.03	2.49±0.03	24.26±0.1	1.07±0.03
4 mM	1.97±0.03	3.82±0.04	24.73±0.1	1.04±0.01
8 mM	2.18±0.04	4.23±0.04	27.25.08	1.09±0.004
12 mM	2.59±0.03	4.42±0.03	29.45±0.1	1.13±0.004
16 mM	1.98±0.04	3.56±0.02	30.57±0.1	1.24±0.002
20 mM	1.59±0.03	3.29±0.03	29.7±0.07	0.99±0.001

Table 29: Effect of different concentrations of Veratryl Alcohol on ligninolytic enzyme activity

All the ligninolytic enzymes were enhanced with the influence of veratryl alcohol (Table 29). In case of MnP enzyme, enhancement was observed from 4mM to 16mM concentration but in 20mM concentration production of enzyme declined and was lesser than the control (Fig. 33 A). But the highest activity was found in the 12mM concentration which was 49.71% higher than the control. While LiP was enhanced in all the concentrations of veratryl alcohol (Fig. 33 B), with the maximum enhancement in 12 mM concentration which is 77.51% higher than the control. Similar to LiP, AAO was also enhanced in all the concentration (Fig. 33 C). With the maximum enhancement in 16 mM concentration (i.e. 26 % enhanced compare to control). Laccase production enhanced only in the range of 8 mM to 16 mM concentrations (Fig. 33 D). Here enhancement of LiP was highest and laccase was lowest with 16 mM concentration.

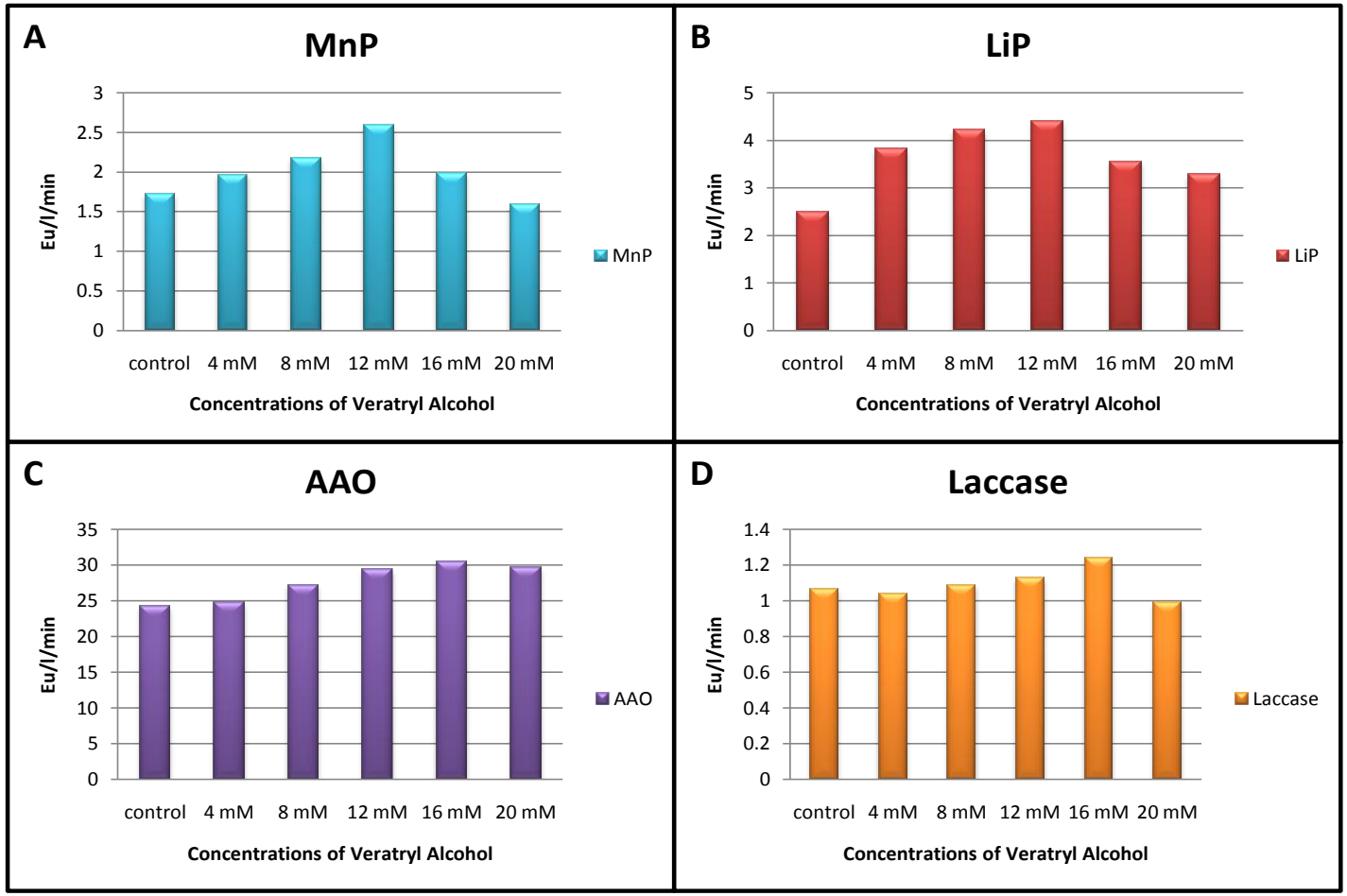


Figure 33: Effect of different concentrations of veratryl alcohol on ligninolytic enzyme activity of *Daedaleopsis confragrosa*

c) Influence of Xylidene (XY) on enzyme production

To determine the effect of xylidene 3 % MEA medium was utilized. Xylidene was added to the MEA media to obtain a final concentration of 4 µM, 7 µM, 10 µM, 20 µM, 30 µM. The control treatment did not include xylidine in the MEA medium.

Concentration of Xylidene	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)
Control	1.73±0.03	2.49±0.03	24.26±0.1	1.07±0.03
4 µM	1.28±0.03	2.59±0.09	18.99±0.07	0.71±0.001
7 µM	1.37±0.01	2.64±0.003	19.69±0.2	0.73±0.01
10 µM	1.39±0.02	2.77±0.01	24.19±0.05	0.76±0.002
20 µM	1.76±0.01	2.89±0.01	24.59±0.04	0.76±0.01
30 µM	2.03±0.03	3.56±0.03	47.22±0.08	0.76±0.01

Table 30: Effect of different concentrations of Xylidine on ligninolytic enzyme activity

Xylidene showed significant enhancing effect on the MnP, LiP and AAO but it did not show significant enhancement in the case of laccase (Table 30). 20µM to 30µM concentrations were responsible for the enhancing the MnP activity (Fig. 34 A), and its production was maximum at 30µM concentration (17.34%). LiP was enhanced in almost all the concentrations of the xylidine (Fig. 34 B) but most significant enhancement was seen with 30 µM concentration.

As seen in figure 34 C AAO enzyme showed the most significant enhancement in 30µM concentration of xylidine (94.64%). Laccase showed no enhancement with xylidine (Fig. 34 D). Enhancement of AAO was very significant (94.64%), but not in MnP (17.34%).

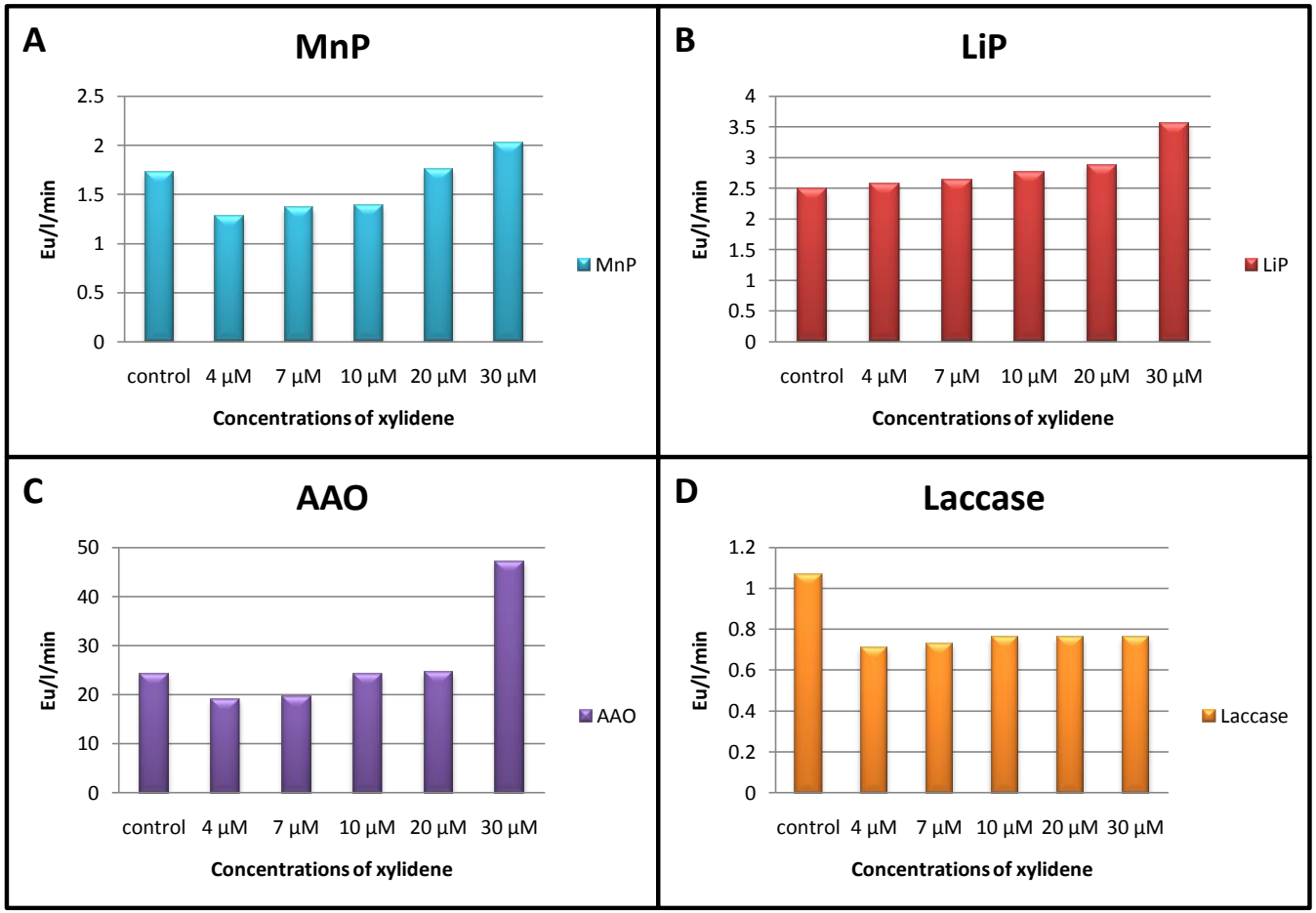


Figure 34: Effect of different concentrations of xylidene on ligninolytic enzyme activity of *Daedaleopsis confragrosa*

d) Influence of Yeast Extract (YE) on enzyme production

To determine the effect of Yeast Extract it is substituted in the MEA medium. Yeast extract is added in MEA medium in the concentration of 0.1 g/l, 0.2 g/l, 0.4 g/l, 0.6 g/l, and 0.8 g/l. In the control the yeast extract is not added.

Concentration of Yeast Extract	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)
control	1.73±0.03	2.49±0.03	24.26±0.1	1.07±0.03
0.1 gm/l	1.37±0.01	2.55±0.03	22.69±0.04	0.58±0.01
0.2 gm/l	1.43±0.01	2.66±0.04	24.15±0.007	0.61±0.005
0.4 gm/l	1.47±0.03	2.73±0.04	24.98±0.03	0.62±0.007
0.6 gm/l	2.15±0.02	3.58±0.007	27.77±0.04	0.69±0.005
0.8 gm/l	1.36±0.03	2.44±0.007	25.73±0.08	0.64±0.01

Table 31: Effect of different concentrations of Yeast Extract on ligninolytic enzyme activity

With the effect of enhancer yeast extract MnP, LiP and AAO activity was enhanced (Table 31). In MnP enzyme showed the enhancement only at the 0.6 gm/l concentration which was 24.28%. In all other concentrations MnP showed low activity than the control (Fig. 35 A). In case of LiP all the concentrations from 0.1 gm/l to 0.6 gm/l showed enhancement (Fig. 35 B), and in 0.6 gm/l concentration LiP show 3.58% enhancement.

AAO enzyme activity was more than control in the concentrations from 0.4 gm/l to 0.8 gm/l, but significant enhancement was observed in the concentration 0.6 gm/l (Fig. 35 C). AAO showed only 14.46% enhancement. Laccase enzyme activity did not enhance with the different concentration of yeast extract (Fig. 35 D) as the substrate. Enhancement of LiP was maximum and AAO was lowest.

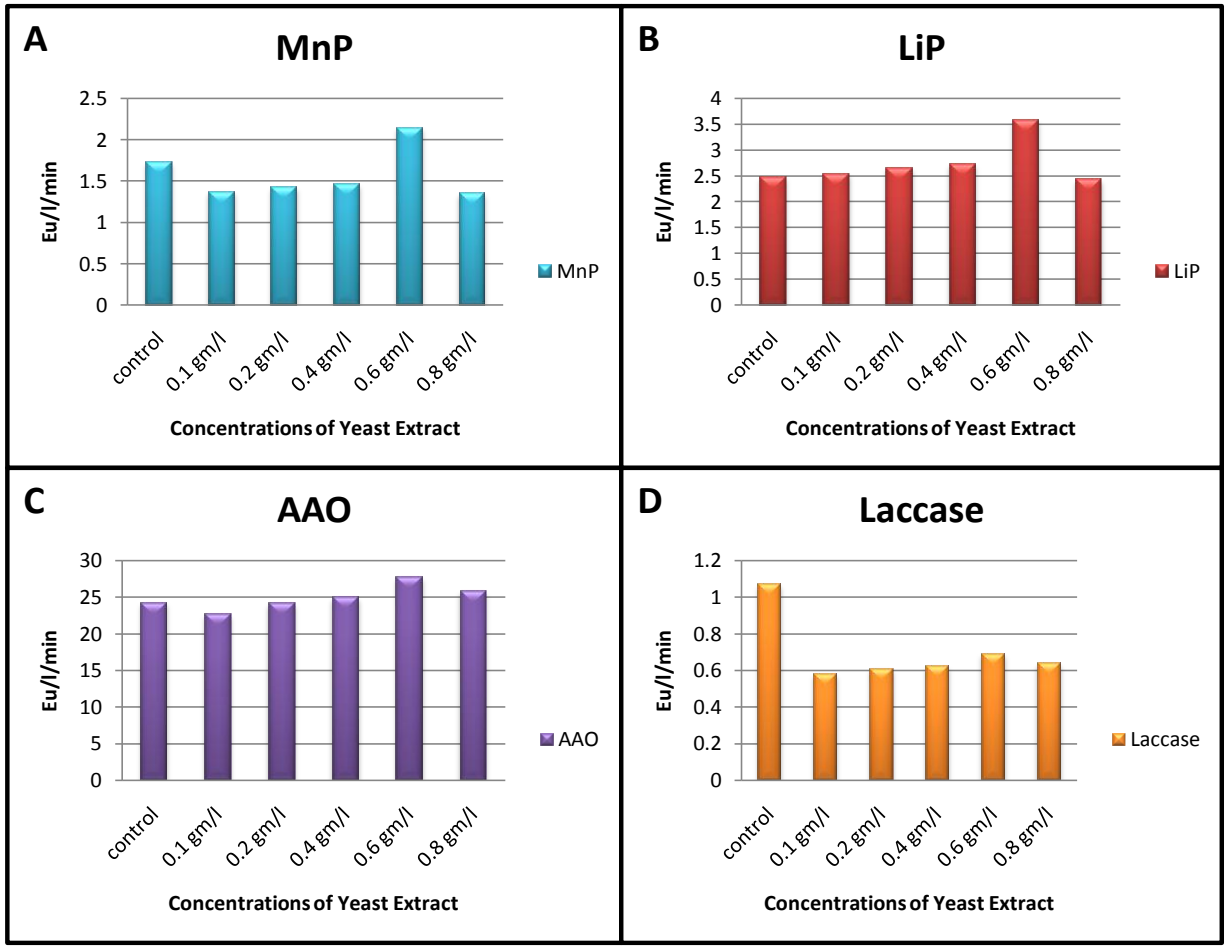


Figure 35: Effect of different concentrations of yeast extract on ligninolytic enzyme activity of *Daedaleopsis confragrosa*

e) Influence of Peptone (PE) on enzyme production

In the experiment to determine the optimal concentration of peptone for induction of biomass yield and enzyme production MEA medium was used. Peptone was added to MEA media to obtain concentration of 0.1 g/l, 0.2 g/l (Kanwal and Reddy 2011), 0.4 g/l, 0.6 g/l, and 0.8 g/l. A control was included in the experiment that contained no peptone.

Concentration of Peptone	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)
control	1.73±0.03	2.49±0.03	24.26±0.1	1.07±0.03
0.1 gm/l	1.36±0.03	2.76±0.02	23.16±0.1	0.6±0.01
0.2 gm/l	1.46±0.03	2.86±0.03	23.32±0.03	0.66±0.01
0.4 gm/l	2.24±0.03	3.02±0.01	26.33±0.05	0.68±0.002
0.6 gm/l	1.54±0.03	2.8±0.03	24.53±0.1	0.7±0.002
0.8 gm/l	1.37±0.01	2.59±0.04	23.49±0.09	0.67±0.003

Table 32: Effect of different concentrations of Peptone on ligninolytic enzyme activity

Table 32 shows the results of the influence of Peptone on the ligninolytic enzymes activity. Here the significant enhancement was observed in MnP, LiP and AAO. Laccase did not show enhancement. In MnP only 0.4 gm/l concentration shows enhancement (29.47%), while other concentrations did not show activity more than control (Fig. 36 A). MnP enzyme activity in all the concentrations of peptone was enhanced (Fig. 36 B). But it was optimum at 0.4 gm/l concentration, which is 21.29 % higher than the control.

AAO activity was more than control in two concentrations of 0.4 gm/l to 0.6 gm/l (Fig. 36 C). And it is highest in 0.4 gm/l concentration, which is 8.53%. Laccase activity was not influenced by the peptone (Fig. 36 D). Here the maximum enhancement is seen in the case of MnP and least in AAO.

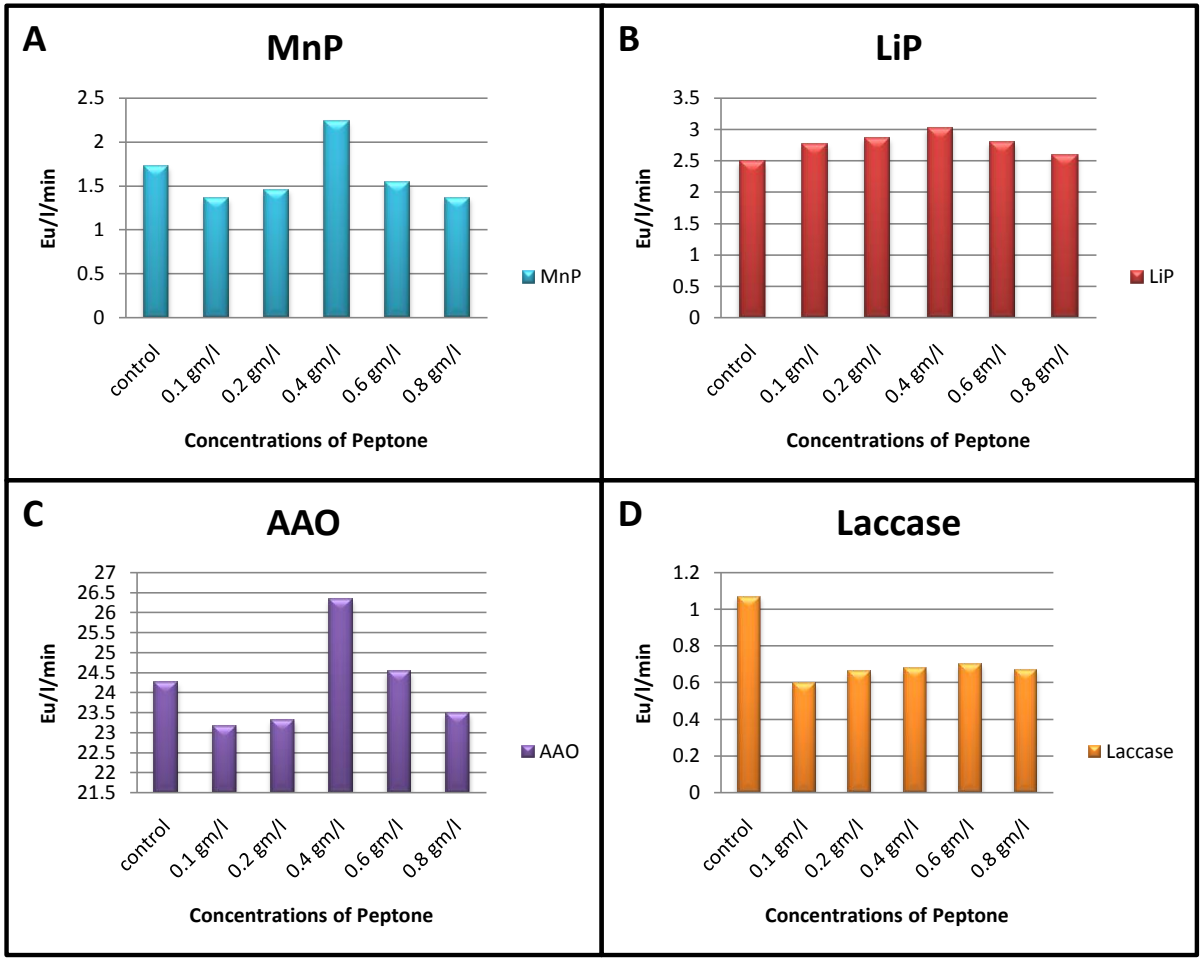


Figure 36: Effect of different concentrations of peptone on ligninolytic enzyme activity of *Daedaleopsis confragrosa*

4) Optimization of enzyme activity in biopulping using different raw Materials

From the results of experiments conducted to study the effect of different conditions (pH, temperature) and different chemicals used as substrate (Ethanol, Veratryl alcohol, Xylidine, Yeast Extract, Peptone) to enhance the enzyme activity, the best optimum conditions and the substrate concentrations were further used, to evaluate and optimize the enzyme activity in biopulping, using different raw materials. The best concentration of the chemicals selected on the basis of experiment on enhancement in enzyme activity 1 % of Ethanol, 12 mM and 16 mM of Veratryl alcohol, 30 µM of Xylidine, 0.4 g/l of Yeast Extract, 0.6 g/l of Peptone were selected. These enhancer chemicals were each substituted in 3 % MEA medium and five selected substrates *Eucalyptus* wood blocks and wood shaving, *Phragmites karka* culm, pulp, *Sesbania sesban* stem were separately experimented with 5 % concentration. These experimental samples were sterilized by autoclaving at 121˚ C temperature and 15 psi pressure for 20 minutes in flask. After cooling, it was inoculated with 9 mm disc of 10 days old fungal culture in aseptic condition. Then culture was incubated at 24 ±0.5 ˚ C for the desired incubation period. After completion of incubation period the experimented cultures were taken out and mycelium broken with the glass pieces and filtered through Whatmen paper No. 1 disc. These filtrates were used as enzyme solution. And remaining portions of samples were used to analyze lignin and cellulose content.

Following are the abbreviations used to indicate the different optimal concentration of chemicals used in further study.

ETH = 1 % of Ethanol, VA 12 =12 mM of Veratryl alcohol , VA16 = 16 mM of Veratryl alcohol XY = 30 µM of Xylidine, PE = 0.4 g/l of Yeast Extract, YE = 0.6 g/l of Peptone

a) Biopulping experiment using *Eucalyptus* wood blocks as substrate

The best enhancers when added with wooden blocks of *E. globules* all the ligninolytic enzymes showed an increase in the enhancement of enzyme activity as clearly indicated in Table 33.

Chemicals	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)	% Lignin loss	% Cellulose loss
Control	2.19±0.01	2.8±0.04	30.49±0.47	1.48±0.02	31.67±2.89	18.33±2.89
VA 12	2.41±0.03	3.01±0.05	33.43±0.39	1.64±0.01	53.33±5.77	20.67±3.06
VA 16	2.11±0.03	2.81±0.04	43.17±0.3	2.11±0.01	46.67±5.77	11±1.73
XY	2.12±0.05	3.27±0.1	40.49±0.3	1.92±0.01	33.33±5.77	15±3.61
ETH	2.24±0.02	2.82±0.01	35.19±0.1	0.98±0.01	36.67±5.77	17.67±4.04
PE	2.55±0.07	3.02±0.07	34.89±0.4	1.12±0.01	33.33±5.77	16.67±3.06
YE	2.24±0.03	2.87±0.04	35.41±0.19	1.82±0.01	35.67±4.04	13.33±5.77

Table 33: Effect of enhancers + wood blocks of *Eucalyptus globulus* on ligninolytic enzyme activity and lignin, cellulose content

MnP enzyme was produced in the highest amount when treated with0.6 gm/l peptone (2.55 EU/l/min) and 12 mM veratryl alcohol (2.41 EU/l/min). When treated with 1% ethanol and 0.4 gm/l Yeast extract 2.24 EU/l/min was produced. MnP production did not show an enhancement compared to the control when supplemented with 16 mM veratryl alcohol and 30 µM Xylidine (Fig. 37 A).

LiP enzyme activity was highest with 30 µM Xylidine (3.27 EU/l/min), 0.6 gm/l peptone (3.02 EU/l/min) and 12 mM veratryl alcohol (3.01 EU/l/min). Whereas in case of other enhancers moderate amount of enhancement was obtained (Fig. 37 B).

AAO enzyme activity enhanced with the supplementation of all the enhancers (Fig. 37 C). It showed its best activity with the 16 mM veratryl alcohol (43.17 EU/l/min), 30 µM Xylidine (40.49 EU/l/min), 0.4 gm/l Yeast extract (35.41 EU/l/min), 1% ethanol (35.19 EU/l/min), 0.6 gm/l peptone (34.89 EU/l/min), 12 mM veratryl alcohol (33.49 EU/l/min).

Laccase enzyme was significantly enhanced with 16 mM veratryl alcohol (2.11 EU/l/min), 0.4 gm/l Yeast extract (1.82 EU/l/min) and 30 µM Xylidine (1.92 EU/l/min). With 12 mM veratryl alcohol concentration the activity was moderate (0.57 EU/l/min). In 1% ethanol 0.6 gm/l peptone laccase activity showed no enhancement (Fig. 37 D).

Percentage of lignin loss was also found to be increased with the supplementation of enhancers (Fig. 37 E). Significant lignin loss was observed with 12 mM veratryl alcohol (53.33 %) and 16 mM veratryl alcohol (46.67 %). With other enhancers, lignin loss obtained was not very significant. Percent of cellulose loss did not enhanced much. With 16 mM veratryl alcohol and 30 µM Xylidine there was decrease in amount of cellulose loss when compared to that of the control (Fig. 37 F).

b) Biopulping experiment using *Eucalyptus* wood shaving as substrate

Wood shavings of *E. globules* were also evaluated for its biopulping potentiality, with the best optimal conditions and concentrations of enhancers. The results are presented in Table 34.

Chemicals	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)	% Lignin loss	% Cellulose loss
Control	2.19±0.05	2.89±0.02	30.28±0.1	1.69±0.01	31±3.61	14.33±5.13
VA 12	2.44±0.02	3±0.01	45.4±0.37	1.94±0.01	39.33±3.06	25.67±3.79
VA 16	2.39±0.05	3.03±0.02	63.49±0.5	2.42±0.02	45.67±3.79	14±2
XY	2.32±0.01	2.96±0.02	42.17±0.12	2.11±0.004	42.67±4.62	13.33±5.7
ETH	1.94±0.02	2.9±0.02	36.42±0.28	1.97±0.03	32.33±6.81	18.67±3.21
PE	2.4±0.02	3.11±0.03	38.68±0.19	2.16±0.01	46.33±3.51	18.33±1.53
YE	2.38±0.01	3.04±0.03	33.34±0.26	1.78±0.01	32.67±2.08	20.33±0.58

Table 34: Effect of enhancers + wood shaving of *Eucalyptus globulus* on ligninolytic enzyme activity and lignin, cellulose content

All the enhancers experimented showed an increase in MnP enzyme production except for 1 % ethanol which showed lesser activity than the control (i.e. 2.19 EU/l/min) (Fig. 38 A). Though lesser the control it was found to be greater than the enzyme produced by the fungus under normal condition without enhancers or substrate on the 25th day of incubation (Table 22). MnP enzyme activity was highest with the 12 mM veratryl alcohol (2.44 EU/l/min), 16 mM veratryl alcohol (2.39 EU/l/min), 0.6 gm/l peptone (2.4 EU/l/min), 0.4 gm/l Yeast extract (2.38 EU/l/min) and 30 µM Xylidine (2.32 EU/l/min).

Lip enzyme activity enhanced with substrate *Eucalyptus globulus* of wood in the form of wood shavings (2.89 EU/l/min). LiP enzyme activity showed enhancement with all enhancers (Fig. 38 B). It was found to be maximum with 0.6 gm/l concentration of peptone (3.11 EU/l/min), 0.4 gm/l Yeast extract (3.04 EU/l/min), 16 mM veratryl alcohol (3.03 EU/l/min) and in 12 mM veratryl alcohol (3.0 EU/l/min). 30 µM Xylidine (2.96 EU/l/min) and 1% ethanol (2.9 EU/l/min) showed the least enhancement.

AAO enzyme activity enhanced in the control (30.28 EU/l/min). Highest enhancement was observed in 16 mM veratryl alcohol (63.49 EU/l/min) which was

more than double (Fig. 38 C). Moderate enhancement was observed in 12mM veratryl alcohol (45.4 EU/l/min), 30 µM xylidine (42.17 EU/l/min) and 0.6 gm/l peptone (38.68 EU/l/min), while it was lowest in 1% ethanol (36.42 EU/l/min) and 0.4 gm/l Yeast extract (33.34 EU/l/min).

Laccase enzyme activity in control was 1.69 EU/l/min. Significant enhancement could be obtained in 16 mM veratryl alcohol (2.42 EU/l/min), 0.6 gm/l peptone (2.16 EU/l/min) and 30 µM Xylidine (2.11 EU/l/min). Enhancement was moderate by 1% ethanol (1.97 EU/l/min) and 12 mM veratryl alcohol (1.94 EU/l/min). Lest amount of enhancement was found with 0.4 gm/l Yeast extract (1.78 EU/l/min) (Fig. 38 D).

A significant increase in the loss of lignin was obtained with 0.6 gm/l peptone (46.33 %), 16 mM veratryl alcohol (45.67 %) and 30 µM xylidine (42.67 %). All other enhancers also, good amount of lignin loss was observed (Fig. 38 E). Cellulose loss increased in 12 mM veratryl alcohol (25.67 %). It did not show much increase with other enhancers (Fig. 38 F).

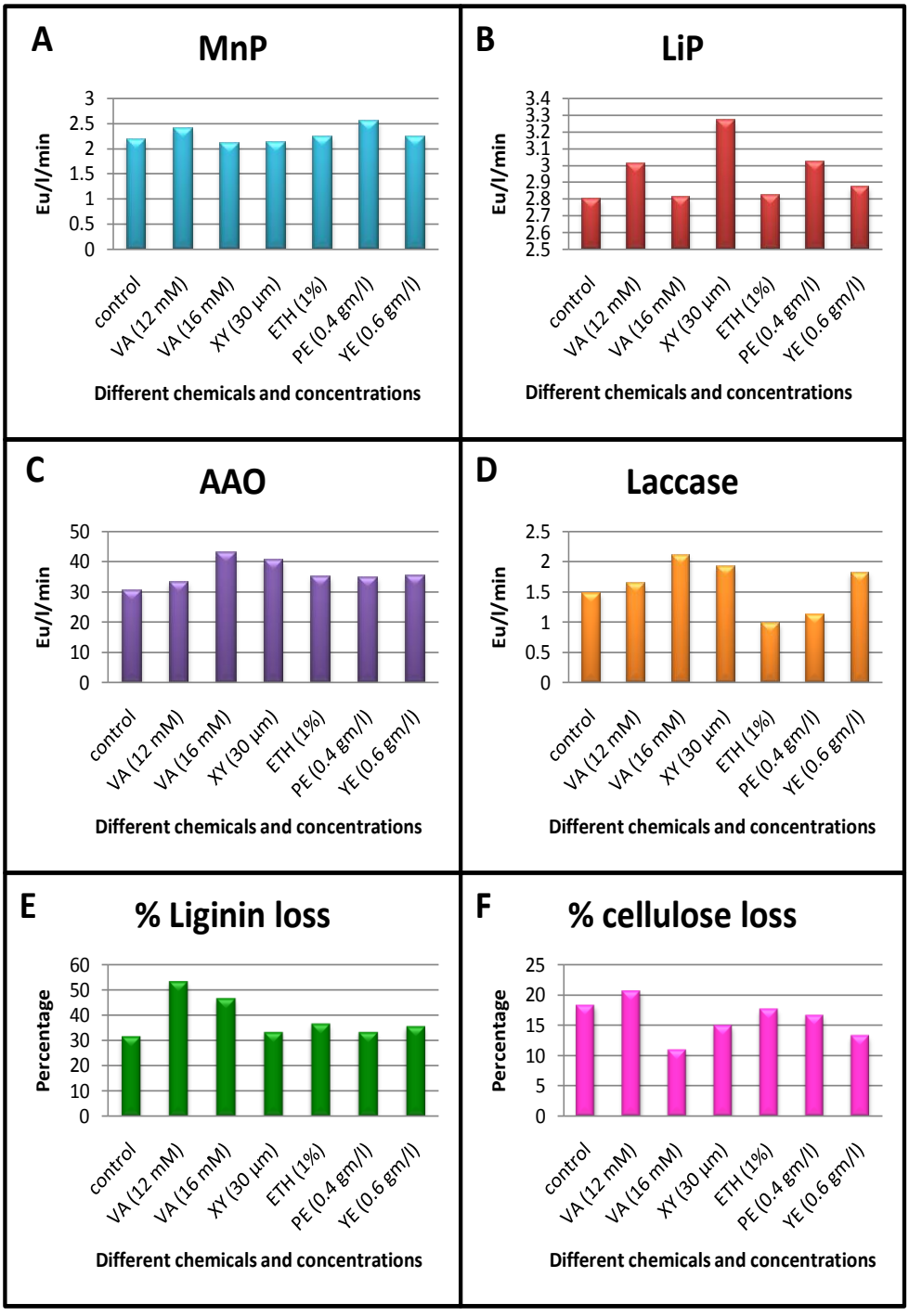


Figure 37: Effect of enhancers + wood blocks of *Eucalyptus globulus* on ligninolytic enzyme activity

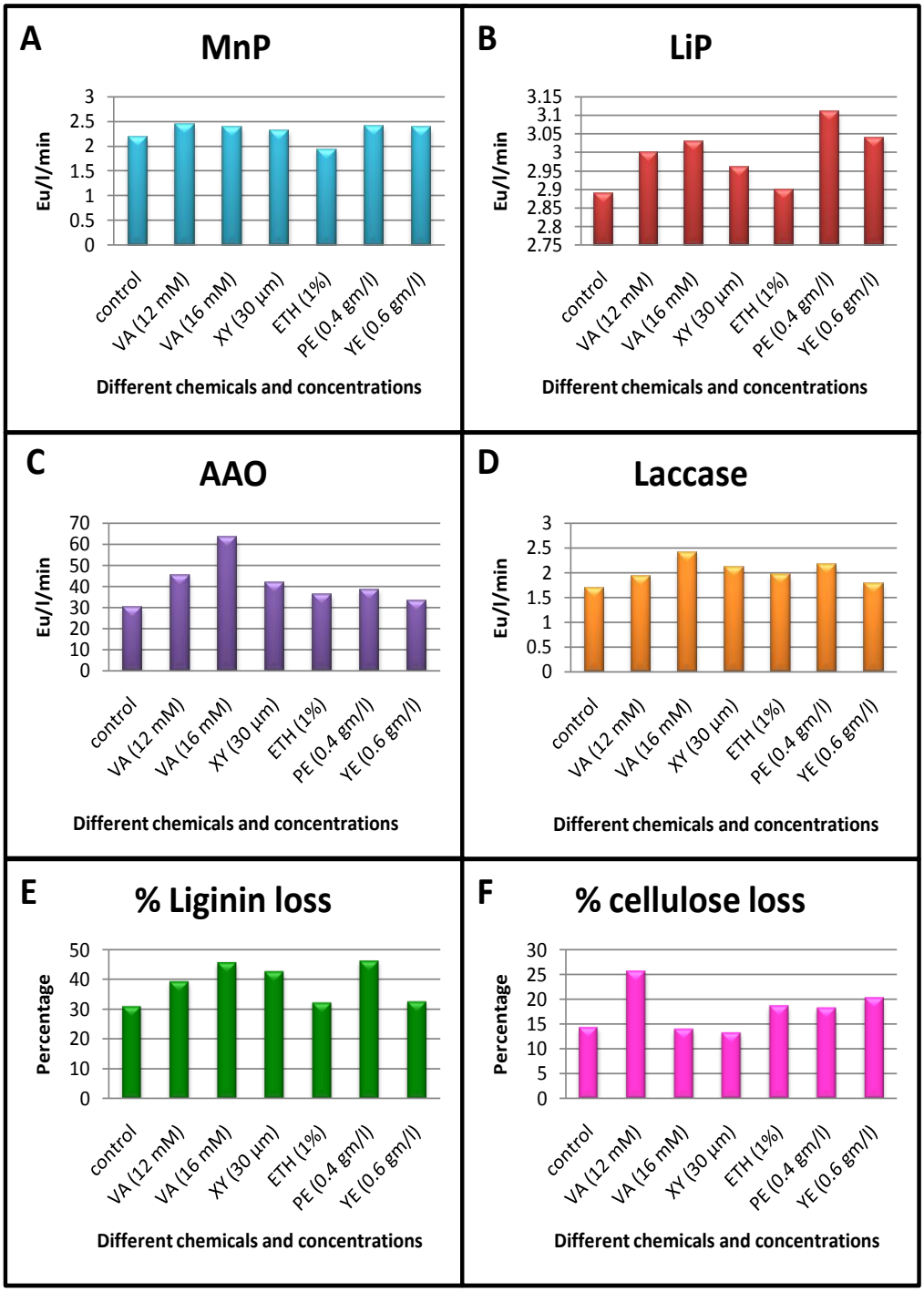


Figure 38 : Effect of enhancers + wood shavings of *Eucalyptus globulus* on ligninolytic enzyme activity

c) Biopulping experiment using pulp as substrate

Pulp from the J.K. Paper industry was a substrate having lesser amount of lignin compared to other substrates because the pulp was obtained after oxygen dependent delignification stage. When different enhancers were added for experimenting the enhancement of enzyme activity with the pulp, almost all the enzymes and loss in lignin content enhanced significantly (Table 35).

Chemicals	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)	% Lignin loss	% Cellulose loss
Control	2.02±0.05	2.78±0.02	38.38±0.4	1.38±0.02	18.06±2.4	10.78±1.35
VA 12	2.12±0.02	3.01±0.03	44.95±0.2	1.93±0.01	34.03±1.2	18.22±1.68
VA 16	2.21±0.01	2.82±0.04	49.46±0.21	2.24±0.01	42.64±2.8	15.56±1.9
XY	2.68±0.02	3.64±0.02	44.04±0.22	1.78±0.01	36.53±5.69	11.11±1.9
ETH	2.73±0.02	3.66±0.03	39.06±0.3	1.32±0.02	44.44±2.4	4.44±1.9
PE	2.61±0.03	3.24±0.04	41.91±0.25	1.26±0.02	30.56±2.4	12.22±1.9
YE	2.52±0.03	3.29±0.07	38.3±0.48	1.17±0.03	22.22±2.4	8.89±1.9

Table 35: Effect enhancers + pulp on ligninolytic enzyme activity and lignin, cellulose content

Mnp enzyme activity was found to be 2.02 EU/l/min, without enhancers. MnP enzyme activity was highest when it was treated with the 1% ethanol (2.73 EU/l/min), 30 µM xylidine (2.68 EU/l/min), 0.6 gm/l peptone (2.61 EU/l/min) and with 0.4 gm/l Yeast extract (2.52 EU/l/min). Enzyme activity did not show much enhancement with 12 mM veratryl alcohol and 16 mM veratryl alcohol (Fig. 39 A).

In the control set up LiP activity was 2.78 EU/l/min. Maximum enhancement was observed with 1% ethanol (2.73 EU/l/min) and 30 µM xylidine (2.68 EU/l/min). Whereas enhancement was moderate with 0.4 gm/l yeast extract (2.52 EU/l/min) and 0.6 gm/l peptone (3.02 EU/l/min). A concentration of 12 mM veratryl alcohol and 16 mM veratryl alcohol showed least amount of enhancement (Fig. 39 B).

With pulp as the substrate AAO activity was found to be 38.38 EU/l/min. AAO enzyme activity was highly enhanced with 16 mM veratryl alcohol (49.46 EU/l/min). Significant enhancement is also observed with 12 mM veratryl alcohol (44.95 EU/l/min), 30 µM xylidene (44.04 EU/l/min), and 0.6 gm/l peptone (41.91 EU/l/min). enhancement was very less with 1% ethanol and 0.4 gm/l Yeast extract (Fig. 39 C).

Laccase enzyme activity in the control showed 1.38 EU/l/min. Laccase enzyme significantly enhanced with 16 mM veratryl alcohol (2.24 EU/l/min) while moderate amount of enhancement was observed with 12 mM veratryl alcohol (1.93 EU/l/min) and 30 µM Xylidine (1.78 EU/l/min). With 1% ethanol, 0.6 gm/l peptone and 0.4 gm/l Yeast extract laccase activity was not enhanced (Fig. 39 D).

With all the enhancers good amount of loss in lignin content was obtained with the highest amount in 1% ethanol (44.44 %) and 16 mM veratryl alcohol (42.64 %). Loss of lignin content was observed to be the least with 0.4 gm/l Yeast extract (Fig. 39 E). Loss of cellulose was found to be enhanced with a few enhancers but it was not a very significant loss with 0.4 gm/l Yeast extract (8.89 %) and 1% ethanol (4.44 %) a decrease (Fig. 39 F).

d) Biopulping experiment using *Sesbania sesban* stem as substrate

The stem of *Sesbania sesban* a legume the flower of which are edible but the stem consider as a agro waste was used as substrate for the biopulping experiment in the combination with the different enhancers so as to enhance the ligninolytic enzyme production and loss of lignin (Table 36).

Chemicals	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)	% Lignin loss	% Cellulose loss
Control	2.16±0.03	3.22±0.03	32.93±0.2	1.39±0.08	7.53±1.86	5.4±1.1
VA 12	2.71±0.04	3.49±0.07	40.68±0.2	1.43±0.03	14.62±1.4	10.16±1.1
VA 16	2.62±0.1	3.29±0.04	42.99±0.14	1.71±0.014	17.2±1.86	6.35±2.75
XY	2.27±0.02	3.16±0.09	33.16±0.2	1.44±0.013	13.66±0.8	8.57±2.08
ETH	2.03±0.03	3.22±0.07	32.94±0.2	1.34±0.01	9.89±0.37	12.7±2.75
PE	2.37±0.03	3.18±0.03	33.1±0.15	1.06±0.022	7.96±1.6	6.35±2.75
YE	2.3±0.03	3.09±0.07	34.23±0.26	1.28±0.02	10.86±1.1	6.51±3.02

Table 36: Effect of enhancers + stem of *Sesbania sesban* on ligninolytic enzyme activity and lignin, cellulose content

Mnp enzyme activity was enhanced with almost all the enhancers. (Fig. 40 A) In the control the activity was found to be 2.16 EU/l/min. Enhancement in MnP enzyme activity was highest 12 mM veratryl alcohol (2.71 EU/l/min) and 16 mM veratryl alcohol (2.62 EU/l/min). It was moderate with 0.6 gm/l peptone (2.37 EU/l/min), 0.4 gm/l Yeast extract (2.3 EU/l/min) 30 µM Xylidine (2.27 EU/l/min). Least enhancement of enzyme activity was observed with 1% ethanol. With substrate *S. sesban* stem Lip enzyme activity showed enhancement (3.22 EU/l/min). LiP enzyme activity showed enhancement with all enhancers (Fig. 40 B), with the maximum in 12 mM veratryl alcohol (3.49 EU/l/min), moderate in 16 mM veratryl alcohol (3.29 EU/l/min) and 1% ethanol (3.22 EU/l/min). In all other enhancers it did not show enhancement in the enzyme activity.

AAO enzyme activity in the control set up was 32.93 EU/l/min. Highest enhancement was found with 16 mM veratryl alcohol (42.99 EU/l/min) and 12mM veratryl alcohol in (40.68 EU/l/min) (Fig. 40 C). Moderate enhancement was found with 0.4 gm/l Yeast extract (34.23 EU/l/min), 30 µM xylidine (33.16 EU/l/min), 0.6 gm/l peptone (33.1 EU/l/min) and 1% ethanol (32.94 EU/l/min). Laccase enzyme activity with *S.sesban* stem is 1.39 EU/l/min. Significant enhancement could be

obtained with 16 mM veratryl alcohol (1.71 EU/l/min). Moderate enhancement was observed in xylidine 30 µM (1.44 EU/l/min) and 12 mM veratryl alcohol (1.43 EU/l/min). Other enhancers did not show enhancement in the activity (Fig. 40 D).

Loss of lignin is enhanced in almost all combinations (Fig. 40 E). Significant increase was observed in the lignin loss which is 17.2 % with 16 mM veratryl alcohol, 14.62% veratryl alcohol 12 mM, 13.66 % in xylidine 30 µM, 10.86 % in 0.4 gm/l Yeast extract, 9.89 % in 1% ethanol and 7.96 % in 0.6 gm/l peptone. Loss of cellulose was found to be less with enhancers 16 mM veratryl alcohol (6.35 %), 30 µM xylidine (8.57 %), 0.6 gm/l peptone (6.35 %), 0.4 gm/l and yeast extract (6.51 %) (Fig. 40 F).

e) Biopulping experiment using culm of *Phragmites karka* stem as substrate

Enhancement of enzyme activity with addition of *Phragmites karka* culm as the substrate with different enhancers, and loss of lignin and cellulose content were evaluated and the results obtained are represented in Table 37.

Chemicals	MnP (Eu/l/min)	LiP (Eu/l/min)	AAO (Eu/l/min)	Laccase (Eu/l/min)	% Lignin loss	% Cellulose loss
Control	2.11±0.03	2.91±0.03	43.7±0.2	1.41±0.01	14.33±5.13	11.11±2.41
VA 12	2.37±0.03	3.1±0.03	49.68±0.43	1.49±0.01	22.33±2.08	11.94±0.96
VA 16	2.44±0.05	3.18±0.04	51.37±0.2	1.54±0.01	34±5.29	12.92±3.56
XY	2.16±0.07	3.04±0.04	45.91±0.12	1.42±0.005	28±3.46	18.06±2.41
ETH	1.59±0.09	2.7±0.2	50.06±0.17	1.93±0.008	19.33±5.03	8.47±2.71
PE	2.41±0.03	3.26±0.04	50.66±0.17	1.5±0.01	28±3.46	16.11±0.96
YE	2.66±0.03	3.26±0.07	51.42±0.19	1.76±0.009	37.33±3.06	15.28±2.41

Table 37: Effect of enhancers + *Phragmites karka* culm on ligninolytic enzyme activity and lignin, cellulose content

2.11 EU/l/min of MnP enzyme was secreted in the control condition (only with the Culm of *P. karka*). MnP enzyme produced was enhanced in almost all the substrate

except 1 % ethanol. Highest amount of enhancement was obtain with the 0.4 gm/l Yeast extract (2.66 EU/l/min) and veratryl alcohol 16 mM (2.44 EU/l/min). Veratryl alcohol 12 mM (2.37 EU/l/min) and peptone 0.6 gm/l (2.41 EU/l/min) showed the moderate amount of enhancement. Lest amount of enhancement was observed in 30 µM xylidine (2.16 EU/l/min) (Fig. 41 A).

In the control set of experiment Lip enzyme activity measured was found to be 2.91 EU/l/min. Enhancement in LiP enzyme activity was highest with the 0.6 gm/l peptone (3.26 EU/l/min) and 0.4 gm/l Yeast extract (3.26 EU/l/min), moderate with 16 mM veratryl alcohol (3.18 EU/l/min), 12 mM veratryl alcohol (3.1 EU/l/min) and 30 µM xylidine (3.04 EU/l/min) and lowest enhancement was observed with 1 % ethanol (Fig. 41 B).

AAO showed 43.7 EU/l/min activity in the control. AAO enzyme activity enhanced with all the enhancers (Fig. 41 C) 0.4 gm/l Yeast extract (51.42 EU/l/min), 16 mM veratryl alcohol (51.37 EU/l/min), 0.6 gm/l peptone (50.66 EU/l/min), 1% ethanol (50.06 EU/l/min), 12 mM veratryl alcohol (49.68 EU/l/min) and 30 µM xylidine (45.91 EU/l/min).

Laccase enzyme activity with the culm of *P. karka* showed 1.41 EU/l/min. Laccase enzyme activity was significantly enhanced with 1% ethanol (1.93 EU/l/min) and 0.4 gm/l Yeast extract (1.76 EU/l/min). With all other enhancers moderate amount of enhancement was observed (Fig. 41 D).

There was a significant increase in the lignin loss with different enhancers (Fig. 41 E). The significant lignin loss was observed with 0.4 gm/l Yeast extract (37.33 %), 16 mM veratryl alcohol (34 %), 30 µM xylidine (28 %), 0.6 gm/l peptone (28 %), 12

mM veratryl alcohol (22.33 %) and 1% ethanol (19.33 %). Loss of cellulose content is not enhanced much, it is in the range of 8 to 13 % which was lesser than lignin loss. (Fig. 41 F).

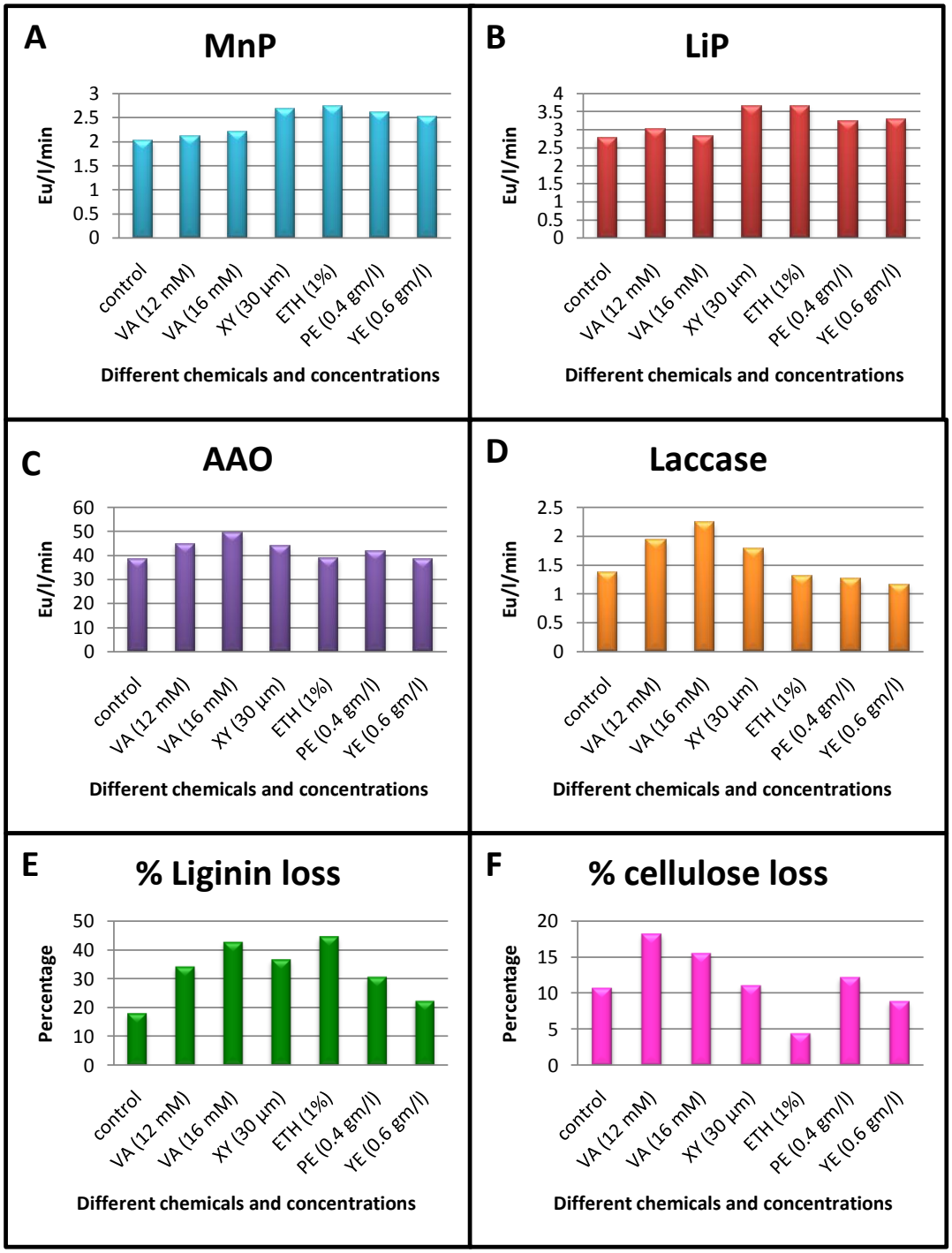


Figure 39: Effect of enhancers + pulp on ligninolytic enzyme activity

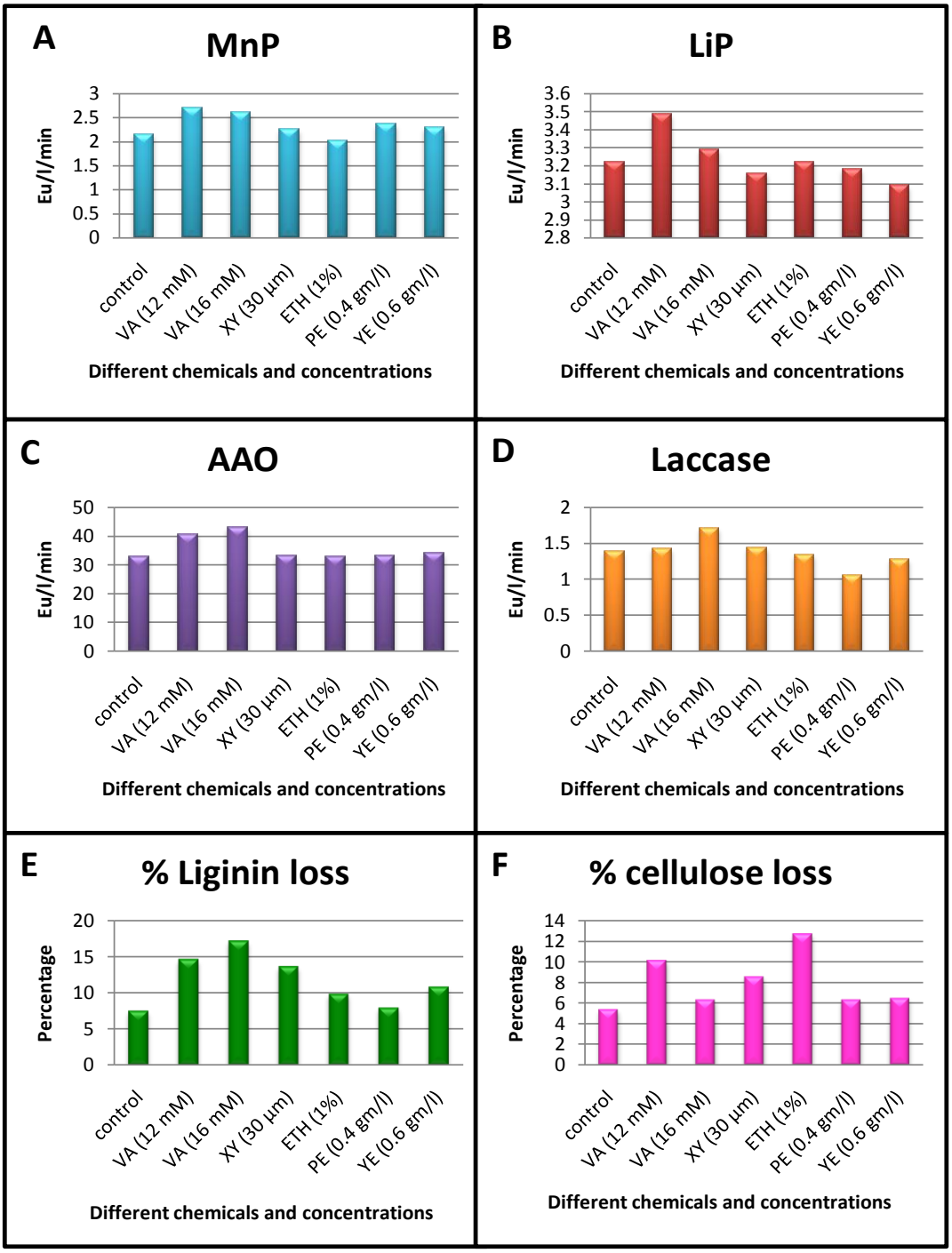


Figure 40: Effect of enhancers + stem of *Sesbania sesban* on ligninolytic enzyme activity

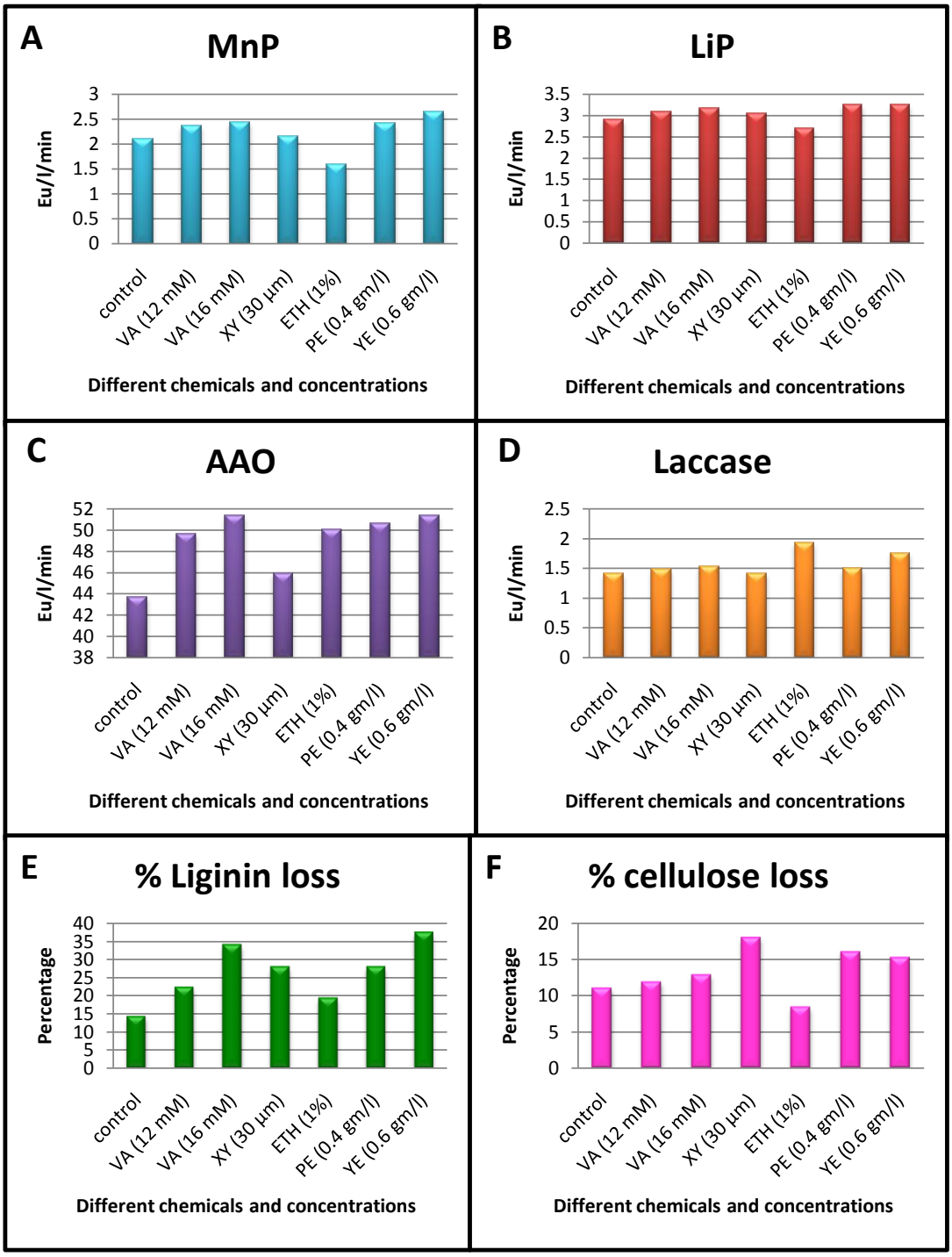


Figure 41: Effect of enhancers + culm of *Phragmites karka* on ligninolytic enzyme activity

II) DISCUSSION

Fungus which can degrade different components of wood are directly associated with the production of different ligninolytic enzymes (Hatakka 1994). These ligninolytic enzymes can be used for biopulping (Ahuja *et al.* 2004). It not only reduces the requirement of chemicals and energy but also reduces the pollution due to the use of chlorine in bleaching process (Akhtar *et al*. 1992).

The aim of the study was to evaluate the ability of two different basidiomycetes fungi to produce ligninolytic enzymes (MnP, LiP, AAO, Laccase). Capacity of fungal strain to degrade lignin can be judged by its enzyme activity. Thus the ligninolytic activity of a fungus is one of the important aspects for biopulping.

Fungal biomass produced by the *D. confragosa* was higher than that of *P. pectinatus*. Both the fungi showed its maximum activity on the 25^{th} day. *D. confragosa* showed less production of AAO and Laccase compared to *P. Pectinatus. P. pectinatus* did not secrete enzymes MnP and LiP. So *D. Confragosa* was considered to be a more efficient fungi in the secretion of ligninolytic enzymes.

D. confragosa demonstrated the simultaneous production of all the ligninolytic enzymes but *P. pectinatus* produced only two groups of enzymes (AAO and Laccase). As described by De Jong *et al.* (1994) most of the wood degrading fungi produce three groups of enzymes but others produce only one or two groups as observed in *P. pectinatus* in the present study, also indicating that it does not require all three enzymes at one time during the lignin degradation process. MnP which is the most common lignin modifying peroxidase enzyme produced by almost all wood colonizing white rot and litter decomposing fungi (Hofrichter 2002) was not produced by *P. pectinatus*.

Phellinus igniaricus and another species of *Phellinus* from Andhra Pradesh screened for the production of these enzymes (Goud *et al.* 2011) showed positive results. All the three enzymes were produced by these species indicating that the production of 1, 2 or 3 groups of ligninolytic enzymes also varied from species to species and strains of the same fungi. Elisahvili *et al.* (2009) reported an absence of MnP production by few ligninolytic basidiomycetes fungi and production of these enzymes also differed with the strains. Of the two strains of *Fomes forment* (1BB9 and 1BB38) and three strains of *T. versicolor* (1BB5, 1BB13, 1BB16), *Fomes forment* 1BB38 and *T. versicolor* 1BB13, 1BB16 produce MnP but *Fomes forment* 1BB9 and *T. versicolor* 1BB5 do not produce MnP.

According to Pela´ ez *et al.* (1995) and Tekere *et al.* (2001), in white rot fungi production of LiP is a rare. *Phelinus* sp. produced 0.044 U/ml laccase enzymes within 14 days of incubation and *P. igniaricus* produced 0.044 U/ml laccase enzyme which agrees with our results for both the fungi. It also produced MnP (0.051 U/ml) and LiP (0.17 U/ml).

Pant and Adholeya (2007) has studied ligninolytic enzyme activity of *Penicillium pinophilum, Alternaria gaisen* and *Pleurotus florida.* In this study they found that the laccase was produced by *P. florida* (0.806 U) within 10 days of incubation but in the present study same amount of the laccase was produced by *D. confragosa*, 25 days, of incubation with absence of MnP and LiP enzymes. MnP production of *P. pinophilum* is approximately same as that of *D. confragosa* but LiP production is much higher than the *D. confragosa*.

Studies on the lignin degrading enzyme Aryl-Alcohol Oxidase (AAO) and its enhancement are few (Ruiz-Duenas *et al*. 2006; Liers *et al*. 2011). AAO is an FAD-containing extracellular enzyme (Guillen *et al.* 1992). Liers *et al.* (2011) has found that *Phlebia radiate* (34 mU/g), *Bjerkandera adusta* (218 mU/g) and *Pleurotus eryngii* (13 mU/g), which agrees with AAO enzyme production in present study by both of fungi. Ruiz-Duenas *et al.* (2006) has also found AAO enzyme activity using strain *Escherichia coli.*

Enzymes are very sensitive to the different factors (like cultivation condition, pH and temperature). In the present study we have studied the effect of pH on the activity of all ligninolytic enzymes. We have tested the stability of enzymes from pH 1 to 8.

AAO enzyme in *D. confragosa* was stable in the pH range of 3 to 5, while in *P. pectinatus* it was stable in the pH range of 5 to 6. Laccase produced by both the fungi showed maximum activity in the same pH range. For both the fungi pH 5 was found to be optimal for stability of all ligninolytic enzymes.

The optimum pH for laccase activity has been found to vary from one substrate to another when compared with the data obtained from other sources. The ligninolytic enzyme activity of purified laccase and MnP from *Pleurotus ostreatus* were found to be completely stable in large pH range (4-8) with an optimum range at pH value of 4 (Aslam and Ashger 2011). Xin and Geng, 2011 evaluated the effect of different parameters on the production of laccase by *Trametes versicolar,* and observed that the optiemum yield of laccase (range of pH 5-9) at initial pH value of 7 was 1.2 fold of that obtained at initial pH 4.

Gianfreda *et al.* (1999) earlier reported laccase from *Trametes versicolar* to be three times more active with DMP than 2, 2'-azino-bis (3-ethylbenziazoline-6-sulphonic acied) diammonium salt. Also Nyanhongo *et al.* (2002) reports laccase activity by a strain of *Trametes modesta* to be optimum at pH 4 using 2,6 dimethoxyphenol (DMP) as the substrate. In the present study with ABTS as substrate both fungi *D. confragosa* and *P. pectinatus* produced an optimal laccase enzyme production and activity at pH range 3-5 with maximum at pH.

Michniewicz *et al.* (2006), observed that the pH optima for the laccase enzyme production by *Cerrena unicolor* to be high in the acidic range (pH 2.5 to 3) with the ABTS as the substrate.

LiP enzyme activity was stable in the pH range of 3 to 5 produced by the fungus *Phanerochaete chrysosporium* (Bosco *et al.* 2010), which is in accordance with the results of the *D. confragosa.*

After the pH one of the major factor affecting the stability of the fungus is temperature. Optimum temperature varies with the variation in the strain (Farnet *et al.* 2000). During the pulping process the pulp is exposed to the high temperature and at high temperature the enzymes become denatured and inactive. So the aim of this study was chalk the ability of fungus to produce the ligninolytic enzymes which remain stable at a higher temperature.

In the present study all ligninolytic enzymes become inactive at the temperature 75˚C except MnP produced by the *D. confragosa*, which became inactive at 65˚C. 25˚C was found to be the optimum temperature for the stability of all the enzymes. The

stability remained continuous and constant till 45˚C and after that it started showing decrease in the enzyme activity.

Hossain and Anatharaman (2006) reported a similar observation in the ligninolytic enzyme activity of *Trametes versicolor*. For many enzymes denaturation of proteins begins to occur at 45 to 50˚C temperatures. Sensitivity of a protein to denaturation at elevated temperatures can vary widely with medium pH and the influence of various temperatures – pH combinations may differ tremendously from enzyme to enzyme (Shuler and Kargi 1992; Pelczar *et al.* 1993). In the present study all the ligninolytic enzymes optimally increased at a pH value of 5 and temperature 25˚C with the stability remaining continuous till 45˚C.

Farnet *et al.* (2000) reported laccase produced by *Marasmius quercophilus* to be were also stable at 60˚C. Monterio *et al.* (1998) could use the fungal laccase in the pre bleaching stages at 55˚C successfully.

Laccase produced by the white rot fungus *Cerrena unicolor* increased with an increase in the temperature and is highest at 60˚C and after which it suddenly declined with increase in temperature (Michniewicz *et al.* 2006). In the present study laccase produced by both the fungi *D. confragosa* and *P. pectinatus* remained stable till only 55˚C.

Using a strain of *Phanerochaete chrysosporium* Bosco *et al.* (2010), studied the effect of temperature on the stability of the LiP enzyme. They observed that LiP produced by *P. chrysosporium* was stable at 40˚C. Lip produced by *D. confragosa* was found to be stable till 55˚ C. which indicates that the temperature stability of LiP enzyme produced by the different fungal species can differ from each other.

Aslam and Asgher (2011), conducted a experiment to evaluate temperature stability of laccase and MnP produced by the *Pleurotus ostreatus* and they found that till 55˚C both the enzyme remained active and after that their activity showed reduction a result completely in accordance with the present study.

Laccase enzyme produced by most of the fungi were shown to be stable below 50˚C (Yaropolov *et al.* 1994; Wood 1980; Goncovales 1996). *D. confragosa* and *P. pectinatus* produce thermostable enzymes so both are the suitable strains for being used in paper pulp industry.

Further the present study was aimed to observe the effect of different chemicals on the enhancement of ligninolytic enzymes, and to find out the best chemical and best concentration for the enhancement of the enzyme activity. Lee *et al.* (1999), stated that higher concentration of laccase enzymes could be obtained by the use of different supplements to the media. Enhancer molecules resembles the molecules of lignin (Marbach *et al.* 1985; Farnet *et al.* 1999). There are many inducers which can enhance the production of enzymes (Assavanig *et al.* 1992; Grazillo *et al.* 1998; Lu *et al.* 1996; Nyanhongo *et al.* 2002; Aslam and Ashger 2011).

Ligninolytic enzymes enhancement would lead to more de-lignification. Ligninolytic enzymes are secreted during the secondary metabolic phase of white rot fungus, frequently regulated by nitrogen depletion (Keyser *et al.* 1978; Sun *et al*. 2004; Zakariashvili and Elisashvili 1993). But Leatham and Kirk (1983) and Buswell *et al.* (1995) observed that few strains of fungi do not show any effect of nitrogen concentration on the production of ligninolytic enzymes. So it is very clear that the

effect of different enhancers and inducers on the enzyme activity varies with the variation in the fungal strain.

In present study five different chemicals (Ethanol, Veratryl alcohol, Xylidine, Yeast Extract, Peptone) were analyzed for their effect on the enhancement of ligninolytic enzyme (MnP, LiP, AAO and laccase) activity. These inducers were able to enhance as well as induce the ligninolytic enzyme activities. The positive inductive effect of some of these inducers on laccase has earlier been reported in many organisms including fungi (Ulmer *et al.* 1984; Niladevi and Prema 2008), but there are only few reports on the effect of inducers on LiP, MnP and AAO. Ethanol in the concentration of 1%, Veratryl alcohol in the concentration of 12 mM (MnP, LiP) and 16mM (AAO, Laccase), Xylidine in the concentration of 30µM, Yeast extract in the concentration of 0.6 gm/l, and Peptone in the concentration of 0.4 gm/l is best for enhancement of ligninolytic enzymes. Laccase activity did not enhance by all the enhancers, but with Veratryl alcohol (16 mM) it enhanced up to 15.88%.

MnP activity enhancement was found to be highest in 1% ethanol (71.09 %) and in 12 mM veratryl alcohol (49.71 %), whereas it was found to be least in the 30 µM xylidine (17.34 %), while in the 0.6 gm/l yeast extract (24.28 %) and 0.4 gm/l peptone (29.47 %) enhancement was moderate.

Enhancement of LiP enzyme activity was highest in 1% ethanol (79.11 %) and in 12 mM vertryl alcohol (77.51 %), and the enhancement was minimum in the 0.4 gm/l peptone (21.29 %). In 0.6 gm/l yeast extract (43.77%) and 30 µM xylidine (42.97 %) enhancement was found to be intermediate.

AAO enzyme activity enhancement was highest with 30 µM xylidine (94.64 %) which completely contrasted with the MnP and LiP. Enhancement of enzyme activity was least in the 16 mM veratryl alcohol (15.88 %), 0.6 gm/l yeast extract (14.46 %), 1% ethanol (14.38 %) and in 0.4 gm/l peptone (8.53 %). Thus the chemicals and their selected concentrations which enhanced the ligninolytic enzyme activity were used for the further biopulping experiment using the different raw materials.

Almost all the ligninolytic enzymes significantly increased with 1 % or 2% concentration of ethanol. Higher concentration of ethanol resulted in a decrease of the enzyme production which is probably due to the toxicity of ethanol (Lee *et al.* 1999). Using *Pycnoporus cinnabarinus* the production of laccase was found to be much higher when ethanol was used as laccase inducer (Lomascolo *et al.* 2003). This was completely contrasting with the present study. Laccase enzyme did not enhance with ethanol, while MnP, LiP and AAO enzymes showed significant enhancement with 1 % concentration of ethanol.

Barbosa *et al.* (1996) has compared enzyme activity of *Botryosphaeria* species grown on glucose and in the presence of veratryl alcohol (40 mmol 1^{-1}). He found that laccase activities enhanced 115 fold higher when grown in glucose supplemented with veratryl alcohol. In present study *D. confragosa* fungi showed an enhancement of 15.88 % with veratryl alcohol which was very low. *Botryosphaeria* sp an ascomycetes fungus shows increase in the production of Laccase up to 25 fold when grown in the presence of veratryl alcohol at 40 mM concentration (Dekker and Barbosa 2001). *D. confragosa* showed an enhancement of 15.88 % laccase activity in presence of 16 mM concentrationa of veratryl alcohol.

Eggert *et al.* (1996) reported xylidine to be the most effective inducer for the production of laccase by *Pycnoporus cinnabarinus.* All the ligninolytic enzyme activity assessed in the present study (MnP, LiP, AAO) showed significant enhancement when supplemented with different concentrations of xylidine except for laccase by *D. confragosa*. Laccase activity enhanced when supplemented with veratryl alcohol.

In *Coriolopsis rigida* and *Trametes modesta* Alcantara *et al.* (2007) and Nyanhonogo *et al.* (2002) found xylidine more effective in enhancing the enzyme activity instead of veratryl alcohol.

AAO enzyme activity by *Pleurotus eryngii* was not affected by the addition of different aromatic compounds like Veratryl alcohol Xylidine etc. (Mun˜oz *et al.* 1997). AAO enzyme activity by *D. confragosa* showed 26 % enhancement with veratryl alcohol and 94.46 % with xylidine.

Many of the previous studies have shown that the nature and concentration of nitrogen source are powerful nutrition factors regulating lingocellulolytic enzyme production by wood rotting basidiomycetes (Zakariashvili & Elisashvili 1993; Sun *et al.* 2004)

Morchella crassipes exhibited different growth patterns according to the different carbon and nitrogen sources used (Kanwal and Reddy 2011). Sodium nitrate and peptone served as the best nitrogen source for the growth of the fungus. Peptone exhibited significant influence on laccase production. In the present study peptone and yeast extract was used as nitrogen source for the enhancement of enzyme activity and maximum increase in all the ligninolytic enzymes could be obtained at concentration of 0.6 g/l and 0.4 g/l respectively. Adejoye and Fasidi (2009) in their study assessed

enhancement in laccase activity in chemically defined media containing 1 % glucose and supplemented with different nitrogen sources (Yeast extract, peptone, urea and $NaNo_3$). All the nitrogen sources used significantly promoted biomass yield and laccase production by *Schizophyllum commune*. The best stimulatory nitrogen source for fungal mycelia biomass yield was achieved using urea while the highest laccase yield was induced with the yeast extract.

Yeast extract showed less effect on the laccase enzyme enhancement in the species *Morchella crassipes* (Kanwal and Reddy 2011) which resembles our study, but this strain did not show presence of MnP and LiP enzymes which are present in *D. confragosa* and enhanced significantly with the yeast extract.

The lignocellulolytic enzymes of basidiomycetes are of fundamental importance for efficient bioconversion of plant residues and they are prospective for various biotechnological applications in pulp and paper, food, textile and dye industries, bio remediation and many others.

Kachlishvili *et al.* 2005 studied the effect of additional nitrogen sources on lignocellulolytic enzyme production by four species of white rot fungi in solid state fermentation of wheat straw and beech tree leaves.

An effort was made to analyze the effect of enhancement on the degradation of different substrates using selected white rot fungi *D. confragosa*. Effect of supplementing the optimal conditions of concentration of enhancers (1 % of Ethanol, 12 mM and 16 mM of Veratryl alcohol, 30 µM of Xylidene, 0.4 g/l of Yeast Extract, 0.6 g/l of Peptone) with different substrates (*Eucalyptus* wood blocks and wood shaving, pulp, *Sesbania sesban* stem, *Phragmites karka* culm) and the enhancement of activity in

the different ligninolytic enzymes were compared with the normal enzyme production (Table 20). The enzyme activity obtained with different substrates has been given in Table No. 31 to 35.

When different substrates (*Eucalyptus* wood blocks and wood shaving, pulp, *Phragmites karka* culm, *Sesbania sesban* stem) were subjected for the bio-pulping experiment with optimal obtained condition, all the ligninolytic enzymes showed enhancement in its enzyme activity in comparison to the enzyme activities in MEA medium without the different substrates.

Normally fungus *D. confragosa* secretes 1.78 EU/l/min of MnP in MEA medium but with addition of *Eucalyptus* wood blocks and wood shavings it produced 2.19 EU/l/min, which was the highest activity obtained among all the experimented substrates. With *Phragmites karka* culm and *Sesbania sesban* stem, the activity measured was 2.11 EU/l/min and 2.16 EU/l/min respectively, while in case of pulp the least amount of activity was obtained (2.02 EU/l/min).

LiP activity without the substrate was 2.49 EU/l/min, but it enhanced to 3.22 EU/l/min with *Sesbania sesban* stem, 2.91 EU/l/min with *Phragmites karka* culm, 2.89 EU/l/min with *Eucalyptus* wood shaving, 2.8 EU/l/min with *Eucalyptus* wooden blocks and 2.78 EU/l/min with pulp.

AAO production without any substrate was 24.26 EU/l/min but AAO activity showed a drastic enhancement with *Phragmites karka* culm (43.7 EU/l/min), and also with all other substrates AAO enzyme activity was enhanced significantly.

1.07 EU/l/min of laccase enzyme activity was measured with MEA medium and it enhanced with the different substrates. Using *Eucalyptus* wood blocks enzyme

activity was 1.48 EU/l/min. With *Eucalyptus* wood shavings the enzyme activity enhanced the highest (1.69 EU/l/min). With pulp, *Phragmites karka* culm *and Sesbania sesban* stem, enzyme activity enhanced up to 1.38 EU/l/min, 1.41 EU/l/min, and 1.39 EU/l/min respectively.

Ligninolytic enzyme MnP showed its highest activity with the substitution of substrates pulp, *Sesbania sesban* stem and *Phragmites karka* Culm. The highest activity in pulp was with the enhancers like 1% ethanol (2.73 EU/l/min), xylidine 30 µM (2.66 EU/l/min), and peptone 0.6 gm/l (2.61 EU/l/min). While in *Sesbania sesban* highest activity was observed with the veratryl alcohol 12 mM (2.71 EU/l/min), veratryl alcohol 16 mM (2.62 EU/l/min) and in *Phragmites karka* it was with 0.4 gm/l Yeast extract (2.66 EU/l/min). *Phragmites karka* with 1% ethanol did not show enhancement. *Eucalyptus* wood shavings and with *Sesbania sesban* stem, 1 % ethanol, and *Eucalyptus* wood blocks with veratryl alcohol 16 mM and xylidine 30 µM showed enhancement in comparison with the medium without any substrate but it did not show enhancement in comparison to the medium with substrate.

Ligninolytic enzyme LiP activity was enhanced with the best substrates being the pulp and *Sesbania sesban* stem. In case of pulp the activity enhanced with 1 % of ethanol (3.66 EU/l/min) and 30 µM of xylidine (3.64 EU/l/min) while in *S. sesban* activity enhanced with 12 mM of veratryl alcohol (3.49 EU/l/min). In *P. karka* with 1 % of ethanol (2.7 EU/l/min) and *S. sesban* with 30 µM of xylidine (3.16 EU/l/min) showed enhancement compared to the normal enzyme activity (Table 20) but it did not show enhancement in comparison with the substrate (*P. karka*).

AAO enzyme activity was enhanced with all substrates experimented but it is maximum with *Eucalyptus* shaving, *Phragmites karka* culm and pulp. *Phragmites karka* culm showed the best enhancement with all most all the enhancers, 0.4 g/l yeast Extract (51.42 EU/l/min), 16 mM veratryl alcohol (51.37 EU/l/min), 0.6 g/l peptone (50.66 EU/l/min), 1 % ethanol (50.06 EU/l/min), 12 mM veratryl alcohol (49.68 EU/l/min) and 30 µM xylidine (45.91 EU/l/min). Maximum enhancement was found with *Eucalyptus* wood shavings with 16 mM veratryl alcohol (63.49 EU/l/min).

Laccase enzyme activity was maximum enhanced with *Eucalyptus* wood shaving, pulp and *Eucalyptus* wood blocks as substrate. Laccase enzyme with the *Eucalyptus* wood shaving showed highest enhancement with the enhancers like 16 mM veratryl alcohol (2.42 EU/l/min), 0.6 g/l peptone (2.16 EU/l/min) and 30 µM xylidine (2.11 EU/l/min). With the substrate pulp the best suitable enhancers were 16 mM veratryl alcohol (2.24 EU/l/min) and 12 mM veratryl alcohol (1.93 EU/l/min). In *Eucalyptus* wood blocks16 mM veratryl alcohol (2.11 EU/l/min) and 30 µM xylidine (1.92 EU/l/min) were the best enhancers for the laccase.

All the lignocellulosic substrate and pulp tested in this study promoted an excellent growth of the fungi and with the optimal conditions and enhancers all the ligninolytic enzyme activity increased. In general the enzyme activity appeared after 3 days of cultivaton and gradually increased achieving a maximum values in 25 days of incubation period. Veratryl alcohol in the concentration of 16 mM was proved best for significant enhancement in delignification in all the selected substrates (*Eucalyptus* wood blocks and wood shaving, pulp, *Phragmites karka* culm, *Sesbania sesban* stem).

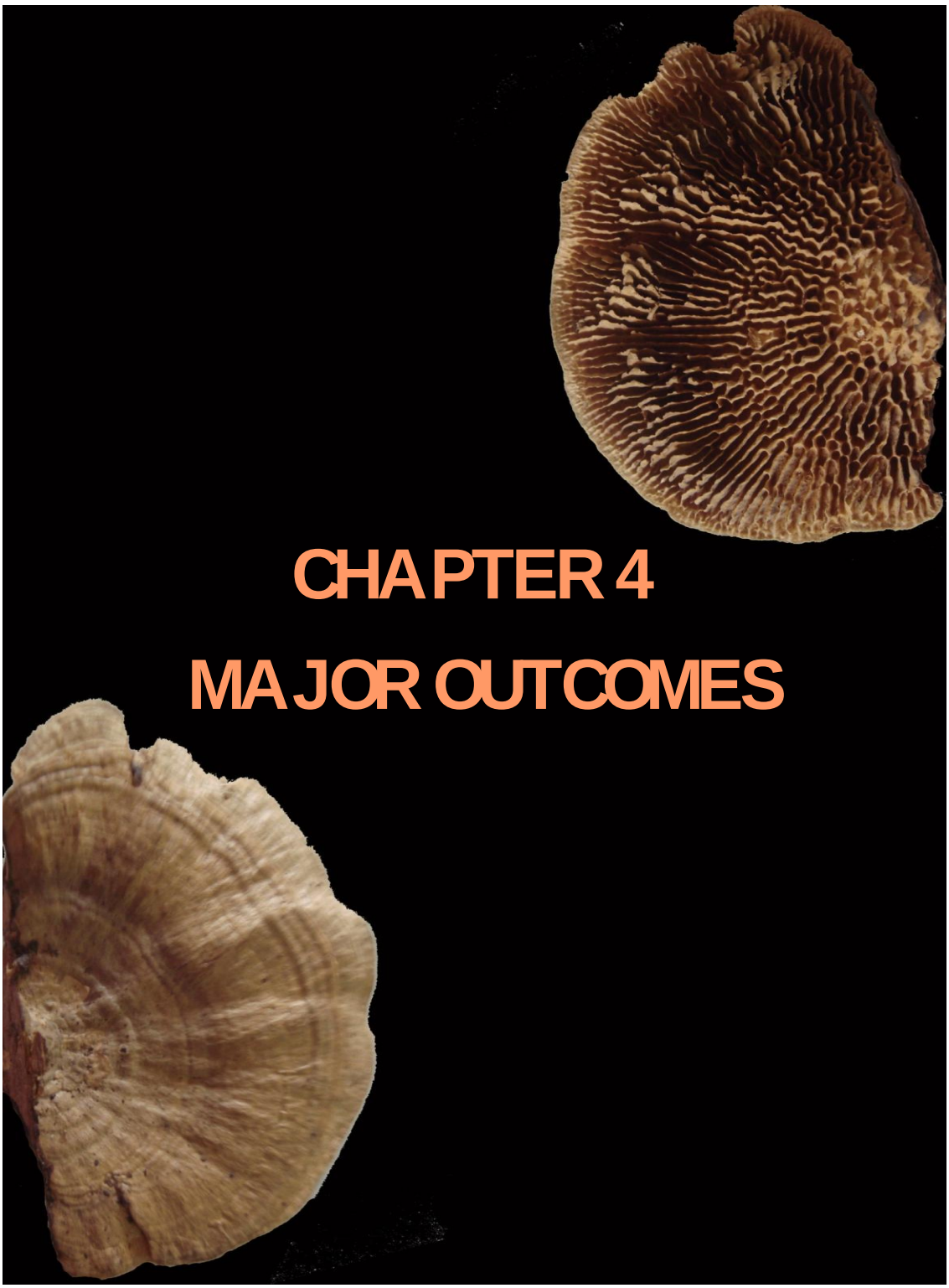
The maximum activity of ligninolytic enzymes expressed by tested fungi varied 2-4 fold with substitution of growth substrates (lignocellulosic substrate). Although different enzymes have shown their enhancement with different substrates and different enhancers, they have their cumulative effect on the lignin degradation. And so lignin degradation was better parameter for evaluating the effect of the enhancement of ligninolytic enzyme activity on the biopulping.

Eucalyptus wood blocks showed the highest lignin degradation with 12 mM veratryl alcohol (53 %) and 16 mM veratryl alcohol (46.67 %). Highest lignin degradation in *Eucalyptus* wood shaving was observed with 0.4 g/l yeast Extract (46.33 %) and 16 mM veratryl alcohol (45.67 %). In the substrate pulp highest lignin degradation was observed with 16 mM veratryl alcohol (42.64 %) and 1 % ethanol (44.44 %). Highest delignification in case of *Sesbania sesban* stem was observed with 16 mM veratryl alcohol (17.2 %) and 12 mM veratryl alcohol (14.62 %). *Phragmites karka* culm showed highest delignification with enhancers like 0.6 g/l peptone (37.33 %) and 16 mM veratryl alcohol (34 %).

To conclude one of the major achievements in the present study was that with a concentration of 16 mM veratryl alcohol and optimal condition, *D. confragosa* is a very potential white rot basidiomycetes fungi which can be applied with the different substrates (*Eucalyptus* wood blocks and wood shaving, pulp, *Sesbania sesban* stem, *Phragmites karka* culm) for the process of biopulping in pulp and paper industry. Application of these cheap 'crude' enzyme mixtures can be more effective than isolated enzymes. Agro industrial residues including crop residues, carpentry residues and grass

can be effectively used for paper production. These can be utilized in mixture with wood fibers in the production of pulp, particle boards and fiber boards.

So veratryl alcohol in the concentration of 16 mM can be used for the better biopulping with all the substrate with *D. confragosa*.



CHAPTER 4
MAJOR OUTCOMES

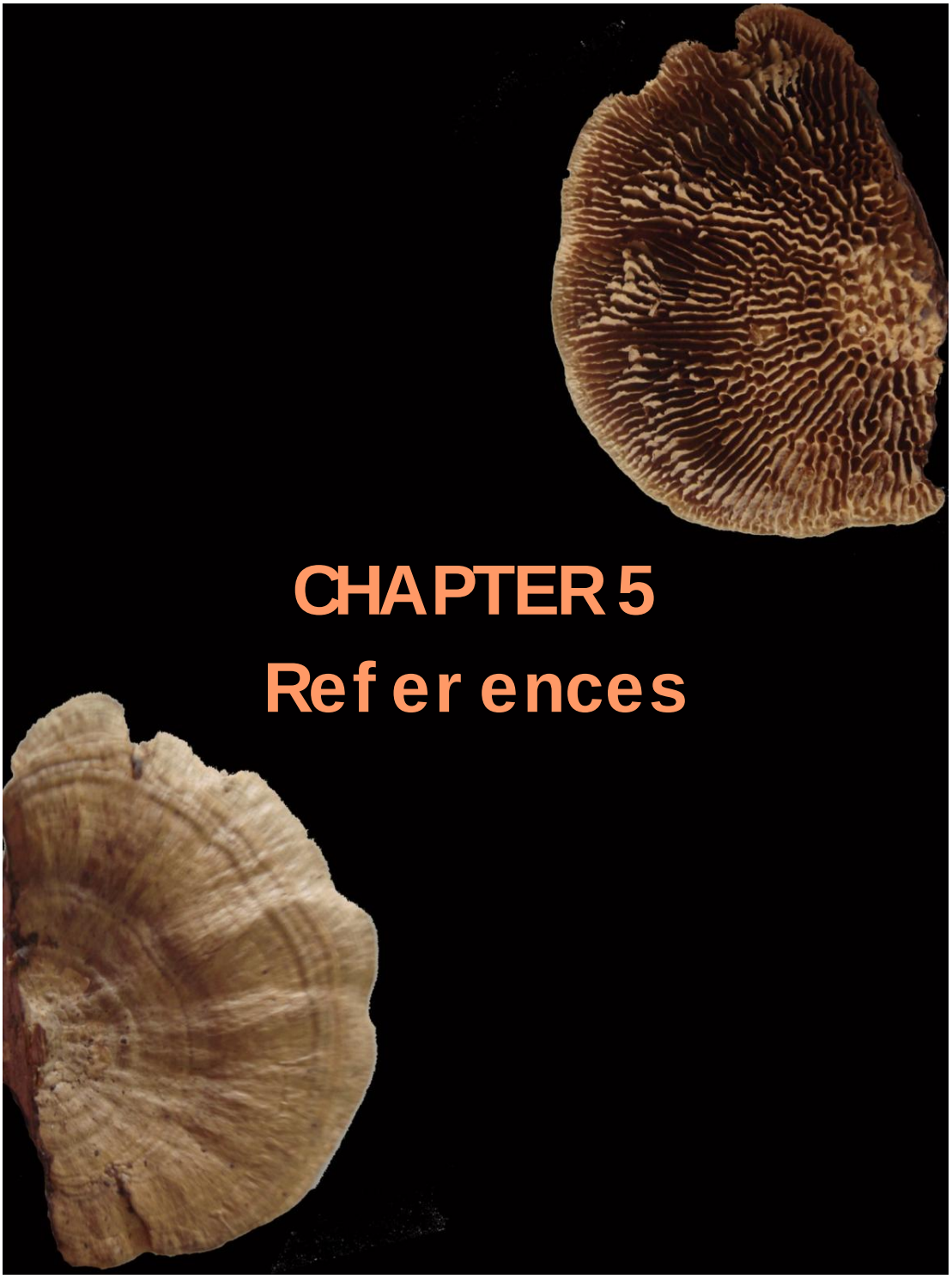
Following are some of the major achievements obtained from the present study:

- On the basis of evaluated fiber properties of commonly growing unpalatable grass species, *Phragmites karka, Vetivaria zizanioides and Saccharum spontaneum* were found to be suitable to be admixed with other commonly used raw material (*Eucalyptus*) for paper making. This is one of the most important achievements of the project because grasses are the annuals and easily available source of raw material. Among the wood species studied *Eucalyptus globulus, Bombax ceiba* are good raw material for paper industry. These are commonly growing trees of Gujarat. They are widely used for other commercial purposes like furniture, packing boxes etc. The lignocellulosic waste obtained can be effectively used for paper production if not at a very large scale at least for small scale paper industry.
- Alternative sources of cellulose raw materials could be obtained. Unpalatable grass which cannot be utilized by the cattle can be utilized in making paper making mixed with the wood. Thus unpalatable agro waste would be utilized. Grasses occur abundantly in every climatic region and possibilities of their non-traditional application are evident.
- Different forms of the raw materials (Saw dust, Wood shavings, Wood blocks) were experimented to evaluate the best suitable form for biopulping. For the fungal pretreatment the wood substrate in the form of saw dust is more efficient to have a high enzyme activity and greater loss of lignin. But from the point of view of efficiency of fungal pretreatment in pulp and paper industry the use of wood chips as pretreatment substrate is more applicable. The reason for this is the breakage of fibers when the substrate is utilized in form of saw dust. Banana pseudo stem and pulp experimented for

efficiency of fungal pretreatment in its biopulping proved that soaking them before pretreatment was beneficial for fungal degradation. Addition of MEA to the raw material fastened the process.

- In biopulping process good amount of lignin is removed by the selected white rot fungi (*D. confragosa* and *P. pectinatus*). A fungal pretreatment of the raw materials would certainly help in reducing the use of costly and harsh chemicals used for the pulping.
- The degradation pattern of the two fungi on different woods (*Mangifera indica, Syzygium cuminii, Bombax ceiba, Eucalyptus globulus, Pithecellobium dulce*) were studied. Both fungi proved to be selectively delignifying, dissolving the middle lamella separating out the cells and at later stage of decay begins to bore holes in the cells. So the information on the events of initiation, spread, penetration mechanisms and response of the raw material/wood samples to the two fungi *D. confragosa* and *P. pectinatus* at cellular and subcellular level has been generated.
- Of all the cultures screened ligninolytic enzyme activity was found to be maximum in fungus *D. confragosa* indicating its potentiality in production of the commercially important enzymes. All ligninolytic enzyme production by *D. confragosa* and *P. pectinatus* white rot fungi were analyzed for stability of enzyme at different pH and temperature. In this study it is observed that all the enzymes secreted by both the fungi were stable at 45˚C. *P. pectinatus* does not produce MnP and LiP enzyme.
- From the experiments for enhancement of enzyme activity, many different enhancers with varying concentrations were proved best for enhancing the enzyme activity. 1 % of Ethanol, 12 mM and 16 mM of Veratryl alcohol, 30 µM of Xylidine, 0.4 g/l of Yeast Extract, 0.6 g/l of Peptone were found to be the best enhancers.

- Biopulping experiment with different substrates along with the enhancer was evaluated for degradation. In this study veratryl alcohol in the concentration of 16 mM was proved best for enhancing the lignin degradation.
- Due to fungal pretreatment (biopulping) separation of the cellulosic fibers and loss of lignin is effectively brought about. The requirement of chemicals used for removal of lignin would thus be reduced and pollution due to the harsh chemicals used for pulping and bleaching is reduced.
- Biopulping process would be very cost effective also because it reduces the chemical energy as well as electricity uses. White rot fungi is known to increase the effective diffusion rate of cooking chemicals through the wood cell wall by chemical modification of the wood cell wall. If the effective diffusion rate is increased, uniform pulp could be obtained from larger chips. Larger chip sizes would save mills money decreasing chipping costs and reducing fiber losses during the chipping process.
- The information generated from the present study will have academic as well as application value. Broadening our knowledge of biodegradation process and enhancement in enzyme activities contributes to the development of new biocatalyst of industrial interest in these organisms and their enzymes. Native enzymes produced on the raw materials would be more economical then using purified enzymes which would be time consuming and expensive. The potential of these fungi in other aspects like waste management, degradation of dyes and production of other commercially important enzymes can be further explored.



CHAPTER 5
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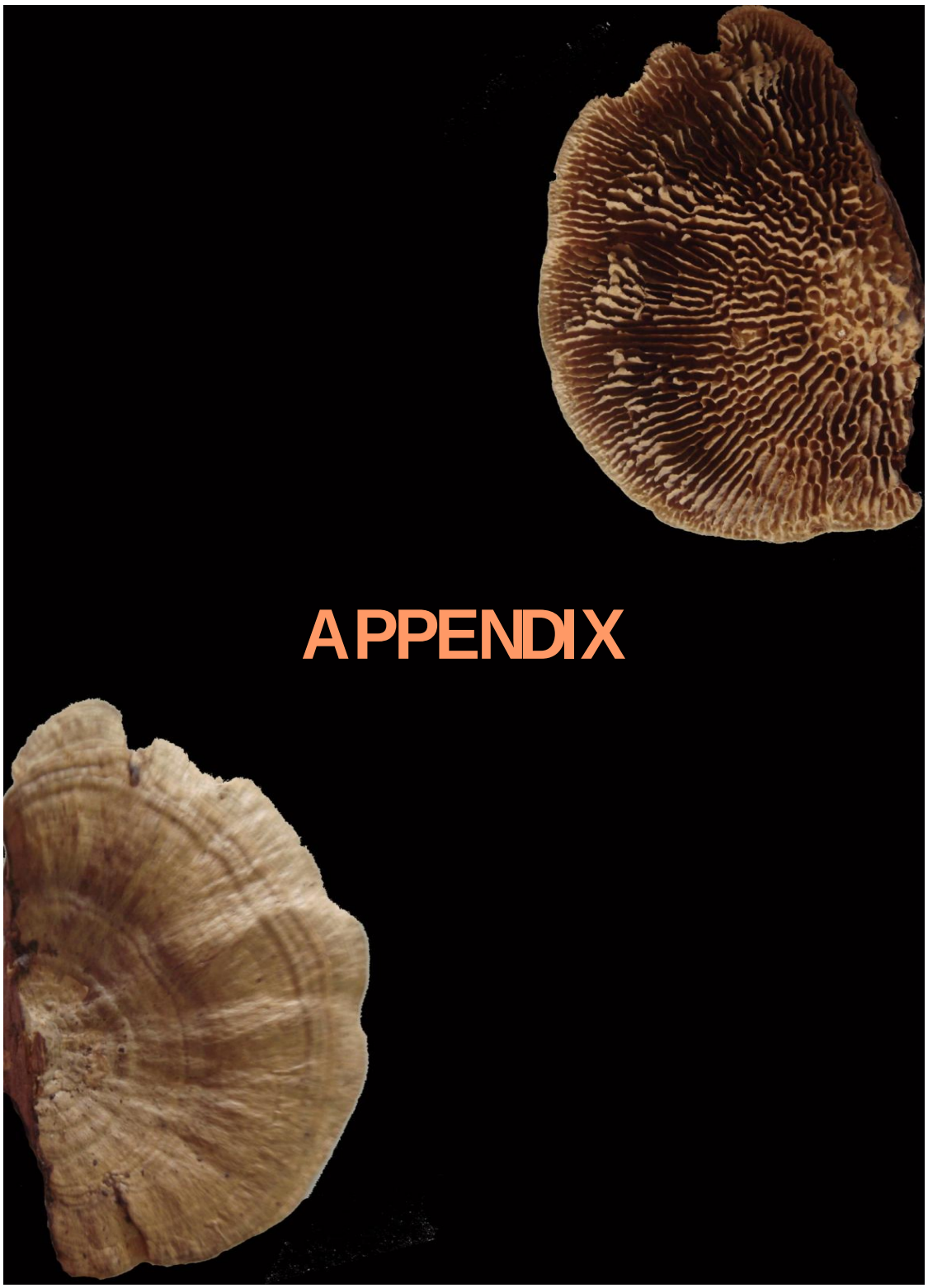

APPENDIX

Synonyms of the fungi

***Flavadon flavus* (Klotzsch) Ryvarden**

1) *Irpex flavus* Klotzsch, Linnaea, 8: 488, 1833 [MB#153240]
2) *Xylodon flavus* (Klotzsch) Kuntze, Revisio generum plantarum, 3: 541, 1898 [MB#472116]
3) *Hirschioporus flavus* (Klotzsch) Teng, Zhong Guo De Zhen Jun [Fungi of China]: 761, 1963 [MB#331988]
4) *Polyporus flavus* Jungh., Praemissa in floram cryptogamicam Javae insulae: 46, 1838 [MB#124452]
5) *Irpex flavus* Jungh., Praemissa in floram cryptogamicam Javae insulae: 46, 1838 [MB#473461]
6) *Polyporus crenatoporus* Rostr., Botanisk Tidsskrift, 24: 360, 1902 [MB#440363]
7) *Coriolopsis melleoflava* Murrill, Bulletin of the Torrey Botanical Club, 35: 393, 1908 [MB#210086]
8) *Daedalea rhodesica* Van der Byl, South African Journal of Science, 22: 167, 1925 [MB#276404]

***Daedaleopsis confragosa* (Bolton) J. Schröter**

1) Boletus confragosus *Bolton, An History of Fungusses, Growing about Halifax, App.: 160, t. 160, 1791 [MB#204177]*
2) *Daedalea confragosa (Bolton) Pers., Synopsis methodica fungorum: 501, 1801 [MB#182702]*
3) *Trametes confragosa (Bolton) Rabenh., Deutschlands Kryptogamenflora, 1: 416, 1844 [MB#261662]*
4) *Polyporus confragosus (Bolton) P. Kumm., Der Führer in die Pilzkunde: 59, 1871 [MB#507963]*
5) *Striglia confragosa (Bolton) Kuntze, Revisio generum plantarum, 2: 871, 1891 [MB#471720]*
6) *Lenzites confragosa (Bolton) Pat., Essai taxonomique sur les familles et les genres des Hyménomycètes: 89, 1900 [MB#469724]*
7) *Agaricus confragosus (Bolton) Murrill, Bulletin of the Torrey Botanical Club, 32 (2): 86, 1905 [MB#255752]*
8) *Daedalea confragosa f. bulliardii (Fr.) Domanski, Orlos & Skirg., Flora Polska Grzyby (Mycota), 3: 249, 1967 [MB#348041]*
9) *Ischnoderma confragosum (Bolton) Zmitr., Mycena, 1 (1): 92, 2001 [MB#466376]*
10) *Ischnoderma confragosa (Bolton) Zmitr., 2001 [MB#266973]*
11) *Daedaleopsis confragosa var. confragosa [MB#417553]*
12) *Boletus suaveolens Bull., Herbier de la France, 7: t. 310, 1787 [MB#220258]*
13) *Boletus angustatus Sowerby, Coloured Figures of English Fungi, 2: 81, t. 193, 1799 [MB#157363]*

14) *Daedalea rubescens Alb. & Schwein., Conspectus Fungorum in Lusatiae superioris: 238, t. 11:2, 1805 [MB#145391]*
15) *Daedalea corrugata Klotzsch, Linnaea, 8: 481, 1833 [MB#182420]*
16) *Daedalea discolor Klotzsch: 325, 1833 [MB#182481]*
17) *Lenzites crataegi Berk., London Journal of Botany, 6: 323, 1847 [MB#140093]*
18) *Lenzites ungulaeformis Berk. & M.A. Curtis, Hooker's Journal of Botany and Kew Garden Miscellany, 1: 101, 1849 [MB#491919]*
19) *Lenzites unguliformis Berk. & M.A. Curtis, Hooker's Journal of Botany and Kew Garden Miscellany, 1: 101, 1849 [MB#241532]*
20) *Daedalea pruinata Secr.: 481, 1855 [MB#469019]*
21) *Lenzites atropurpurea Sacc., Nuovo Giornale Botanico Italiano, 5: 271, 1873 [MB#197235]*
22) *Lenzites cookei Berk., Grevillea, 4 (32): 161, 1876 [MB#236261]*
23) *Lenzites proxima Berk., Grevillea, 4 (32): 162, 1876 [MB#220964]*
24) *Trametes purpurascens Berk. & Broome, Annals and Magazine of Natural History, 3: 210, 1879 [MB#205573]*
25) *Polyporus purpurascens Berk. & M.A. Curtis, 1879 [MB#473667]*
26) *Trametes erubescens Schulzer: 179, 1882 [MB#156768]*
27) *Trametes zonata Wettst., Verhandlungen der Zoologisch-Botanischen Gesellschaft Wien, 35: 561, 1885 [MB#231026]*
28) *Lenzites sinensis Cooke, Grevillea, 17 (84): 75, 1889 [MB#247126]*
29) *Lenzites sibirica P. Karst., Öfvers. finska VetenskSoc. Förh., 46 (11): 3, 1904 [MB#246838]*
30) *Daedalea favoloides Murrill, Bulletin of the New York Botanical Garden, 8: 151, 1912 [MB#469002]*
31) *Daedalea ochracea Velen., Ceske Houby, 4-5: 693, 1922 [MB#272672]*
32) *Lenzites queletii var. jappii Velen.: 18, 1930 [MB#469736]*
33) *Tyromyces subradiatus Corner, Beihefte zur Nova Hedwigia, 96: 199, 1989 [MB#136586]*

***Coriolopsis telfarii (KL). Ryv.* (Klotzsch) Ryvarden**

1) *Polyporus telfarii Klotzsch, 1833 [MB#375002]*
2) *Polyporus telfairii Klotzsch, Linnaea, 8: 484, 1833 [MB#201692]*
3) *Coriolopsis telfairii (Klotzsch) Ryvarden, Norwegian Journal of Botany, 19: 230, 1972 [MB#532673]*
4) *Trametes telfarii (Klotzsch) Corner, 1989 [MB#132050]*
5) *Trametes telfairii (Klotzsch) Corner, Beihefte zur Nova Hedwigia, 97: 167, 1989 [MB#532675]*
6) *Funalia telfarii (Klotzsch) A. David & Rajchenb., 1992 [MB#358934]*
7) *Funalia telfairii (Klotzsch) A. David & Rajchenb., Mycotaxon, 45: 146, 1992 [MB#532674]*
8) *Trametella telfairii (Klotzsch) M. Pieri & B. Rivoire, Bulletin de la Société Mycologique de France, 123 (1): 62, 2008 [MB#532814]*
9) *Polyporus zeylanicus Berk., Annals and Magazine of Natural History, 10: 377, 1843 [MB#473460]*
10) *Trametes cristata Cooke, Grevillea, 10 (56): 132, 1882 [MB#471892]*

11) *Hexagonia dybowskii Pat., Bulletin de la Société Mycologique de France, 8 (2): 54, 1892 [MB#122377]*
12) *Hexagonia wildemanii Bres., Annales Mycologici, 9: 269, 1911 [MB#240575]*
13) *Hexagonia expallida Bres., Annales Mycologici, 9: 270, 1911 [MB#147866]*

***Oxyporus cervino-gilvus* (Jungh.) Ryvarden**

1) Polyporus cervinogilvus Jungh., Praemissa in floram cryptogamicam Javae insulae: 45, 1838 [MB#183675]
2) Polystictus cervinogilvus (Jungh.) Fr., Nova Acta Regiae Societatis Scientiarum Upsaliensis, 1: 94, 1851 [MB#158427]
3) Microporus cervinogilvus (Jungh.) Kuntze, Revisio generum plantarum, 3: 495, 1898 [MB#469989]
4) Trichaptum cervinogilvum (Jungh.) G. Cunn., Bulletin of the New Zealand Department of Industrial Research, 164: 96, 1965 [MB#340263]
5) Flabellophora cervinogilvum (Jungh.) Corner, Beihefte zur Nova Hedwigia, 86: 58, 1987 [MB#469186]
6) Flavodon cervinogilvum (Jungh.) Corner, 1987 [MB#131224]
7) Flavodon cervinogilvus (Jungh.) Corner, Beihefte zur Nova Hedwigia, 86: 58, 1987 [MB#263196]
8) Rigidoporus cervinogilvus (Jungh.) Teixeira, Revista Brasileira de Botânica, 15 (2): 126, 1992 [MB#360686]
9) Trametes dermatodes Lév., Annales des Sciences Naturelles Botanique, 2: 196, 1844 [MB#167475]
10) Polyporus personatus Berk. & Broome, Botanical Journal of the Linnean Society, 14: 51, 1875 [MB#472765]
11) Polyporus peradeniae Berk. & Broome, Botanical Journal of the Linnean Society, 14: 51, 1875 [MB#207224]
12) Polyporus diversiporus Berk. & Broome, Botanical Journal of the Linnean Society, 14: 53, 1875 [MB#472539]
13) Polyporus chrysoleucus Kalchbr., Grevillea, 4 (30): 72, 1875 [MB#193393]
14) Trametes bombycina Pat., Journal de Botanique (Morot), 3: 166, 1889 [MB#202882]

***Schizophyllum commune* Fries**

1) *Schizonia vulgaris Pers., Mycologia Europaea, 3: 14, 1828 [MB#432129] ≡Daedalea commune (Fr.) P. Kumm., Der Führer in die Pilzkunde: 53, 1871 [MB#507835]*
2) *Merulius communis (Fr.) Spirin & Zmitr., Novosti Sistematiki Nizshikh Rastenii, 37: 182, 2004 [MB#366358]*
3) *Agaricus alneus L., Species Plantarum: 1176, 1753 [MB#207608]*
4) *Scaphophoeum agaricoides Ehrenb., 1820 [MB#450725]*
5) *Schizophyllum alneus (L.) Kuntze, Revisio generum plantarum, 3: 478, 1898 [MB#523460]*

***Ganoderma colossus* (Fr.) Baker**

1) *Polyporus colossus Fr., Nova Acta Regiae Societatis Scientiarum Upsaliensis, 1: 56, 1851 [MB#185173]*
2) *Tomophagus colossus (Fr.) Murrill, Torreya, 5: 197, 1905 [MB#455283]*
3) *Dendrophagus colossus (Fr.) Murrill, Bulletin of the Torrey Botanical Club, 32 (9): 473, 1905 [MB#469046]*
4) *Ganoderma obokensis Pat., 1887 [MB#141561]*
5) *Ganoderma obockense Pat., Bulletin de la Société Mycologique de France, 3 (2): 119, 1887 [MB#536212]*
6) *Polyporus hollandii Massee, Bulletin of Miscellaneous Informations of the Royal Botanical Gardens Kew, 1901: 163, 1901 [MB#162547]*

***Ganoderma lucidum* (Curtis) P. Karsten**

1) Boletus rugosus Jacq., Flora Austriaca, 2: 44, f. 169, 1774 [MB#473292]
2) Boletus lucidus Curtis: 72, t. 224, 1781 [MB#269837]
3) Polyporus lucidus (Curtis) Fr., Systema Mycologicum, 1: 353, 1821 [MB#449530]
4) Grifola lucida (Curtis) Gray, A natural arrangement of British plants, 1: 644, 1821 [MB#362180]
5) Fomes lucidus (Curtis) Cooke, Grevillea, 13 (68): 118, 1885 [MB#159723]
6) Placodes lucidus (Curtis) Quél., Enchiridion Fungorum in Europa media et praesertim in Gallia Vigentium: 170, 1886 [MB#472359]
7) Phaeoporus lucidus (Curtis) J. Schröt., Kryptogamen-Flora von Schlesien, 3-1(4): 491, 1888 [MB#470439]
8) Boletus flabelliformis Leyss., Flora halensis: 219, 1761 [MB#255032]
9) Agaricus pseudoboletus Jacq., Miscellanea austriaca ad botanicum, chemiam et historiam naturalem spectantia, 1: 26, t.41, 1773 [MB#468090]
10) Boletus obliquatus Bull., Herbier de la France, 1: t. 7, 1781 [MB#194313]
11) Boletus vernicosus Bergeret, 1783 [MB#468342]
12) Agaricus lignosus Lam., Encyclopédie Méthodique, Botanique, 1-1: 51, 1783 [MB#468048]
13) Boletus dimidiatus Thunb.: 348, f. 39, 1784 [MB#468264]
14) Boletus castaneus Weber: 13, 1787 [MB#473713]
15) Boletus laccatus Timm, Flora megapolitanae Prodomus exhibeus plantas ductatus Megapolitano: 269, 1788 [MB#468291]
16) Boletus crustatus J.J. Planer, Index Plantarum quas in Agro Erfurtensi sponte provenientes: 280, 1788 [MB#468261]
17) Agarico-igniarium trulla Paulet, Traité des champignons, 2: 95, pl. 10:1-2, 1793 [MB#467960]
18) Boletus verniceus Brot., Flora Lusitanica, 2: 468, 1804 [MB#468341]
19) Ganoderma ostreatum Lázaro Ibiza: 110, 1916 [MB#141406]
20) Ganoderma nitens Lázaro Ibiza: 104, 1916 [MB#451357]

***Ganoderma applanatum* (Persoon) Patouillard**

1) Boletus applanatus Pers., Observationes mycologicae, 2: 2, 1799 [MB#172019]
2) Boletus fomentarius var. applanatus (Pers.) Pers., Synopsis methodica fungorum: 536, 1801 [MB#498162]

3) Polyporus applanatus (Pers.) Wallr., Flora Cryptogamica Germaniae, 2: 591, 1833 [MB#177005]
4) Fomes applanatus (Pers.) Gillet, Les Hyménomycètes ou Description de tous les Champignons qui Croissent en France: 686, 1878 [MB#163010]
5) Placodes applanatus (Pers.) Quél., Enchiridion Fungorum in Europa media et praesertim in Gallia Vigentium: 171, 1886 [MB#416525]
6) Phaeoporus applanatus (Pers.) J. Schröt., Kryptogamen-Flora von Schlesien, 3-1(4): 490, 1888 [MB#416503]
7) Elfvingia applanata (Pers.) P. Karst., Bidrag till Kännedom av Finlands Natur och Folk, 48: 334, 1889 [MB#431619]
8) Friesia applanata (Pers.) Lázaro Ibiza: 587, 1916 [MB#469258]
9) Boletus lipsiensis Batsch, Elenchus fungorum. Continuatio prima: 183, t. 25:130, 1786 [MB#235082]
10) Polyporus merismoides Corda, Deutschlands Flora, Abt. III. Die Pilze Deutschlands, 3-14/15: 139, 1837 [MB#196149]
11) Polyporus stevenii Lév.: 91, 1844 [MB#472849]
12) Polyporus leucophaeus Mont., Sylloge generum specierumque plantarum cryptogamarum: 157, 1856 [MB#176834]
13) Polyporus leucophaeum Mont., 1856 [MB#277589]
14) Polyporus incrassatus Berk., Botanical Journal of the Linnean Society, 16: 41, 1878 [MB#472642]
15) Polyporus concentricus Cooke, Grevillea, 9 (49): 13, 1880 [MB#472497]
16) Fomes gelsicola Berl., Malpighia, 3: 373, 1889 [MB#200270]
17) Fomes nigriporus Lázaro Ibiza, Revista de la Real Academia de Ciencias Exactas Fisicasy Naturales Madri, 14: 662, 1916 [MB#229943]
18) Ungularia subganodermica Lázaro Ibiza, Revista de la Real Academia de Ciencias Exactas Fisicas y Naturales Madri, 14: 674, 1916 [MB#177747]
19) Fomes longoporus Lloyd, Mycological Writings, 6 (62): 940, 1920 [MB#269765]

***Phellinus pectinatus* (Klotzsch) Quélet**

1) Polyporus pectinatus Klotzsch, Linnaea, 8: 485, 1833 [MB#211232]
2) Fomes pectinatus (Klotzsch) Gillet, Les Hyménomycètes ou Description de tous les Champignons qui Croissent en France: 686, 1878 [MB#216835]
3) Scindalma pectinatum (Klotzsch) Kuntze, Revisio generum plantarum, 3: 519, 1898 [MB#471477]
4) Xanthochrous pectinatus (Klotzsch) Pat., Essai taxonomique sur les familles et les genres des Hyménomycètes: 101, 1900 [MB#472065]
5) Pyropolyporus pectinatus (Klotzsch) Murrill, Bulletin of the Torrey Botanical Club, 34: 479, 1907 [MB#102399]
6) Boudiera pectinata (Klotzsch) Lázaro Ibiza, Revista de la Real Academia de Ciencias Exactas Fisicas y Naturales Madri, 14: 837, 1916 [MB#468349]
7) Porodaedalea pectinata (Klotzsch) Aoshima, Transactions of the Mycological Society of Japan, 7: 89, 1966 [MB#337528]
8) Phylloporia pectinata (Klotzsch) Ryvarden, Synopsis Fungorum, 5: 196, 1991 [MB#460306]

9) Fulvifomes pectinatus (Klotzsch) Bondartseva & S. Herrera, Mikologiya i Fitopatologiya, 26 (1): 13, 1992 [MB#358540]
10) Polyporus hasskarlii Lév., 1844 [MB#173260]
11) Polyporus haskarlii Lév., Annales des Sciences Naturelles Botanique, 2: 190, 1844 [MB#534786]
12) Polyporus capucinus Mont., Annales des Sciences Naturelles Botanique, 5: 369, 1856 [MB#374245]
13) Polyporus xerampelinus Kalchbr., Grevillea, 4 (30): 72, 1875 [MB#472914]
14) Polystictus substygius Berk. & Broome ex Cooke, Nuovo Giornale Botanico Italiano, 10: 17, 1878 [MB#236004]
15) Polystictus hasskarlii (Lév.) Cooke, 1886 [MB#248610]
16) Trametes liliputiana Speg., 1889 [MB#184822]
17) Trametes lilliputiana Speg., Boletín de la Academia Nacional de Ciencias en Córdoba, 11 (4): 440, 1889 [MB#516482]
18) Polyporus bonianus Pat., Journal de Botanique (Morot), 5: 311, 1891 [MB#182474]
19) Phaeoporus ferrugineus Romell, Bihang till Kungliga svenska Vetenskaps-Akademiens Handlingar, 26 (16): 26, 1901 [MB#174298]
20) Pyropolyporus subpectinatus Murrill, North American Flora, 9 (2): 109, 1908 [MB#151619]
21) Polyporus oroniger Lloyd, Mycological Writings, 6 (65): 1044, 1920 [MB#273080]

***Phellinus gilvus* (Schweinitz) Patouillard**

1) Boletus gilvus Schwein., Schriften der Berlinische Gesellschaft Naturforschender Freunde, 1: 96, 1822 [MB#451971]
2) Polyporus gilvus (Schwein.) Fr., Elenchus Fungorum, 1: 104, 1828 [MB#172277]
3) Mucronoporus gilvus (Schwein.) Ellis & Everh., Journal of Mycology, 5 (1): 28, 1889 [MB#185929]
4) Fomes gilvus (Schwein.) Speg., Anales del Museo Nacional de Historia Natural Buenos Aires, 6: 165, 1898 [MB#469212]
5) Hapalopilus gilvus (Schwein.) Murrill, Bulletin of the Torrey Botanical Club, 31 (8): 418, 1904 [MB#469393]
6) Fomes gilvus (Schwein.) Lloyd: 6, 1912 [MB#469213]
7) Fuscoporia gilva (Schwein.) T. Wagner & M. Fisch., Mycologia, 94 (6): 1013, 2002 [MB#384767]
8) Boletus membranaceus Sw., Nova genera et species Plantarum seu prodromus descriptioneum vegetabilium maximam parte incognitorum qua sub itinere in Indiam Occidentalem annis 1783-1787 digessit Olof Swartz M.D.: 148, 1788 [MB#240864]
9) Polyporus scruposus Fr., Epicrisis Systematis Mycologici: 473, 1838 [MB#182224]
10) Polyporus rubiginosus Berk., Annals and Magazine of Natural History, 3: 324, 1839 [MB#142457]

11) Polyporus calvescens Berk., Annals and Magazine of Natural History, 3: 390, 1839 [MB#192890]
12) Polyporus licnoides Mont., Annales des Sciences Naturelles Botanique, 13: 204, 1840 [MB#374899]
13) Polyporus omalopilus Mont., Annales des Sciences Naturelles Botanique, 17: 128, 1842 [MB#204447]
14) Polyporus flabellum Mont., Annales des Sciences Naturelles Botanique, 17: 126, 1842 [MB#233684]
15) Polyporus isidioides Berk., London Journal of Botany, 2: 515 ['415'], 1843 [MB#152666]
16) Polystictus membranacea (Sw.) Berk., 1843 [MB#119252]
17) Polyporus trachodes Lév., Annales des Sciences Naturelles Botanique, 2: 192, 1844 [MB#472879]
18) Polyporus spurcus Lév., Annales des Sciences Naturelles Botanique, 5: 135, 1846 [MB#472845]
19) Polyporus connexus Lév., Annales des Sciences Naturelles Botanique, 5: 135, 1846 [MB#261767]
20) Polyporus bonplandianus Lév., Annales des Sciences Naturelles Botanique, 5: 301, 1846 [MB#182654]
21) Polyporus bonplandensis Lév., Annales des Sciences Naturelles Botanique, 5: 301, 1846 [MB#492885]
22) Polyporus holosclerus Berk., London Journal of Botany, 6: 501, 1847 [MB#162489]
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List of publication
PUBLICATION FROM THE THESIS

Sr. No.	Title of Paper/Report/Book	Author(s)	Name & Vol. of Journal & Year	Page No.	
				From	To
1.	Morphology, Anatomy and cultural characters of two wood decaying fungus *Schizophyllum commune* Fries and *Flavadon flavus* (Klotzsch) Ryvarden.	Amee Padhiar, Praveen Kumar Nagadesi ,Susy Albert and Arun Arya	*Journal of Mycology and Plant Pathology*. 39 (1): 2009:	27	31
2.	Evaluation of the Ligninolytic Effect of *Flavadon flavus* (Klotzsch) Ryvarden and *Schizophyllum commune* Fries on *Mangifera indica* and *Syzgium cumini* wood blocks	Amee Padhiar, Susy Albert, Arun Arya and Praveen Kumar	Journal of Wood Chemistry and Technology. 30(2): 2010	129	139
3.	Pictorial Floristic Diversity of Grasses and associated vegetation from Randhikpur Forest Range, District Panchmahals, Gujarat	S. N. Tyagi, Amee Padhiar, Susy Albert, Neeta Pandya,Gandhi Dhara and Krishna Panchal	The Indian Forestor 10 : 2010	1581	1592
4.	Morphological and anatomical variation in *Ganoderma* species.	Amee Padhiar, Susy Albert, and Dhara Gandhi	Bioscience Guardian, 1(1): 2010	245	247
5.	Characterisaton of Biologically Pretreated Raw Materials for Biopulping Process	Susy Albert and Amee Padhiar,	*International Journal of Applied Biology and Pharmaceutical Technology* 3 (4): 2012	369	375
6.	Fiber Properties of *Sorghum halepense* and its suitability for Paper Production	Susy Albert, Amee Padhiar, and Dhara Gandhi	*Journal of Natural Fibers*, 8 : 2011	263	271
7.	Anatomical studies on decaying wood of Mangifera indica by two white rot fungi Schizophyllum commune and Flavadon flavus	Amee Padhiar, Susy Albert	*J Indian Acad Wood Sci* 9(2): (2012)	143	153

OTHER PUBLICATIONS

1.	Screening of *Trichoderma* Spp. As Potential Fungal Partner in Co-Culturing with White Rot Fungi for Efficient Bio-Pulping.	Susy Albert, Dhruvina Chauhan, Bhavika Pandya and Amee Padhiar	*Global Journal of Biotechnology & Biochemistry*. 6 (3) 2011	95	101

2.	Anatomical changes in *Syzygium cumuini* Linn. wood decayed by two white rot fungi *Schizophyllum commune* Fries.a nd *Flavodon flavus* (Klotzsch) Ryvarden.	Amee Padhiar, Susy Albert	*J Indian Acad Wood Sci* 8(1): 2011	11	20
3.	Morphological, anatomical and biochemical studies on the foliar galls of Alstonia scholaris (Apocynaceae)	Susy Albert, Amee Padhiar,Dhara Gandhi and Priyanka Nityanand	*Revista Brasil. Bot.* 34 (3): 2011	343	358
4.	Evaluation of colony characteristics and enzymatic activity of some fungi for potential use in co-culture for bio pulping	Susy Albert, Bhavika Pandya and Amee Padhiar	*Asian Journal of Biological and Life Sciences* 1 (2): 2012	83	89
5.	Screening of some white rot fungi for potential application in remediation of effluent from pulp and paper industry	Bhavika Pandya, Susy Albert and Amee Padhiar	*Poll. Res.* 31 (2): 2012	301	305

PRESENTATIONS

1.	Degradability of lignocellulose in *Imperata cylindrica* by *Daedaleopsis Confragosa.*	Amee Padhiar, Susy Albert and Dhara Gandhi.	Presented in XXIVGujarat science congress held on 21-03-2010 Ahemdabad.
2.	Characterisation of biologically pretreated raw materials for biopulping process	Amee Padhiar and Susy Albert	International Conference On Mycology And Plant Pathology Biotechnological Approaches Held on 27-29 Fab 2012
3.	Evaluating The Potential Use of Some Grass Species As Paper Making Raw Material	Amee Padhiar and Susy Albert	World Congress on Biotechnology-Hyderabad held on 4 to 6 May 2012.
4.	Determination of fiber properties of some unpalatable brasses for biopulping	Amee Padhiar and Susy Albert	35th All India Botanical Conference and National Symposium on Role of Plants and Microbes for the Betterment of Mankind in the Changing Climate held on 8 to 10th Dec 2012
5.	Pattern of the Invitro Decay in the wood of P*ithecellobium dulce* Caused by *Daedaleopsis confragosa*	Amee Padhiar and Susy Albert	National conference on Current Trends in Science and Technology to be held on 28-30 Nov. 2013.
6.	A Comparative Account Of Biochemical Changes In *Pithecellobium dulce* Wood Decayed By Two White Rot Fungi	Amee Padhiar and Susy Albert	National seminar on Climate Change in the Indian Context 14th Dec. 2013