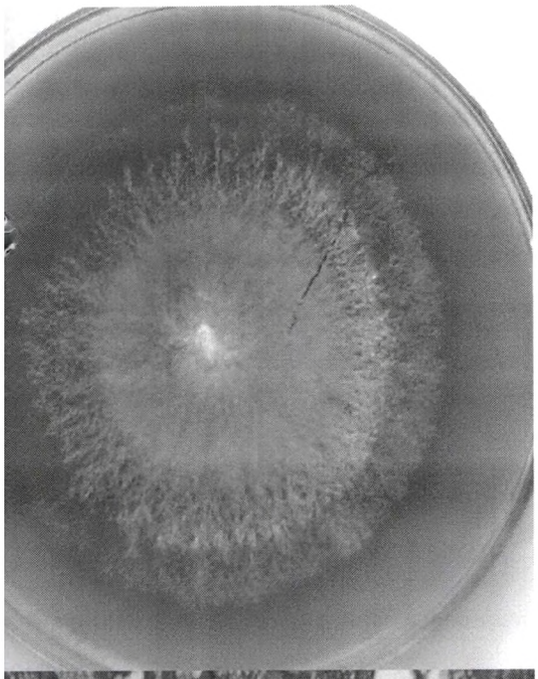
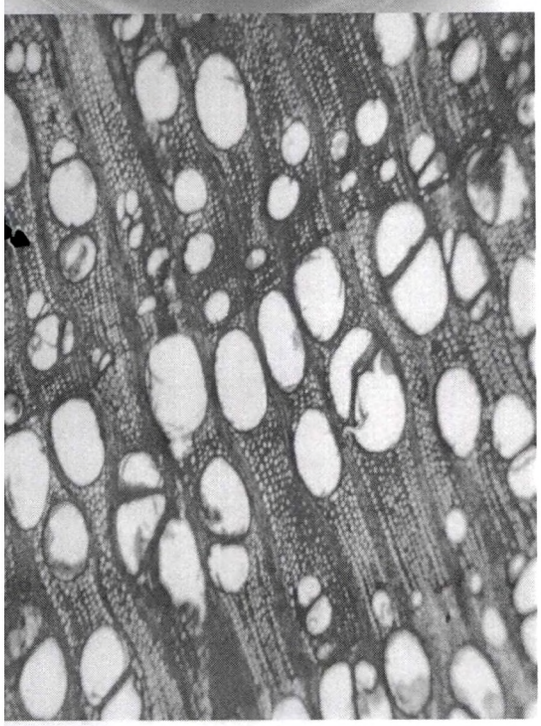
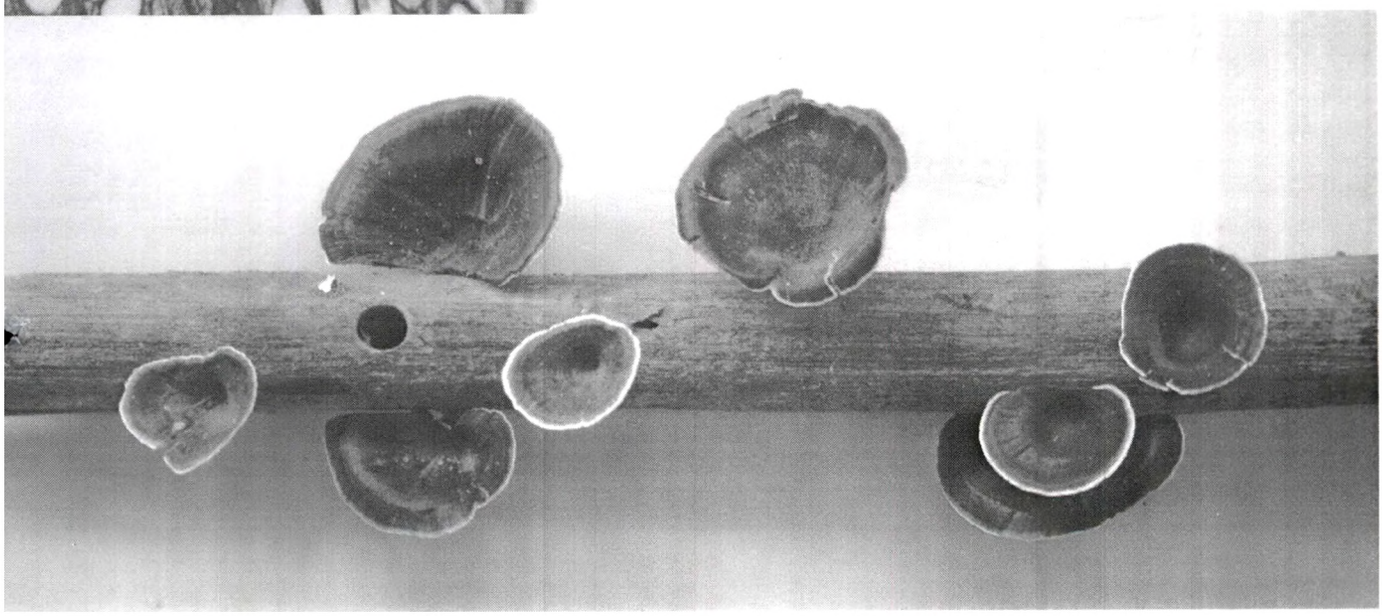




Introduction



Wood is a very important byproduct produced as a result of a series of biological processes in tree. It is an important natural resource, one of the few that are renewable. It is prevalent in our day to day life and the economy, construction of wood-frame houses and for furniture. Forests can contribute greatly to meet the challenges of poverty, disease, access to clean water, climate change and biodiversity conservation (Kaimowitz, 2003). Forests represent approximately 27% of the worlds land area and wood is the predominant commercial product from forests. Global wood consumption is around 3500 million m^3/ year, and has increased over 65% since 1960. More than half of this consumption is for fuel. The remainder of the global sound wood consumption is largely for pulp and paper products, building materials and other wood in service (Martinez *et al.*, 2005). Wood and wood products are also a store house of carbon, thus, helping to minimize carbon dioxide in the atmosphere. Deforestation, fire and diseases have resulted in the loss of forest cover in 1990s between 12 and 15 million ha/yr (Mathews, 2001).

The total forest cover in India according to the latest State of Forest Report 2003 is 67.83 m ha and this constitutes 20.64% of the geographic area. The state of Gujarat is one of the progressive states in the western part of India with an area of 196,024 sq. km. While the recorded forest area is 19,393 sq.km. which is 9.89% of the total geographical area. The forest area which produces timber and fuel wood is only 63.5% of the recorded area. Ninety percent of forest raw material is processed by 25,000 saw mills and a larger number of cottage units, who would also lay claims on forests.

Four different types of agents may be responsible for the timber decay are:

1) Biological agents: Fungi (dry rot, wet rot, moulds and others), bacteria, actinomycetes, lichens, mosses and algae, wood-boring insect larvae (wood worm, death watch beetle) carpet beetle, moths, book lice silverfish, termites and etc.
2) Chemical agents: Acids, bases, Corrosion by salts and solvents.
3) Physical agents: Mechanical abrasion, general handling and others, Decomposition by physical agents such as prolonged heating, fire and moisture.
4) Radiation: Ultraviolet light.

Timber degrading fungi belonging to Aphyllophorales are economically important as many of these are pathogens of forest trees and cause serious damage. These wood-rotting fungi are also important in the forest ecosystem as they are active

decomposers of organic matter (Natarajan and Kolandavelu 1998). They can be a valuable resource for few pharmaceuticals, food production, bioremediation of toxic chemical spills (Kirk *et al.* 1992a), biopulping and other industrial uses (Akhtar *et al.* 1993, Kirk *et al.,* 1992b). Timber decay is caused primarily by enzymatic activities of microorganisms.

Members of Aphyllophorales constitute a cosmopolitan group, fruiting mostly on dead woods and wood products throughout the world under different environmental conditions. The sufficient substratum provided both by angiospermic and coniferous tree species, and coupled with a great diversity in ecological habitats provide rich environmental conditions for the growth of these fungi particularly in the temperate Himalayan zones (Sharma 1995). The wood decay of fungi as mentioned by Berkeley (1850,1854) was the first systematic information on survey and study of wood rotting mycoflora of Himalaya and this was further enhanced by Thind (1961), Bakshi (1971); Rattan (1977); Rattan and Khurana (1978) and Sharma (1995, 1999a and 1999b). Other incident and scattered reports on these fungi from the temperate forests are also found in Bakshi and Bagchee (1950); Bagchee and Bakshi (1954); Bagchee *et al.* (1954); Imazeki *et al.* (1966), Khara (1977a and 1977b); Mass Geasteranus (1971), Reid *et al.* (1958, 1959); Rehill and Bakshi (1965,66); Rattan (1974), Sharma (1996,1997), Thind and Adlakha (1956) Thind and Khara (1968, 1975); Thind and Rattan (1968, 1973); Bose (1919-28) gave a comprehensive account on Indian Polypores collected from Bengal in a series of papers. Sundararaman and Marudarajan (1925) also reported several polypores from Madras. Sarbhoy *et al.,* (1984) listed more than 500 Aphyllophorales. Bakshi (1971) published a book on Indian Polyporaceae and Sharma (1995) on Hymenochaetaceae of India. Thind (1973) explored the mycoflora in Himalayas. He put forth the tissue concept for Indian species of Polypores as proposed by Corner.

Sabnis and Amin (1992) reported Aphyllophorales from Sardar Sarovar Environs in Gujarat. Bakshi (1971) reported *Polyporus luteo-umbrinus* Romell on ground attached to buried wood or root and dead leaves of *Heritiera minor* in Baroda. Arya (2004) reported *Ganoderma lucidum* (Fr.) Ryv., *Phellinus nilgheriensis* (Mont) Cunn, *Trametes cingulata* Fr. and *T. varians* Van der Bij. from Baroda and Shoolpaneshwar wildlife sanctuary. Arya *et al.* (2008) reported that *Lenzites sterioides* was recorded for the first time on *T. grandis*. Two other basidiomycetous fungi *N. floccosa* and *C. aspera* are reported for the first time from India by them.

In India *Phellinus* was studied by Bagchee (1950, 1961), Bakshi (1955, 1976), Ganesh and Leelavathy (1986), Natarajan and Kolanduvelu (1998), Roy (1979), Sharma (1995, 1999a) Singh (1966) and Thind and Dhanda (1980a), Three hundred and sixty seven *Phellinus* spp. have been reported in the CBS (http://www.punenvis.nic.in/bd_list.htm). Fifty-three species have been already reported from India (Sharma 1999a). Eighteen species have been reported from Kerala (Ganesh and Leelavathy, 1986). A new species of *Phellinus* was reported from Gujarat i.e. *Phellinus nilgheriensis* (Mont.) Cunn. by Arya (2004). The aphyllophorales is an order of the Basidiomycetes, these are roughly charactrarized by non – septate basidia and persistant gymnocarpous fruitbodies, which usually are not lamellate.

Many species of Aphyllophorales grow on wood, the majority is saprophytes, while some are pathogenic for example *Heterobasidion annosum* and *Phaeolus schweinitzii*. Their economic importance is considerable as they may cause decay in the living or dead trees, stored wood or timber. Based on degradation of wood the wood rotting fungi are differentiated into three types white rot fungi, brown rot fungi, soft rot fungi

White rot fungi: These fungi degrade all the major wood components (cellulose, hemicellulose and lignin) more or less simultaneously, so that the wood becomes progressively more fragile, but remains white as the decays progresses. White rots are caused by two major root rot pathogens of trees, *Armillaria mellia* and *Heterobasidion annosum* and also by many saprotrophic fungi including the common colonizers of stumps *Coriolus versicolor* and the common wood rotting Ascomycetous members *i.e. Xylaria hypoxylon* and *X. polymorpha.*

Brown rot fungi: Brown rot fungi degrade the cellulose and hemi cellulose but leave the lignin more or less intact às a brown frame work. Only about 6% of wood decay fungi cause brown rots and all these fungi are members of Basidiomycota. They include *Serpula lacrimans* dry rot fungus and common birch polypore, *Piptoporus betulinus.*

Soft rot fungi: These fungi degrade only the cellulose and hemi cellulose and typically occur in wood of high water content and high nitrogen content. They are most commonly found in rotting window frames, wet floor boards and fence posts etc., where nitrogen is recruited from the soil or from atmospheric contamination. Some of these fungi are common decomposers of cellulose in soil (*e.g. Chaetomium* species) and they are the least specialized form of the wood rot fungi.

Aphyllophorales in general do not form basidiocarps in pure culture. As the taxonomy of this order is based on characters of the basidiocarps. Identification of cultures with the conventional keys is almost impossible. On the other hand attacked wood often do not show basidiocarps and the identity of the responsible fungus can only be ascertained by pure cultures derived from wood. The keys are subdivided into diagnostic and descriptive sections. Both theses parts may be traced backwards along the number given between parentheses, so that a full description of any species can be found. The keys are generally dichotomous, but when a group of species is so little distinct that the species have only gradational or minor difference then more than two entries may be present. At the end of each description species code is given. This is a series of numbers, each number representing a character. It was found necessary to incorporate as many characters as possible in the numerical code to make it workable as synoptic key. As the most extensive existing key (53 characters,) Nobles, (1965) did not include characters like texture of the mycelial mat and hyphal width. It was found necessary to design a new and more comprehensible code (96 characters). In the present studies certain wood rotting fungal cultures have been identified and maintained as pure cultures.

Basidiomycetous members are mainly classified on the morphology of fruit bodies. However, many strains of *Basidiomycete* species do not form fruit bodies readily, if not at all, under experimental conditions. The development of a simple method for distinguishing species or strains with vegetative mycelia, therefore, is important. Recently, molecular markers, such as isozymes Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) have been used to detect genetic differences in the species and strains of basidiomycetous. Among these molecular markers, RAPD which was introduced by the use of polymerase chain reaction (PCR) with arbitory 10 mer primers (Williams *et al.* 1990) can express DNA variations for distinguishing basidiomycetes species and strains with less labor and high reliability (Ito *et al.* 1998). The application of DNA markers enable to achieve genotype identification and molecular tagging of gene isolation as well as to identify various agronomic traits. RAPD is especially useful for analyzing a large number of marker loci and this method was used for the construction of genetic maps in filamentous fungi also (Williams *et al.* 1990, Xu and Leslie 1996, Kerrigan *et al.* 1993). Two genetic maps have been reported for *Lentinula edodes* using RAPD Markers (Kwan and Xu, 2002) and AFLP Markers

(Terashima *et al.* 2002). Ten pure cultures of different genera of the aphyllophorales were molecularly characterized by using RAPD and AFLP methods. Wood decaying organisms are classified into three types based on colonization; they are Pioneer Decayers, Decay at Later Stages and Final Stage Decayers.

Pioneer Decayers

The basic trends in the community development of wood rotting fungi are mainly governed by the physical and chemical properties of a host tree species and microclimate of the growth site (Rayner and Boddy 1988, Boddy 1992). A large number of primary decayers like *Fomitopsis pinicola* and *Heterobasidion annosum* on coniferous trees, *Piptoporus betulina* on oaks and birches do not suffer much due to the death of the host tree as the pathogen usually continues to live on the fallen tree trunks for several years or decades. A few number of species like *Phellinus pini, Inonotus circinatus* and *I. tomentosus* on pines, *I. radiatus* on *Abies* and *Betula* growing as primary decayers seems to suffer indirectly of the death and fall down of the primary host.

Decay at Later Stage

The wood rotting fungi become more complex and the amount of species involved also increase as the decomposition proceeds. However, certain species stand out as more dominant, more extensive and more persistent than others. Such second stage dominants have been seen in natural forests in which dead and fallen trees are removed. *Fomitopsis rasea; Glaeophyllum subferrugineum, G. sepiarium; Antradia xanthan,* species of *Hypodantia, Phlebiella vaga, Oligaparus sericeomollis, Phellinus laevigatus* and other members of the *P. igniarius* group are common as second stage dominant decayers in the temperate forests. It has also been noted that brown rot causing species are more dominants at this main decomposition stage. At this some initial decayers like *Fomitopsis pinicola* (both conifers and hardwoods), *Fomes famentarius, P. igniarius, Piptaparus betulina* (on hardwoods) may also co-occur regularly and in abundance. The fallen trunks become decorticated and covered with epiphytes, sink down against the ground and eventually become fragmented towards the end of the stage.

Final Stage Decayers

In temperate boreal zones, the complete decomposition of a fallen big coniferous trunk may even last for decades or even more (Hofgaard, 1993). Certain groups of wood rotting fungi are specialists in utilizing the tree trunks at the final stage of decay. Among

such species *Odonticium romellii; Serpula himantiodies*; species of *Tubulicrinis; Antorida crassa* and *Diplomitoporus lenis* are dominant and inhabit extensively decayed trunks of coniferous trees, which are covered by moss, soft and about to fall apart. There seems to be a competition for space between fungi and mosses. Decayed trunks are frequently covered with them. Many larger species with great vigour ignore the presence of mosses and override their mechanical barriers, but small corticoid fungi do not fruit well, where mosses occur in abundance. Mosses may exclude those fungi by depleting available space for fungal growth on fallen trees. Two types of wood rotting fungi are found in the final stages of decay. In the first category are a great deal of *Corticiaceous* and *Polyporaceous* fungi which are ephemerals, appearing quickly on suitable pieces of wood, while in the second category are the species which establish slowly and evidently survive only in circumstances staying stable for a longer time.

Wood decay

A primary function of fungi in any ecosystem is depolymerisation of important plant biopolymers such as cellulose, hemicelluloses and lignin. This function is very critical for volatilization of C, H and N as well as elemental release during decomposition and formation of soil and thus wood rotting fungi are the likely candidate for important function category. The wood inhabiting members of Aphyllophorales form a cosmopolitan group and are capable of utilizing components of wood cell walls for their growth and reproduction. Although, all wood basically is composed of the structural polymers cellulose, lignin and hemicelluloses but there is considerable variation which is particularly evident in the heartwood of living trees in which a wide array of non structural extraneous materials (extractives) are deposited as the maturing cells die (Rowe and Conner 1978). Cellulose is most consistent of the structural components varying minimally between wood species. Lignin and hemicelluloses, however, vary both in composition and amounts not only between hard woods and conifers but also among hardwoods (Timell, 1967). The conifer wood is far more homogenous.

Cellulose is a long chain polymer of glucose anhydride units joined by β 1-4 linkages. In general, cellulose components of wood are light in colour, have strong affinity for water and are soft and tough. Hemicelluloses consist of similar polymers of glucose joined by other linkages or polymers of monosaccharides other than glucose. Lignin is quite different from celluloses and hemicelluloses and is also the most resistant

to biodegradation. It is three dimensional amorphous, branched polymer of phenylpropane units joined by a variety of interunits link ages (Alder, 1977). It is formed by a random free radical oxidative copolymerization of three different oxy-cinnamyl alcohols which occur in various ratios depending upon the tree species. The greatest differences are between coniferous and hardwoods. It differs from all polymers in being largely nonhydrolizable, forming a protective layer around the wood polysaccharides and limiting the cellulose accessibility within the cell walls. Conifer wood in general has a higher lignin content (27-35%) than wood of angiosperms (19-24%). Unlike cellulose, lignin components are darker, have no affinity for water and are harder and brittle.

The wood rotting fungi are grouped into two categories i.e. white rot fungi and brown rot fungi depending upon the way in which they decay wood. Since their substances differ markedly in their physical characters, so the effect of wood decaying fungi is different depending upon which substance is removed. The wood that has been acted upon by a lignin dissolving fungus contains a relatively high remainder of cellulose, becoming soft and spongy in texture and is whitened in contrast to normal wood. A decay of this type is called white rot. A wood from which the cellulose component has been removed, is darker than the normal wood, will be dry, brittle and of charcoal consistency. The decay of this sort is called brown rot.

Brown rot fungi utilize the hemicellulose of the cell walls leaving lignin essentially undigested, but slightly modified (Kirk, 1975; Kirk and Alder 1970). Evidently the differences between the conditions in culture and decaying wood profoundly affect the lignin degrading ability of brown rot fungi. The mechanism of hemicellulose break down by brown rot fungi appears similar to that of white rot fungi (Highley 1976, Keilich *et al.* 1970) but these fungi evidently employ a different mechanism than white rot fungi for attacking the cellulose in the wood (Cowling and Brown 1969, Highley 1977; Koening 1974). Hyphae of brown rot fungi like those of white rot fungi grow inside the lumina in contact with the tertiary wall, into the capillaries of which the secreted enzymes are able to diffuse (Bailey *et al.* 1968, Liese 1970, Wilcox 1970). Unlike the white rot fungi, the enzyme attack is not localized near the hyphae but is widespread and deeply diffused. As the decay proceeds, the cellulose and hemicelluloses are gradually destroyed at approximately the same relative rate. Brown rotted wood tends to shrink abnormally when dried giving rise to a characteristic cubical pattern of checking. The brown rot fungi reduce the strength of wood much more

than the white rot fungi and at the advanced stages the wood is reduced to a residue of amorphous crumbly brown cubical pieces which excessively vertical and horizontal splitting (Brown Cubical rot) composed largely of slightly modified lignin. Brown rot fungi do not produce extra cellular phenol oxidases and generally give negative oxidase test on gallic and tannic acid media and with gum guaic and syringaldazine reagents. Brown rot residues are extremely stable and are important organic components in forest soils (Gilbertson, 1981).

White rot fungi degrade cellulose and hemicelluloses at approximately the same rate, relative to the original amounts present (Kirk and Highley 1973), whereas, the lignin is decomposed at a similar rate or usually somewhat at faster rate on a relative basis (Blanchette 1980; Setiff and Eudy 1980). Hyphae of the white rot fungi are concentrated in the ray cells and vessels although, other cells are invaded very early in decay. The hyphae initially invade other cells from ray cells and vessels via pits or directly by penetration of cell walls (Wilcox, 1970; Liese 1970). White rot fungi have cellulose and lignase enzymes systems secreted at hyphal tips and on lateral surfaces. The enzymes assist cell wall penetration and enlarge bore holes to perforations. Along the young hyphae, lysis furrows are produced. The degradation products of various cell wall layers are completely absorbed by the hyphae. White rot fungi successively de-polymerize cell wall substances only to the extent that the products can be utilized consecutively for metabolism (Cowling, 1961). The action of enzymes system of white rot fungi is restricted to the cell wall layers in the immediate vicinity of the hyphae. A rather specialized type of white rot is caused by some species of *Phellinus,* where the end result is a series of oval holes (about 1.5 cm x 0.5 cm) distributed evenly throughout the infected zone. This distinctive type of decay is called a white pocket rot.

The wood rotting fungi that grow and produce basidiocarps on living trees are either restricted to interior, primarily nonliving portions of living tree (heartwood) or are capable to decay fungi unless it is outer living sapwood. In living trees, the sapwood is quite immune to decay fungi unless it is exposed by a wound of some sort. Though, the decay is not likely to progress much beyond the limits of wounded area but these fungi (*e.g. Aurificaria shorea, Phellinus gilvus* etc.) are true pathogens and are capable of invading and killing living sapwood resulting in killing of the tree in many cases. These fungi generally do not attack the heartwood except a limited attack in certain cases.

Of the several thousand wood decaying fungi, only a small number like *Phaceolus schweinitzii* can cause degradation in the wood of the hearts of living trees (Wagner and Davidson, 1954) and are known as necrotrophs or heart rot fungi. They usually invade trees through dead branch stubs (Boyce, 1961) and can tolerate the chemical and physical constraints within the tree trunk. The constraints which are obviously suspect in this regards are levels of O_2, CO_2 variation of moisture content and pH of heart tissue, concentration and nature of volatile organic compounds, interaction with other microbes and above all the host response (Shigo and Hillis, 1973; Shortle and Cowling, 1978; Shain 1971; Fries, 1973). These factors are probably involved in determining the host specificity of heart rot fungi. The heart rot fungi do not normally attack the sapwood. As such the infected tree is not killed but continues to grow and present all outward signs of a healthy tree with vigorous growth. However, the decay is progressive and with increasing years more and more sound heartwood becomes decayed resulting in a considerable volume loss in standing timber particularly in old growth stands. Besides basidiocarps, other symptoms like stem canker (*Phellinus laevigatus, P. nigricans*) and punk knots (*P. caryophyllii* Ryv and *P. pini*) indicates heart rot decay. In a few cases symptoms like wounds, trunk or butt swells, dead or broken tops, crooked or forked trees, fire scars are also indicative of hear rots.

Members of white-rot *Basidiomycetes* include microorganisms able to degrade lignin efficiently. However, the degree of lignin degradation with respect to other wood components largely depends on the environmental conditions and the fungal species involved. Endoglucanases are produce by brown rot fungi have been isolated from *L. trabea* (*G. trabum*) (Herr *et al.* 1978). It was used as bioassay fungus in the degradation of wood preservatives (Duncan and Deverall, 1964). The physiology of *L. sepiaria* its special reference to enzymatic activity was studies by Zeller (1916). During decay of timber the *L. trabea* produced lignocellulose degrading enzymes (Gadd, 2001). Some microorganisms produce a complex set of enzymes capable of efficient degradation of native cellulose for example *Schizophyllum* has efficient cellulase system to degrade the native cellulose. Lignolytic action of *S. commune* was studied by Jurasek (1968) and influence of extractive on cellulase and xylanase activities of *S. commune* was studied by Sopko (1968). The mechanisms of lignin degradation by fungi have revealed the complexity of the enzymatic systems because there is more than one path to lignin degradation and the enzymatic machinery of the various microorganisms is different

(Hatakka, 1994). Two classes of oxidative enzymes, namely laccases (phenoloxidases) (Thurston, 1994, Leonowicz *et al.* 2001) and peroxidases (lignin and manganese peroxidase) (Farrell *et al.* 1989, Datta *et al.* 1991, Reddy, 1993, Reddy and D'Souza, 1994, Cullen, 1997) have received the greatest attention. The role of laccases recently has been reevaluated because new information on their biodegradative mechanisms has been obtained in several fungal species (Bourbonnais and Paice 1990, 1992, Archibald and Roy, 1992, Leonowicz *et al.* 2001).

Studies on the relative rates of utilization of the structural components of wood by white and brown rot fungi are quite frequent (Santra and Nandi, 1975). Cowling (1961) has reported preferential removal of mannan by brown rot fungi from hardwoods, while neither the major hemicellulose xylan nor the mannan is consistently removed before the glucan by the white rot fungi. Seifert (1968) has reported depletion of cellulose and xylan from pine almost simultaneously by *Coniophora cerebella* a brown rot fungus.

The ability to identify brown rot, white rot and soft rot is essential for assessing biodegradation of structural lumber and utility poles. Light microscopy permits rapid viewing of many cells with minimal specimen preparation. Some decay fungi classification schemes have included Ascomycetes among both white rot (Nilsson, 1985; Eaton and Hale, 1993) and soft-rot categories (Nilsson, 1988; Worrall *et al.* 1997). Members like *Xylaria polymorpha, Daldinia concentrica* and others cause decay with macroscopic features of white rot (Nilsson *et al.* 1989; Eaton and Hale, 1993; Worrall *et al.* 1997). Microscopically, the erosion produced in hardwoods by these *Ascomycetes* is similar to that produced by *Basidiomycetes*, the common feature being erosion channels that form on the lumen surface beneath hyphae. Also, some members of *Basidiomycetes* have been considered to cause soft rot (Daniel *et al.* 1992) because of their ability to form cavities in the S 2 layer. In selectively decayed woods, lignin and hemicelluloses are preferentially removed. Blanchette (1984a) mentioned that fungi that remove lignin selectively without appreciable losses of cellulose are extremely attractive for use in biological pulping processes. Some white rotters can simultaneously degrade all wood components (i.e. lignin, cellulose and hemicellulose) (Blanchette & Reid 1986); while some white-rot fungi are capable of both types of decay in the same wood or in different wood species (Blanchette 1984a, b).

Several higher plants and their constituents have shown success in plant disease control and are proved to be harmless and non-phytotoxic, unlike chemical fungicides. During recent years use secondary metabolites from plant for the control of fungi is gaining importance. Biofungicidal properties of different plant extracts on the growth of *S. commune* were evaluated by Singh and Basu (2004). The effect of heartwood extracts from *Acacia mangium* (heartrot susceptible) and *A. auriculiformis* (heartrot-resistant) was examined on the growth of wood rotting fungi by Mihara *et al.* (2005). *A. auriculiformis* heartwood extracts had higher antifungal activity than *A. mangium*. Wood biodeterioration control potential of *Acalypha hispida* leaf phenolic extract in combination with *Trichoderma viride* culture filtrate was studied by Ejechi (2001). The phenolic extracts of *Acalypha* leaves inhibited growth of *Gloeophyllum sepiarum* and *Pleurotus* sp. *Trichoderma lignorum* was used to control a wood decaying fungus *Lenzites sepiaria* (*Gloeophyllum sepiarium*) (Highley, *et al.* 1994).

The objectives of the following study includes:

1. To survey different forest areas and timber markets of Gujarat to find out association of fungi in wood decay.
2. To study the morphological and anatomical details of Basidiocarps.
3. To study the cultural characters of timber rotting fungi
4. To study the molecular characters of certain wood degrading fungi
5. To study the biochemical events that occur as a consequence of infection in wood.
6. To study the wood degradation, histological events and further events by microtomy
7. To record the morphological changes in xylem tissue.
8. Biochemical analysis will be performed for the presence of total sugars, lignin and enzymes.
9. Besides prevention of timber decay the studies will be undertaken to find out control of wood degrading fungi by Botanical extracts