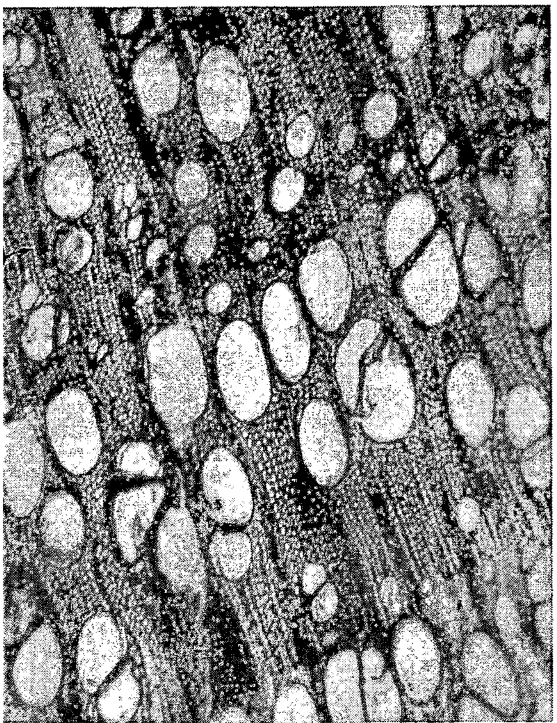
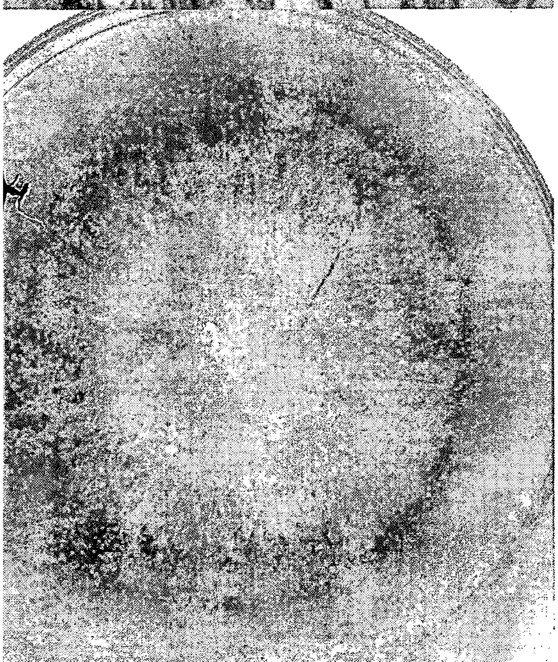
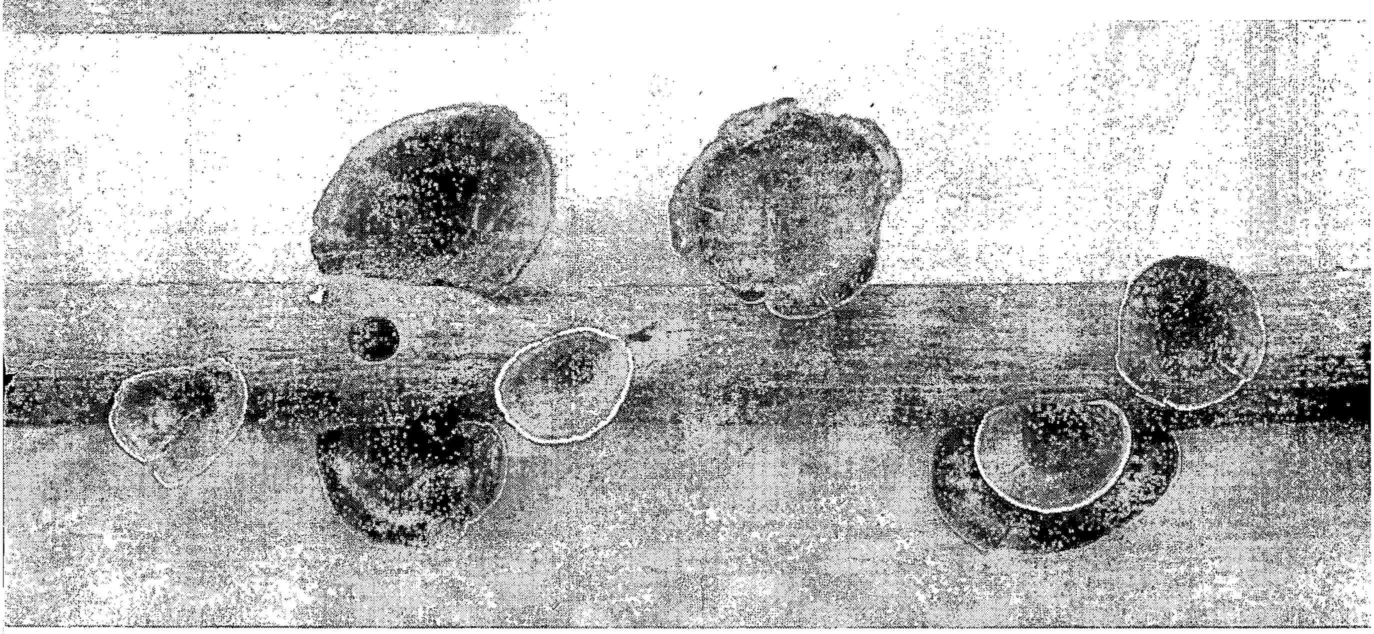




Conclusions



Survey of Saw Mills and different Forest areas

In five districts of Gujarat 94 different sawmills were surveyed. Out of 234 samples collected from different sawmills 32 different fungi were identified. Three spp. of *Xylaria,* 2 spp. each of *Daldinia, Ganoderma,* and *Phellinus* and 3 spp. of *Lanzites* were identified. Seven different types of fungi were found associated with *Tectona grandis* L., followed by four in *Acacia arabica* L. and three each in *Azadirachta indica* A. Juss and *Mangifera indica* L. respectively. The commonly observed timber degrading fungi encountered were *Schizophyllum commune, Flavodon flavus* and *Ganoderma lucidum* belonging to Basidiomycota. The Ascomycota members found associated in theses areas include *Daldinia concentrica* and *Xylaria polymorpha.* Survey of forest areas in 4 different districts resulted into identification of 63 fungi. Of these 39 fungi were belonging to Basidiomcota, 10 to Ascomycota, 4 to Zygomycota, and 9 to Mitosporic fungi.

Studies and Disease cycle of Timber Decay Fungi

46 Basidiomycota and 3 Acomycota members were studied for their morphological and anatomical characters. New and intresting record of Basidiomycetous fungus was *Lenzites sterioides,* which was recorded for the first time on *T. grandis* and second time from the country. *Navisporus floccosa, Coriolopsis aspera Ganoderma curtisii, Microporus alboater, and Phellinus shaferi* were reported for the first time from India. Three different wood decay fungi causing wood rot diseases were studied. The *N. floccosa* heart rot was recorded in living trees of *Ailanthus excelsa* for the first time. The details of *Phellinus hoehnelii* stem rot in live trees was studied for the first time. The new varieties of fungi identified included *Microporus affinis* var. *glabriceps and Aurificaria indica* var. *indica.* The new host records of fungi were *Phellinus badius, P. caryophyllii, P. conchatus, P. hoehnelii, P. pachyphloeus, P. pectinatus, P. rhabarbarinus* and *P. robustus* on *Peltophorum ferrugineum and Irpex hydnoides* on *Tamarindus indica.*

Cultural characters of Timber degrading fungi

The fungal cultures were obtained from the basidiocarps and rotten wood samples. Various cultural and biochemical characters were studied. Cultural characters of 10 different timber degrading fungi were studied. The cultural character of *Flavodon flavus, Hexagonia apiaria Lenzites sterioides Navisporus floccosa,* and *Sterium hirsutum* were studied for the fist time.

Molecular characterization of wood rotting fungi

They were characterized molecularly with markers. Analyses of genetic relationship of different timber degrading Aphyllophorales germplasm were conducted using RAPD and AFLP. Based on RAPD analysis 100 percent polymorphism was observed when primers OPL-1, OPL-5, OPO-7, OPL-14, OPL-18, OPN-10, OPL-4, and

OPQ-15. Use of primer OPL-5 has resulted in lowest number of markers (10) without any common marker and OPO19 has given lowest number of markers (16) with one marker common to all the species studied and showed lowest Percentage Polymorphism (PP) (93.75). On the contrary intrageneric GS was found to be maximum in between *L. exima* and *F. flavus* strain 1 and minimum in between *Pleurotus* sp. and *S. hirsutum*. Over all PP among seven genera through RAPD was found to be 98.81 with 2 markers common in all the genus studied. In the pair wise comparison the mean PP was 98.87.

Based on AFLP analysis The GS was also highest in case of AFLP as observed with RAPD in between *S. commune* and *Pleurotus* sp. and minimum in between *F. flavus* strain 2 and *G. lucidum*. Use of primer set EAGC/MCAT has resulted maximum number of markers (151) with no common marker to all the species indicating 100% polymorphism among various genera studied. Use of primers combination EAGC/MCAT resulted in 100% polymorphic markers. When primers combination EAGG/MCAT was used highest numbers of non polymorphic markers (2) were obtained and resulted in lowest PP (98.52). Mean PP was found to be 99.29.

Physiological studies of different wood rotting fungi

For selection of suitable medium for growth of four wood rotting fungi, Malt Extract Medium, Modified Asthana and Hawkers Medium 'A', Czapek Dox's Medium, Basal Medium and 1 % Malt Extract Medium were used. All the organisms showed maximum growth in Czapek Dox medium.

When 4 fungi were grown on different initial pH, the maximum growth was observed at pH 4.0 for *L. sterioides,* and *H. apiaria*, at pH 4.5 for *T. pini* and at pH 5.0 for *N. floccosa.* To find a suitable temperature for the growth of timber degrading fungi, the temperatures like 5,10,15,20,25,30,35, and 40°C were maintained. The maximum growth was observed at temperature 20°C for *L. sterioides*, and *H. apiaraia*, at 25° C for *T. pini* and *N. floccosa. H. apiaria* showed the maximum growth of mycelium in D – arabinose and D-xylose. *L. sterioides* showed maximum growth in D-xylose, sucrose, and malt extract, in *T. pini* maximum growth of mycelium was observed in case of teak wood sawdust supplemented in the medium as carbon source.

All the four test fungi failed to consume D-xylose completely up to 15 days of incubation. Sucrose was slowly breakdown into monosaccharides, it remained present up to 10 days in case of *T. pini* and *N. floccosa* while it was present up to 8 days in *L. sterioides* and 12 days in *H. apiaria*. Maltose was utilized by the present fungi through a hydrolytic pathway.

The effect of 5 different nitrogen sources was observed in case of 4 wood rotting fungi. The results indicate that potassium nitrite showed better growth for *T. pini* and *N. floccosa,* the sodium nitrate showed better growth in *L. sterioides*, and the ammonium nitrate as sole nitrogen source produced better growth in *H. apiaria.* Based upon growth supporting ability the inorganic nitrogen compounds are grouped as calcium nitrate >Sodium nitrate >Potassium nitrate > Potassium nitrite > ammonium nitrate (35%) for *L. sterioides.*

Biochemical Changes

In biochemical studies the effect of carbon and nitrogen sources on growth and secretion of ligninolytic enzymes like laccase, lignin peroxidase, manganese peroxidase, ary alcohol oxidase and peroxidases enzymes were studied. The ability of timber degrading fungi to secrete the lignocellulolytic enzymes were tested by using enzyme plate method. The biochemical events that occur due to the initiation of decay in xylem and other cells were studied by using the concentration of protein and ligninolytic enzymes in decayed samples.

Laccase activity was more in case of *Adina* wood infected with *T. pini.* Whereas lowest in case of teak wood infected with *H. apiaria.* The highest aryl alcohol oxidase activity was observed in case of *Adina* wood infected with *C. versicolor,* whereas lowest in case of teak wood infected with *T. pini.* The highest peroxidase activity was observed in case of *Adina* wood infected with *C. versicolor,* whereas lowest incase of teak infected with *T. pini.* The highest lignin peroxidase activity was observed incase of *Adina* wood infected with *C. versicolor,* whereas lowest in case of the *T. crenulata* wood infected with *L. sterioides.*

The concentration of protein was highest in case of teak wood infected with *C. versicolor,* whereas lowest in case of *T. arjuna* wood infected with *L sterioides.* It also showed highest concentration of protein in infected *Adina* wood. The C. *versicolor, T. pini* and *H. apiaria* showed highest concentration of protein in infected teak wood. The white rot fungi produce large quantity of lignin degrading enzymes to degrade lignin. By using the lignolytic enzymes, these can be used degrade the low quantity of lignin in pulp. The white rot fungi tested may be used in the biodegradation of the lignocellulosic waste materials. The capacity of white rot fungi to produce lignocellulolytic enzymes can be exploited for industrial use or commercial production of ethanol from lignocellulosic wastes and pulp effluent.

Wood decay test

The wood degrading capacity of timber decay fungi was studied by decay test. In which two methods were followed, i) wood chips method ii) wood block method. The wood block method was best method to study the long duration of decay. In initial stages of decay the percentage of moisture was more, whereas in advanced stages of decay the % moisture was less. As the percentage moisture was less the percentage weight loss was also less, this indicates that the decay capacity of the fungi depend on the % moisture content in wood. After 12 months decay period in block test 90% of wood was decayed in *T. arjuna* due to *L. sterioides*, followed by *T. bellerica* and *T. grandis*. Wood decay was minimum (16.82%) in teak due to *H. apiaria* in 3 months. Based on the weight loss studies different scientists have explained that the woods are resistant to a particular fungi. But in present study it was found that in case of lesser weight loss also the wood was severely degraded by the test fungi. Therefore, on the basis of weight loss studies alone the type of wood decay can not be certainly decided.

Biochemical analyses of certain artificially infected woods

In biochemical analyses the degraded wood samples were analyzed for Water content, pH of samples, Solubility in hot water, Solubility in Ethanol-Benzene, Acid insoluble lignin and Chlorite holocellulose. The artificially infected wood log of Teak, *Adina*, *T. crenulata* and *T. arujna* were chemically analyzed. The percentage of moisture loss was highest in case of *T. crenulata* wood decayed by *L. sterioides*, whereas lowest in case of teak wood decayed by *C. versicolor*. The percentage loss in hot water soluble substrates was more in case of *T. crenulata* due to *L. sterioides* for 5 months, whereas lowest in case of teak wood decayed by *H. apiaria* for 5 months. The percentage loss in ethanol benzene soluble substrate was more in case of *Adina* wood decayed by *C. versicolor* for 5 months. Whereas lowest in case of teak wood infected with *L. sterioides* for 3 months. The chemical analysis of artificially inoculated wood blocks for 1 year was done. As the incubation period increased the percentage loss in acid soluble lignin was more in case of all infected woods reaching to almost 50%. Whereas the percentage loss of holocellulose was up to 20% only.

Histological and morphological changes

Anatomical studies were conducted to study the normal wood structure and pattern of decay caused by white rot fungi in selected timbers i.e. *T. grandis, A. cordifolia, T. crenulata* and *T. arjuna*. The wooden logs were infected with certain white rot fungi like *L. sterioides, C. versicolor, H. apiaria* and *T. pini*. For changes in morphology of infected wood and cell wall alteration due to initiation of decay were studied by light microscopy and by use of selective stains like safranin, fastgreen and toludine blue. Histochemical localization was carried out to detect the histochemical changes taking place in infected woods. The Ultra structural changes in the cell wall layers of different cells in infected teak wood samples were observed by using Scanning Electron Microscopy.

The four fungal species i.e. *Lenzites, Coriolus, Hexagonia* and *Trametes* were selectively delignifying fungi. The pattern of decay caused by four species was found to be almost the same in all the samples. Samples of *T. crenulata* decayed by *C. versicolor* and *H. apiaria* showed both selective as well as simultaneous decay of cells. Selective deleignification process was observed only in the vessel elements, whereas in fiber cells and ray cells simultaneous decay has been observed. In the other wood samples selective delignification was appaearntly noticed by the dissolution of middle lamella, separating the cells from their neighboring ones. Bore holes were noticed to be formed in the walls. All the wood samples except *T. arjuna* were decayed by *T. pini* and the decay showed damage to vessel elements more prominently than the other cells.

Ultra structural changes

Lenzites sterioides showed selective delignification. Discolouration of the ray cells was distinctly observed, which further increased making the walls more or less transparent. Only the regions of pits appeared to be darkly stained. The pectin rich middle lamella was dissolved first as a consequence of which individual cell walls of the horizontal cells completely separated from one another. Then followed the complete degradation of the remaining secondary and tertiary wall, it caused a hole in the middle of the ray, which increased in size during the further course of decay. The region of ray cells were identified as spindle shaped cavities. Sometimes only the outline of ray cells was notice. All the 4 fungi tested for their wood degrading activity were found to be selective lignin degraders.

Biocontrol of Timber degrading fungi

To control damages by fungi scientists have suggested use of chemicals, fumigants, use of γ irradiations and application of UV rays, heat treatments etc. Use of creosote oil to prevent rail, roads and telephone poles and sodium fluoride and zinc chloride (Wolman salts) to treat the wood used in mines had been tried. Turpentine, varnishes and paints provide a protective coating and seal the pores present in wood to imbibe water and this protects wood from decay caused by fungi. The best preventive method for storage of wood was storing them in a dry atmosphere.

Leaf extracts of *Thevetia peruviana* (Pers.) Schum., *Tagetes erecta* L., *Eucalyptus globulus* Labill., *Azadirachta indica* A. Juss., *Prosopis juliflora* (Sw.) DC., *Saraca indica* L., *Lantana camara* L., *Biota sinensis* L., *Cimbopogan citrates* (Nees) Stapf., *Datura metel* L., *Callistemon linearis* DC., and *Parthenium hysterophorus* L. were tried *in vitro* to control wood degrading fungi. Leaf extracts of 10 dicots, 1 monocot and 1 gymnospermous plant were tested against 5 wood degrading fungi. For most of the fungi 25% methanolic extract was more effective than 5 and 10% concentrations. *L. sterioides* was completely inhibited by 5% leaf extract of *Prosopis Juliflora* and 10% leaf extract of *Cymbopogon* and 25% concentration of *Datura. Tagetes, Eucalyptus, Azadirachta,* and *Prosopis* controled all the 5 test fungi completely. Extract of *P. juliflora* and *A. indica* were more effective than other plants. Methanolic extract of *Cymbopogon* showed 100% inhibiting of *S. hirsutum,* while its aqueous extracts was ineffective in all the concentrations tried. Variation in activity observed in methnolic and aqueous extract may be due to presence of different inhibitory compounds in these extracts. Aqueous extracts of *A. indica P. juliflora* and *P. hysterophorus* may be further tried *in vivo* against all the 5 wood decay fungi. Leaf extracts of *Tagetes, Eucalyptus, Azadirachta, Saraca, Cymbopogon, Datura* and *Callistemon* were able to check the growth of all the test organisms completley at 25% concentration.

Oils and gels of *Cymbopogon citrate* (Nees) Stapf., *Anacardium occidentale* L., *Gossypium barbadensis* L., *Linum usitatissimum L., Aloe vera* L., and *Aloe ferox* Mill were used to control the timber degrading fungi. Out of 4 oils cashew nut shell oil was most effective followed by cotton seed oil. Of the two *Aloe* gels tried the *A. ferox* gel showed better results than *A. vera.* Uses of ecofriendly alternative like botanical pesticides and gels may be helpful to control the wood damage in forests and in different wood depose. The leaf extracts, oils and gels were tried as ecofriendly management of certain wood decay fungi. For the first time, use of botanical pesticides have been tried *in vitro* to control white rot fungi. Preliminary studies are indicative of great potential for production, commercialization and the use of botanical pesticides in fungal pest control.