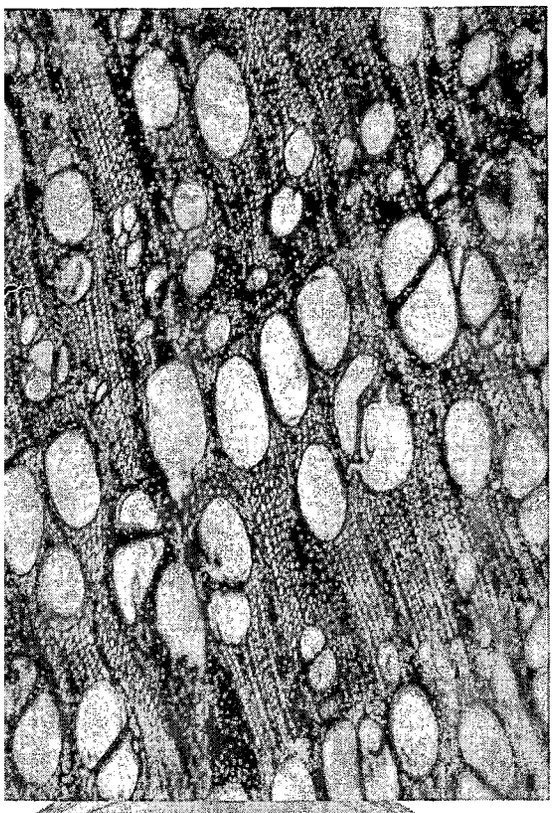
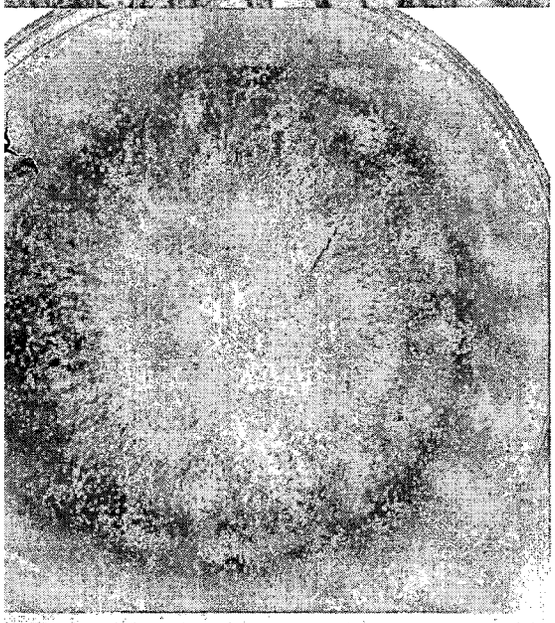
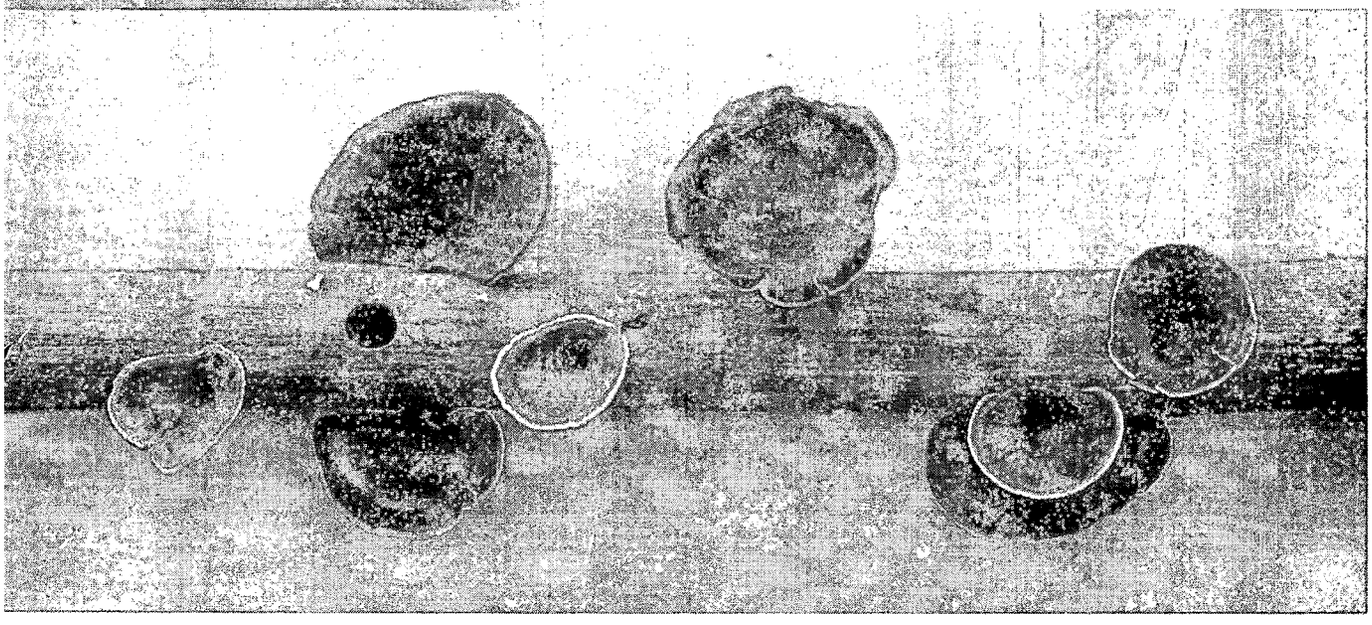




Results and Discussion



1. Survey of Saw Mills

To find out the association of the timber degrading fungi and study the timber decay problems in sawmills a survey was conducted during last 5 years in different sawmills of i) Vadodara, ii) Ahmedabad, iii) Bharuch, iv) Rajkot and v) Jamnagar. In all 94 sawmills were surveyed from the Gujarat state, in which 28 sawmills were from Vadodara, 29 from Ahmedabad, 12 from Bharuch, 21 from Rajkot and 4 from Jamnagar. The location of these 5 districts is depicted in (Figure 1) and results are recorded in Tables 1, 2 and 3. The survey revealed that the economically important woods present in the saw mills included were a) Arjun (*Terminalia arjuna* W.A.), b) Baheda (*T. bellerica* Roxb.), c) Biyo (*Pterocarpus marsupium* Roxb.) d) Devdar (*Cedrus deodara* (Roxb. ex D. Don.) G. Don, e) Haldar (*Adina cordifolia* Roxb), f) Kapur (*Dryobalanops aromatica* Gaertn. F., Nom.), g) Marinty (*S. contorta* Vidal), h) Pine (*Pinus longifolia* Roxb. Sans.), i) Sal (*Shorea robusta* Gaertn. j) Sevan (*Gmelina arborea* Roxb.), k) Sisoo (*Dlabergia sissoo* Roxb.), l) Steam Beech (*Fagus grandifolia* Ehrh.), m) Sycamore (*Platanus occidentalis* L.) n) Teak (*Tectona grandis* L.f.), f.), and. Locally available, common woods present were a) Amli (*Tamarindus indica* L.), b) Aonla (*Emblica officinalis* Gaertn.), c) Babul (*Acacia arabica*), d) Gorus amli (*Pithecellobium dulce* (Roxb.) Benth.), e) Gulmohar (*Delonix regia* (Bojer ex Hook.) Raf), f) Mango (*Mangifera indica* L.), g) Neem (*Azadirachta indica* A.Juss), and these woods are commonly used in making planks for furniture, decorative items, and making packaging boxes etc.

Out of the 94 sawmills surveyed, 84 sawmills showed the presence of timber degrading fungi. The 10 sawmills where no association of any fungi was found included Siva Shakti Sawmill in Vadodara, P. K. Patel and Co and Bhavani saw mill in Ahmedabad, K.K. Patel and Company and Patel A.Y. Lakadawala in Baruch, Silicon Timber Mart, J.M. Patel Sawmill, Bagavan Timber Mart, Narayan Traders and Maruti Timber Mart in Rajkot. Only one timber decaying fungi was found in sawmills of Shri Mahadev Sawmill and Shiv Shakti timbers in Vadodara, Pavi Timber Mart, Sreelakshminarayan Saw mill, Shree Ganesh saw mill, Shree Vishnu saw mill and Shree Krishna saw mill in Ahmedabad, Lalit Timber Mart, Haji Abhdul Rahim Mahamadbhai Lakadawala, and Dhanlakshmi Sawmill in Bharuch, Shankar Vijay timber mart, Jyoti timber mart, D.K Patel and Co, Visal Timber Traders and Shankar Vijay Timber mart in Rajkot and Krishna Sawmill in Jamnagar.

Fifteen and thirteen fungal species were observed in two different saw mills situated in Chhani road, Vadodara, followed by 11 fungi in a saw mill of station road Vadodara. Seven different types of fungi were found associated with teak, followed by four in babul and three each in neem and mango respectively. The commonly observed timber decaying fungi were *Schizophyllum commune*, *Flavodon flavus* and *Ganoderma lucidum* belonging to *Basidiomycota*. *Ascomycota* members found associated were *Daldinia concentrica* and *Xylaria polymorpha*.

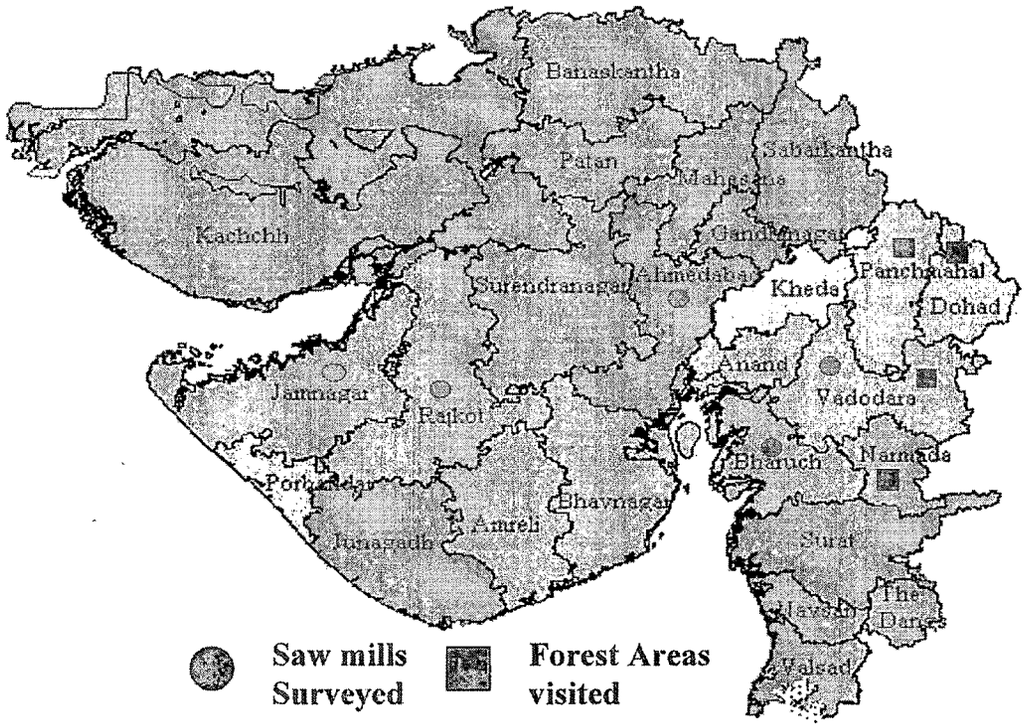
The timber decaying fungi associated with different woods in 94 sawmills produced huge amount of fungal spores dispersed in that area. The workers of sawmills surveyed were facing some respiratory problems. This may be because of the fungal spores inhalation which might be present in the air.

In most sawmills around the world, trees are harvested into logs and stored in the forest or in a log yard for a period of time before being sawn into lumber. These logs may be attacked by various pigment producing fungi. Since hardwood species are used to a great extent in furniture industry and in the making of other valuable wood products, the reduction of wood staining fungi, molds, and decay fungi in these species has a significant economic impact. Studies on airborne fungal spores which are produced from different wood inhabiting fungi are undertaken by various scientists (Burge, 1989; Lacey and Dutkiewicz, 1994; Verhoeff and Burge, 1997). People, who are constantly exposed to an indoor environment where works on organic substances and their by products are dealt with, may often develop respiratory disorders. Sawmill workers may be exposed at work to the inhalation of various allergenic and immunotoxic agents, comprising of wood derivatives (e.g. terpenes, resin acids) and microorganisms associated with timber (Crook and Olenchock 1995, Demers *et al.* 1997, Dennekamp *et al.*, 1999, Enarson and Chan-Yeung, 1990, Halpin *et al.*, 1994, Tatken 1987). Inhalation of fungal spores may result into decrease in lung function, bronchial hyperresponsiveness and respiratory disorders, such as: organic dust toxic syndrome (ODTS), allergic alveolitis, asthma, non-asthmatic chronic airflow obstruction, chronic bronchitis, mucous membrane irritation syndrome (MMI) and rhinitis (Halpin *et al.*, 1994, *Mandryk et al.*, 1999, Mandryk, 2000, Wimander and Belin,1980).

According to Wealth of India, (1971) Kerala has largest number of sawmills. A recent estimate shows that more than 25000 registered sawmill are present in Gujarat. Airborne fungi occurring in different indoor environments such as libraries, markets, flour mills, hospital wards etc., have been reported from different parts of India (Narayan

et al., 1982; Vittal and Glory, 1985; Singh and Singh, 1994, Sharma and Datta, 2001). But no such data is available for number and type of fungi occurring in the environment of saw mills. Few reports of such studies are however available from countries like Scandinavia (Jappinen *et al.*, 1987), France (Simeray *et al.*, 1997), Canada (Duchaine *et al.*, 2000) and Poland (Dutkiewicz *et al.*, 2001). The studies conducted in Poland showed that the greatest concentrations of microorganisms in sawmills processing coniferous wood was noted at debarking stage of pine logs (Dutkiewicz *et al.*, 2001).

Figure 1: Map of Gujarat showing surveyed areas.



1.1. Diversity of fungal flora in saw mills

In the present study *Aspergillus flavus, A. niger, Chaetomium globosum, Colletotrichum gloeosporioides, Fusarium moniliforme, F. pallidoroseum, Lasiodiplodia theobromae, Nectria cinnabarina, Phomopsis salmalica, Trichoderma harzianum,* and *T. viride* were found associated with different timbers present in five district of Gujarat. Most dominant invaders of woods were *A. niger A. flavus, T. harzianum* and *T. viride.* The high risk genera like *Aspergillus* and *Trichoderma* were observed. In the present study it was found in a number of woods which were infected by the timber decaying fungi, dehisces different concentration and composition of fungal spores. The study

revealed high prevalence of predominantly allergenic fungal spores in certain sawmills of the five districts of Gujarat.

Padmanabhan and Naya (2004) found association of *Aspergillus, Penicillium, Cladosporium, Nigrospora, Ganoderma,* 'other basidiospores' and ascospores in air. They further found that *Aspergillus,* and *Penicillium* were the two most dominant spore types in the indoor air, which contributed 51.19% and *Cladosporium,* the most dominant spore type in the outdoor contributed 44.75% of the total spores. In a similar survey conducted in Eastern Canadian sawmills by Caroline and Anne (2000) found that *Penicillium* spp were the most frequently isolated microfungi. Aerobiologists have found that the two high allergy risk genera like the presence of *A. fumigatus* and various species of *Mucor, Trichoderma* and *Phoma* could adversely affect the man power working in these establishments (Simeray *et al.,* 1997). The concentration and composition of airborne microflora in sawmills may vary to a great degree depending on the kind of timber being processed and the technology of production (Mandryk, 2000). The type of wood processed may influence the composition of the mycoflora present in the atmosphere. *Penicillium* spp predominated in conifer and *Cladosporium* spp in hardwood sawmills (Simeray *et al.,* 1997). The pollution of air with microorganisms has been reported from the primary or secondary infection of timber (Dutkiewicz, 1989; Eduard, 1993 and Rask-Andersen 1994). The primary infection develops in timber logs stored in forests and in lumber yards, initially with bacteria (described as "pioneer organisms") and then with fungi which may eventually cause wood decay (Greaves 1971, Käärik 1975, Levy 1975, Rossell *et al.,* 1973).

Table 1: Results of Survey conducted in different sawmills in 5 districts of Gujarat state and Number of various wood degrading fungi associated with different woods.

Vadodara District				
S. No	**Name and Address of shop/ Date of sampling**	**Wood available**	**Occurrence of Fungi**	**Total no of Fungi**
1	Jai Gopla sawmill Timber merchant Gajarawadi Vadodara - 390017 (13-3-2007)	*Shorea robusta* *Dryobalanops* sp. Marina teak (*Tectona grandis*) Nigeria teak " Ghana teak " Panama teak " *Pinus longifolia* *Mangifera indica* *Azadirachta indica* *Acacia arabica*	-- -- -- -- *Schizophyllum commune* -- -- *Trichoderma viride* • *Daldinia concentrica* • *Aspergillus niger* *T. harzianum*	5

2	Shiva Sakti Sawmill Dabhoi road, Vadodara-4 (13-3-2007)	*Dryobalanops* sp. *S. contorta* African Mahogani *Aspidosperma* sp. Nigeria teak (*T. grandis*) Ghana teak " Sudan teak " Burma teak " *Gmelina arborea* *Fagus grandifolia* *Pterocarpus marsupium*	-- -- *S. commune* • *Lenzites betulina* • *Daedaleopsis confragosa* -- -- *Flavodon flavus* *Xylaria plymorpha* -- -- -- -- *T. viride* *Fusarium pallidoroseum* -- -- -- --	7
3	Vishal Sawmill Dabhoi road, Vadodara- 4 (13-3-2007)	*M. indica* *Tamarindus indica* *T. grandis* *A. indica* *Derris pinnata* *A. nilotica*	*F. flavus* *F. moniliforme* -- -- *S. commune* *D. concentrica* -- --	4
4	Siddharth Sawmill Dabhoi road, Vadodara – 4 (13-3-2007)	*P. longifolia* *P.* roxberghii *T. grandis* Burma teak (*T. grandis*) Malaysia teak " *Cedrus deodara*	*S. commune* -- -- *F. flavus* *Phellinus badius* -- -- -- --	3
5	Bhagvati Ply and Timber Agency 4/A, Haribhakti Industrial Estate, Dabhoi road, Baroda – 4 (13-3-2007)	Nigeria teak, (*T. gandis*) Nagpuri teak, " Valsad teak, " C.P. teak, " Malaysia teak " *S. robusta*	-- -- -- -- *S. commune* *L. sterioides* *T. viride* -- --	3
6	Swastik Sawmill Opp. Mahanagar Soc, Nr. Yamuna mill, Dabhoi road, Baroda - 4 (13-3-2007)	Burma teak, (*T. grandis*) Nigeria teak, " Ghana teak, " *C. deodara,* *Terminalia myriocarpa* *S. contorta* *S. robusta*	*S. commune* *X. allantoidea* -- -- *Sterium hirsutum* -- -- *T. viride* -- --	4
7	Mahesh timber mall, Sama road, Sama, Baroda – 8 (21-3-2007)	Nigeria teak (*T. grandis*) *Platanus occidentalis* *Fraxinus americana* *Fagus grandifolia*	• *L. betulina* • *F. flavus* *S. commune* -- -- • *X. polymorpha* • *Aspergillus flavus*	5
8	Shivashakti Sawmill, Sama road, Sama, Baroda-8 (21-3-2007)	*M. indica* *A. arabica* *S. robusta* *T. grandis* Nigeria teak, (*T. grandis*) Ghana teak " *Dryobalanops* sp. *P. longifolia* *P.* roxberghii	*S. commune* *F. flavus* -- -- *S. commune* -- -- -- -- -- -- *T. viride* -- --	4
9	Ganesh Sawmill Sama Road, Baroda (21-3-2007)	*T. grandis* Nigeria teak (*T. grandis*) Ghana teak, " African Mahagoni *Dryobalanops* sp. *S. robusta*	*T. harzianum* -- -- *P. noxius* *X. polymorpha* -- -- -- --	3

10	Sahajahand Sawmill, Near Union Bank of India, Sama road, Vadodara-390008, (21-3-2007)	*T. grandis* *M. indica* *A. nilotica* *A. indica*	-- -- -- -- *Collectotrichum gloeosporioides* *S. commune*	2
11	Bajarang Timber Trading Co. Opp. Sama Jakathnaka , Sama, Vadodara-390008 (21-3-2007)	Nigeria teak (*T. grandis*) Burma teak " Valsad teak " *S. robusta* *P. longifolia* *Olea europaea* *Ficus carica*	-- -- -- -- *F. flavus* -- -- *T. viride* -- -- -- --	2
12	J.K.S. Sawmill Harni, Baroda (16-3-2007)	*T. grandis* *Dryobalanops* sp.	• *F. flavus* • *D. sacchari* • *S. commune* • *X. Allantoidea*	4
13	Kalyan Sawmill, Harni, Baroda (16-3-2007)	*A. indica* *Prosopis juliflora* *M. indica* *A. nilotica*	*T. viride* -- -- *Phomopsis salmalica* *X. polymorpha*	3
14	Jagadesh Sawmill, Harni, Baroda (16-3-2007)	*C. deodara* *P. longifolia* *Bombax ceiba* *A. indica*	-- -- *Lasiodiplodia theobromae* -- -- *Trichoderma* sp.	2
15	Patel Timber Merchant Harrni, Baroda (16-3-2007)	Ghana teak (*T. grandis*) Nigeria teak " *G. arborea* *A. indica*	• *S. commune* • *F. flavus* *D. concentrica* • *A. niger* • *A. flavus* *S. commune*	6
16	Datidar Sawmill, Harni, Baroda (16-3-2007)	*T. grandis* Nigeria teak (*T. grandis*) Malaysia teak " Valsad teak " C.P. teak " Nagpuri teak " *D. sisso* *C. deodara* *T. myriocarpa* *P. longifolia*	*S. commune* *Coriolus versicolor* -- -- -- -- *Nectria cinnabarina* -- -- *D. concentrica* -- -- -- -- *X. polymorpha*	5
17	Jivarai sawmill, Station road, Near Shastri Bridge, Vadodara (8-4-2007)	*F. benghalensis* *T. grandis* *T. indica* *B. ceiba* *A. indica* *M. indica* *P. roxburghii* *C. deodara*	• *S. commune* • *T. harzianum* -- -- • *F. flavus* • *A. flavus* -- -- • *S. commune* • *D. concentrica* • *T. viride* • *X. polymorpha* • *A. niger* • *Ganoderma lucidum* • *D. concentrica* -- --	11
18	Ambhika Sawmill, Near total Gate, Chhani road,	*Hardwickia binnata* *Delonix regia* *A. arabica*	*T. viride* *S. commune* • *S. commune*	13

	Chhani, Baroda (8-4-2007) (5-7-2009)	 *P. longifolia* *T. grandis* *Cordia mixa*	• *G. lucidum* • *A. niger* • *F. flavus* • *X. polymorpha* • *T. haizianum* • *L. sterioides* • *F. flavus* • *D. concentrica* • *A. flavus* • *F. moniliformi*	
19	Warrior Timber Merchant, Near Total gate Chhani road, Chhani, Vadodara (8-4-2007) (5-7-2009)	*Pithecellobium dulce* *M. indica* *A.nilotica* *Emblica officinalis*	• *S. commune* • *T. viride* • *G. applanatum* • *A. flavus* • *S. Commune* • *X. polymorpha* • *F. flavus*	7
20.	Pregathi Timber Mart, Near Total gate, Chhani road, Chhani, Vadodara (8-4-2007) and (5-7-2009)	*T. grandis,* *T. indica,* *A. nilotica,* *S. robusta*	-- -- *G. lucidum* *F. palidoroseum* -- -- -- --	2
21	Patel Timber Mart, Near total gate, Chhani road, Chhani, Vadodara (8-4-2007) (5-7-2009)	*T. grandis,* Burma teak, (*T. grandis*) Nigeria teak, " *S. robusta,* *A. nilotica,* *M. indica* *T. indica*	• *L. sterioides* • *F. flavus* • *G. lucidum* • *C. versicolor* • *Chaetomium globosum* • *S. hirsutum* • *X. polymorpha* • *S. commune* • *T. viride* • *S. commune* • *G. applanatum* • *D. concentrica* • *Auricularia aricula* • *Hypoxylon rubignosa.* • *A. niger*	15
22	Shri Mahadev Sawmill Near Makarpura Road, Vadodara (13-7-2009)	*T. grandis,* *Eucalyptus globulus* *A. nilotica* *Bambusa arundinacea*	*G. lucidum* -- -- -- -- -- --	1
23	Shivamahadeve Saw mill, Muval, Padra thaluka, Vadodara district. (5-7-2009)	*A. indica,* *P. dulce* *Leucinia leucocephala* *A. arabica* *E. globulus*	• *S. commune* • *H. rubignosa* • *G. lucidum* • *T. viride* *F. flavus* *P. badius* -- --	6
24	Pateal wood works Muval, Padra taluka, Vadodara district. (5-7-2009)	*A. indica,* *M. indica,* *L. leucocephala,* *D. pinnata*	*A. niger* -- -- -- -- *S. commune*	2
25	Jalaram Sawmill Station Road, Padra, Dist. Vadodara- 391940	*B. arudinacea* *P. marsupium* *Dryobalanops* sp. *T. grandis*	-- -- -- -- -- -- *F. flavus*	2

	(5-7-2009)	*S. rubusta* *P. longifolia* *A. indica*	-- -- -- -- *S. commune*	
26	Shiv Uma Vijay Sawmill Station Road, Padra, Dis Vadodara- 391940 (13-7-2009)	*D. pinnata* *A. indica* *P. dulce* *E. globulus,* *P. longifolia,* *B. arudinacea* *T. indica* *P. juliflora*	-- -- -- -- -- *S. commune* -- -- -- -- -- -- • *F. flavus* • *Agaricus* sp.	3
27	Shiv Shakti timbers Station Road, Padra, Dist. Vadodara- 391940 (13-7-2009)	*T. grandis* Gana teak (*T. grandis*) *Dryobalanops* sp. *S. rubusta,* *C. deodara,* *A. indica,* *M. indica* *P. juliflora*	-- -- -- -- -- -- -- -- -- -- *S. commune* -- -- -- --	1
28	Siva Shakti Sawmill Station Road, Padra, Dist. Vadodara- 391940 (13-7-2009)	*A. indica,* *Tamarindus indica,* *E. globulus,* *P. pinnata* *A. nilotica* *P. juliflora*	-- -- -- -- -- -- -- -- -- -- -- --	
		Ahmedabad District		
29	Shivam enterprises, Gitamandir road, Latia Bazar, Ahmedabad (7-7-2007)	Ghana teak, (*T. grandis*) Burma teak, Ghana teak, *Balfourodendron roedelianum* *P. longifolia.*	*S. commune* -- -- *D. concnetrica* -- -- *T. harizianum*	3
30	Shri Shakti Timber Mart, Gitamandir road, Ahmedabad (7-7-2007)	*S. robusta,* Ghana teak (*T. gandis*) Burma teak "	-- -- *F. flavus* -- --	2
31	Pavi Timber Mart, Gitamandir road Ahmedabad (7-7-2007)	*P. lingifolia,* *C. deodara,* *S. robusta*	*T. viride* -- -- -- --	1
32	Shree Ambica Sawmill, Citamandir road, Latia Bazar, Ahmedabad (7-7-2007)	*M. indica,* Ghana teak (*T. grandis*) *P. longifolia* *P. roxberghii*	• *G. lucidum* • *A. niger* • *A. flavus* -- -- -- -- -- --	3
33	Lucky Timber Mart, Gitamandir road, Latia Bazar, Ahmedabad (7-7-2007)	*P. longifolia,* *P.* roxberghii *M. indica* Burma teak (*T. grandis*)	-- -- -- -- • *S. commune* • *F. flavus* -- --	2
34	Maruthi Wooden Industry Odhav Bus stand, Near Unipoal plastic industry, Odhav, Ahmedabad. (7-7-2007)	*M. indica* *A. nilotica* *A. indica* *D. pinnata* *P. longifolia* *T. grandis*	-- -- -- -- *S. commune* *D. concentrica* -- -- -- --	2

35	Raghuvamshi Timber Mart, Near Unipoal plastic industry, Odhav road, Odhav, Ahmedabad. (7-7-2007)	*M. indica* *A. nilotica* *A. indica* *P. longifolia*	*S. commune* *G. lucidum* *D. concnetrica* -- --	3
36	Hingraj Timber Mart, Near unipoal plastic industry, Odhav road, Odhav, Ahmedabad (7-7-2007)	*M. indica* *A. indica* *A. nilotica*	*S. commune* -- -- *A. flavus*	2
37	Mahakali Enterprises, Near unipoal plastic industry, Odhav road, Odhav, Ahmedabad (7-7-2007)	*Adina cordifolia* *M. indica* *D. pinnata* *A. nilotica* *E. globulus*	-- -- *S. commune* *D. sacchari* -- -- -- --	2
38	Mahakali Saw mill Near unipoal plastic industry, Odhav road, odhav, Ahmedabad (7-7-2007)	*T. bellerica,* *E. globulus* *P. dulce,* *A. nilotica,*	-- -- *S. commune* *F. flavus* *T. viride*	3
39	Sreelakshminarayan Saw mill, Odhav bus stand, Near unipoal plastic industry, odhav road, odhav, Ahmedabad (7-7-2007)	*M. indica* *A. arabica* *A. indica* *E. globulus*	-- -- -- -- -- -- *L. betulina*	1
40	Sri Siva Vijay saw mill. Near Mangalam talkies Odhav road, opp odhavgam, Ahmedabad. (7-7-2007)	*C. deodara* *A. nilotica* *B. ceiba*	-- -- • *S. commune* • *X. polymorpha* -- --	2
41	Shree Ganesh saw mill, Ganesh industrial estate, Nr. Odhav bus stand, odhav, Ahmedabad-382415 (7-7-2007)	*M. indica* *A. indica* *A. nilotica* *C. deodara*	-- -- *T. viride* -- -- -- --	1
42	Shreelakshmi saw mill, Ganesh industrial estate, Nr. Odhav bus stand, odhav, Ahmedabad- 382415. (7-7-2007)	*M. indica,* *A. indica* *P. pinnata*	*A. niger* *F. flavus*	2
43	Anil Timber Coperator, Ganesh industrial estate, Nr. Odhav bus stand, Odhav, Ahmedabad (7-7-2007)	*A. nilotica* *C. deodara* *T. grandis* *P. longifolia*	• *S. commune* • *T. viride* -- -- -- -- *D. sacchari*	3
44	Shree Patidar saw mill, Near vallab nagar, Odhav road, Odhav, Ahmedabad (7-7-2007)	*M. indica,* *A. nilotica,* *A. indica* *D. pinnata*	*A. niger* *F. moniliforme* -- -- -- -- -- --	2
45	Shiv Packing industry, Besides Rudhraksh appartment, Odhav road, Odhav, Ahmedaba (7-7-2007)	*M. indica* *A. arabica* *P. dulce* *E. globulus* *P. longifolia*	*X. polymorpha* -- -- *S. commune* -- -- -- --	2
46	Mahalakshmi saw mill, C.M. C. India, Odhav road, Odhav, Ahmedabad (7-7-2007)	*A. indica* *P. longifolia* *A. nilotica* *M. indica*	*F. flavus* -- -- -- -- *F. moniliforme*	2

47	Shree Vishnu saw mill, C. M.C.India, odhav road, odhav, Ahmedabad (7-7-2007)	*A. indica* *P. longifolia* *A. nilotica* *M. indica* *E. globulus*	*T. viride* -- -- -- --	1
48	P. K. Patel and Co, Near maruthi popular wheeler, Narol- ishanpur high way, Ishanpur, Ahmedabad-382443 (10-7-2007)	*T. grandis* *A. arbica*	-- --	
49	Shree Krishna saw mill, Near Reliance petrol pump, Narol-Ishanpur high way, Ishan pur, Ahmedabad- 382443 (10-7-2007)	*T. grandis* Valsad teak (*T. grandis*) Gana teak " *Kleinhuvia hospita*	-- *A. niger* -- --	1
50	Shree Ashok wood industries, Narol-Ishanpur high way, Ishan pur, Ahmedabad- 382443 (10-7-2007)	Malaysia teak (*T. grandis*) Gana teak " Valsad teak " *A. nilotica* *D. pinnata* *A. indica* *M. indica*	-- -- -- *A. flavus* *S. commune* -- --	2
51	M/S. V. K. Patel and Co., Rajbai pateal timber market, Opp mahalakshmi mill, Narol, Ahmedabad (10-7-2007)	*M. indica* *P. dulce* *E. globulus* *A. indica* *A. nilotica* *Pterocarpus marsupium*	*T. viride* -- -- *D. concentrica* -- --	2
52	Shree Ashok Trading and Co. Rajbai Patel timber market, opp mahalakshmi mill, Narol, Ahmedabad (15-7-2007)	*M. indica* *A. indica* *A. nilotica*	*X. polymorpha,* • *D. concentrica* • *A. niger* -- --	3
53	L. N. Pateal and Co. Rajbai patel timber market, opp mahalakshmi mill, Narol, Ahmedabad. (15-7-2007)	*D. pinnata* *A. indica* *A. nilotica*	*D. concentrica* *C. globosum* -- --	2
54	Shree Gajanan wood working, Opp. Rajbai patel timber market, Ishanpr high way, Narol post, Ahmedabad - 382405 (15-7-2007)	*T. grandis* *M. indica* *A. nilotica* *F. benghalensis* *P. dulce*	*S. commune* *F. moniliforme* -- -- -- --	2
55	M/S. Vinayaca saw mill, Ishanpur high way, Narol post, Ahmedabad-382405 (15-7-2007)	*T. grandis* *A. indica* *A. nilotica* *M. indica*	*L. sterioides* *F. flavus* *T. harzianum* -- --	3
56	Bhavani saw mill, Ishanpur high way, Narol post, Ahmedabad-382405 (15-7-2007)	African teak Valsad teak Malaysia teak	-- -- --	
57	Bhagwati Timber Merchant, Ishanpr high way, Narol post, Ahmedabad-382405 (15-7-2007)	*A. arabica* *P. dulce* *T. grandis* *P. pinnata* *A. indica* *M. indica*	*S. commune* -- -- -- *X. polymorpha* --	2

	Bharuch District			
58	Shree Shanker Vijay Saw mill, Kavi ring road, Jambusar District Baruch (13-7-2009)	*T.grandis* *A. cordifolia* *T. bellerica* *T. arjuna* *A. indica* *A. nilotica* *M. indica* *P. longifolia*	• *L. sterioides* • *Trametes hirsutum* • *S. commune* -- -- -- -- -- -- *X. polymorpha* -- -- *A. niger* *T. viride*	6
59	Lalith Timber Mart Kavi ring road, Jambusar District Baruch (13-7-2009)	*T. grandis* Valsad teak Nilgiri teak *S. contorta* *A. indica*	*S. commune* -- -- -- -- -- -- -- --	1
60	Shree Ashapura timber Kavi ring road, Jambusar District Baruch (13-7-2009)	*B. arudinacea* *P. longifolia* *T. grandis* Nilgiri teak "	-- -- *S. commune* *D. concentrica* -- --	2
61	K.K. Patel and Company Kavi ring road, Jambusar District Baruch (13-7-2009)	*M. indica* *A. indica,* *T. indica,* *E. globulus* Nilgiri teak (*T. grandis*)	-- -- -- -- -- -- -- -- -- --	
62	Patel A.Y. Lakadawala Mohamadpura Jumbusar Road Bharuch (13-7-2009)	*T. grandis,* Nilgiri teak (*T.grandis*) *B. arudinacea* *P. longifolia*	-- -- -- -- -- -- -- --	
63	Haji Abhdul Rahim Mahamadbhai Lakadawala Mohamadpura Jumbusar Road Bharuch (13-7-2009)	*T. grandis,* *T. crenulata* *A. cordifolia* *B. arudinacea* *Casuarina equisetifolia* Nilgiri teak (*T. grandis*)	-- -- -- -- -- -- *S. commune* -- -- -- --	1
64	RasulBhai HasamBhai Lakadawal Near Maszid, Mohamadpura, Jumbusar Road , Bharuch (13-7-2009)	*P. roxberghii* *B. arudinacea* *T. grandis* *A. cordifolia*	*F. flavus* -- -- -- -- *S. commune*	2
65	Janatha Sawmill Near Maszid, Mohamadpura Jumbusar Road Bharuch. (13-7-2009)	*P. rouxbergii* *B. arudinacea* *T. grandis* Nilgiri teak (*T. grandis*) Valsad teak "	-- -- -- -- -- -- -- -- -- --	
66	Gani Timber Mart Near Maszid, Mohamadpura. Jumbusar Road Bharuch (13-7-2009)	Valsad teak (*T. gandis*) *M. indica,* *A. indica* *P. roxberghii* *B. arudinacea*	-- -- *A. flavus* *S. commune* -- -- -- --	2
67	V.A. Traderes Near Maszid, Mohamadpura Jumbusar Road Bharuch (13-7-2009)	*T. grandis* Valsad teak (*T. grandis*) *M. indica* *A. indica* *P. dulce*	-- -- -- -- -- -- • *S. commune* • *X. feejeensis* *G. lucidum*	3

68	Patel Sawmill Near Maszid, Mohamadpura Jumbusar Road Bharuch (13-7-2009)	*T. grandis* *S. rubusta* *A. indica* *B.arudinacea* *A. nilotica* *P. juliflora*	-- -- *S. commune* -- *T. viride* --	2
69	Dhanlakshmi Sawmill High way road Bharuch (13-7-2009)	*T. grandis* *A. nilotica* *T. indica* *E. globulus* *P. juliflora*	-- -- *S. commune* -- --	1
		Rajkot District		
70	Gita Sawmill Outside Railway crossing, Gondal Road, Rajkot – 360004 (21-7-2009)	*T. grandis* *C. deodara* *S. rubusta* *Dryobalanops* sp. *S. contortus* *B. arundinacea* *M. indica* *A. indica* *E. globulus*	-- -- -- -- -- -- -- *S. commune* *F. flavus*	2
71	Raghuvir Sawmill Outside Railway crossing, Gondal Road, Rajkot – 360004 (21-7-2009)	*C. deodara* *S. rubusta* *E. globulus* *T. indica* *A. nilotica*	-- -- -- *S. commune* --	2
72	Balaji Timber Mart Opp Rajshree Bajaj Showroom, Near Malavia College, Gondal Raod, Rajkot -4 (21-7-2009)	*T. grandis* Valsad teak Nigiria teak Malasia teak *S. contortus* *Dryobalanops* sp. *C. deodara*	*S. commune* • *X. allantoidea* • *T. viride* -- -- -- -- --	3
73	Swasthik Tomber Mart Near Malavia College, Gondal Raod, Rajkot -4 (21-7-2009)	*T. grandis* Nilgiri teak *C. deodara* *Dryobalanops* sp. *P. lingifolia*	*S. commune* -- -- -- *Agaricus* sp.	2
74	Shankar Vijay timber mart Near Malavia College, Gondal Raod, Rajkot -4. (21-7-2009)	*T. grandis* *S. rubusta* *A. indica* *T. indica* *M. indica*	-- -- *S. commune* -- --	1
75	Silicon Timber Mart Gondal Raod, Rajkot -4. (20-7-2009)	*S. rubusta* *A. cordifolia* *T. indica* *M. indica*	-- -- -- --	
76	M/S N. Mehta and Co. timber mart Gondal Road Rajkot – 4 (21-7-2009)	*A. indica* *T. grandis* *Dryobalanops* sp. *S. contortus* *C. deodara* Valsad teak	*S. commune* *X. polymorpha* -- -- -- -- --	2
77	J.M. Patel Sawmill, Gondal Raod, Rajkot -4 (21-7-2009)	*T. grandis* Gana teak " Malasia teak " *S. rubusta* *Dryobalanops* sp.	-- -- -- -- --	

78	Jyothi Timber mart Gondal Raod, Rajkot -4 (21-7-2009)	*T. grandis* *S. rubusta* *M. indica* *A. indica* *P. dulce* *E. globulus*	-- -- -- *S. commune* -- --	1
79	Anjali Timber Mart Gondal Raod, Rajkot -4 (21-7-2009)	*A. indica* *M. indica* *T. indica* *B. aurundenacia* *P. longifolia*	-- *T. viride* *S. commune* -- --	2
80	Bagavan Timber Mart Gondal Road Rajkot -4 (21-7-2009)	Gana teak Malasia teak C.P. teak *D. sissoo* *S. rubusta* *Dryobalanops* sp. *S. contort*	-- -- -- -- -- -- --	
81	Hindhustan Timber Mart Gondal Road Rajkot -4 (21-7-2009)	*A. indica* *M. indica* *A. nilotica* *T. indica* *P. dulce* *E. globulus* *P. juliflora* *D. pinnata*	-- -- *F. flavus* *S. commune* -- -- -- --	2
82	Narayan Traderes Gondal Road Rajkot -4 (21-7-2009)	*P. pinnata* *Ficus benghalensis* *D. sisso* *S. rubusta* *T. grandis*	-- -- -- -- --	
83	D.K Patel and Co Gondal Road Rajkot – 4 (22-7-2009)	*T. grandis* *T. bellerica* *T. arjuna* *D. sissoo* *D. pinnata*	-- -- -- -- *S. commune*	1
84	Srilakshmi Sawmill Gondal Road Rajkot – 4 (22-7-2009)	*A. indica* *M. indica* *E. globulus* *B. aurundenacia* *P. longifolia*	*S. commune* *A. niger* -- -- --	2
85	Srimahalakshmi timber mart Gondal Road Rajkot – 4 (22-7-2009)	Barma teak (*T. grandis*) Malasia teak " C.P teak " Valsad teak " Nigria teak " *C. deodara* *Dryobalanops* sp.	-- -- -- *S. commune* *D. concentrica* *T. viride* --	3
86	Thrishul Timber Mart Gondal road Rajkot – 4 (22-7-2009)	*T. grandis* *S. rubusta* *C. deodara,* *S. contortus* *Dryobalanops* sp. *M. indica* *A. nilotica*	*S. commune* *L. sepiarum* -- -- -- -- --	2
87	Visal Timber Traders Gondal Road Rajkot – 4 (22-7-2009)	*A. nilotica* *M. indiaca* *P. juliflora* *D. sissoo*	-- -- -- *S. commune*	1

88	Maruthi Timber Mart Gondal Road, Rajkot – 4 (22-7-2009)	*T. grandis* Burma teak " C.P teak " *S. rubusta* *D. sissoo* *C. deodara* *S. contortus*	-- -- -- -- -- -- --	0
89	Ganesh timber marat Gondal Road Rajkot- 4 (22-7-2009)	*A. nilotica* *A. indica* *T. indica* *D. pinnata*	*A. flavus* *T. viride* -- --	2
90	Shankar Vijay Timber mart Gondal Road Rajkot- 4 (22-7-2009)	*T. grandis* Valsad teak (*T. grandis*) *C.deodara* *Dryobalanops* sp. *S. rubusta* *A. cordifolia*	*S. commune* -- -- -- -- --	1
		Jamnagar District		
91	Amar Sawmill Jamnagar (23-7-2009)	*T. grandis,* *A. indica* *T. arjuna* *S. rubusta* *P. longifolia*	-- • *S. commune* • *T. viride* -- -- *X. polymorpha*	3
92	Bajarang Sawmill Jamnagar (23-7-2009)	*T. grandis* *S. rubusta* *T. india* *D. pinnata,*	-- -- *S. commune* *D. concentrica*	2
93	Amarnath Sawmill Jamnagar (23-7-2009)	*T. grandis* C.P. teak (*T. gandis*) Valsad teak " Malasia teak "	*S. commune* *F. flavus* • *X. polymorpha* • *A. niger* --	4
94	Krishna Sawmill Jamnagar (23-7-2009)	*A. cordifolia* *D. sissoo* *T. grandis* C.P. teak (*T. grandis*) Valsad teak "	*S. commune* -- -- -- --	1

It is evident from the table 2 and histogram 1, that in 5 different districts of Gujarat, 119 samples from vadodara, 56 samples from Ahmedabad, 29 samples from Rajkot, 20 samples from Bharuch and 10 samples from Jamnagar were associated with timber decaying fungi. 29 species of timber deteriorating fungi were identified from vadaodara in which 13 belonged to group *Basidiomycota*, 8 belonged to group *Ascomycota* and Mitosporic fungi each. Timber deteriorating fungi identified from Ahmedabad, Rajkot, Bharuch, and Jamnagar were 14, 10, 10 and 6 species respectively.

Table 2: Showing occurrence of different wood deteriorating fungi in Saw mills of 5 different districts of Gujarat

			Occurrence of Fungal deteriogens		
Place	**Total No of Saw mills**	**Samples with Associated Fungi**	**Basidiomycota**	**Ascomycota**	**Mitosporic fungi**
Vadodara	**28**	**119**	**13**	**8**	**8**
Ahmedabad	**29**	**56**	**5**	**6**	**3**
Rajkot	**21**	**29**	**4**	**5**	**1**
Bharuch	**12**	**20**	**5**	**4**	**1**
Jamnagar	**4**	**10**	**2**	**3**	**1**

Histogram 1: Showing the occurrence of wood decay fungi and their groups in different sawmills of Gujarat

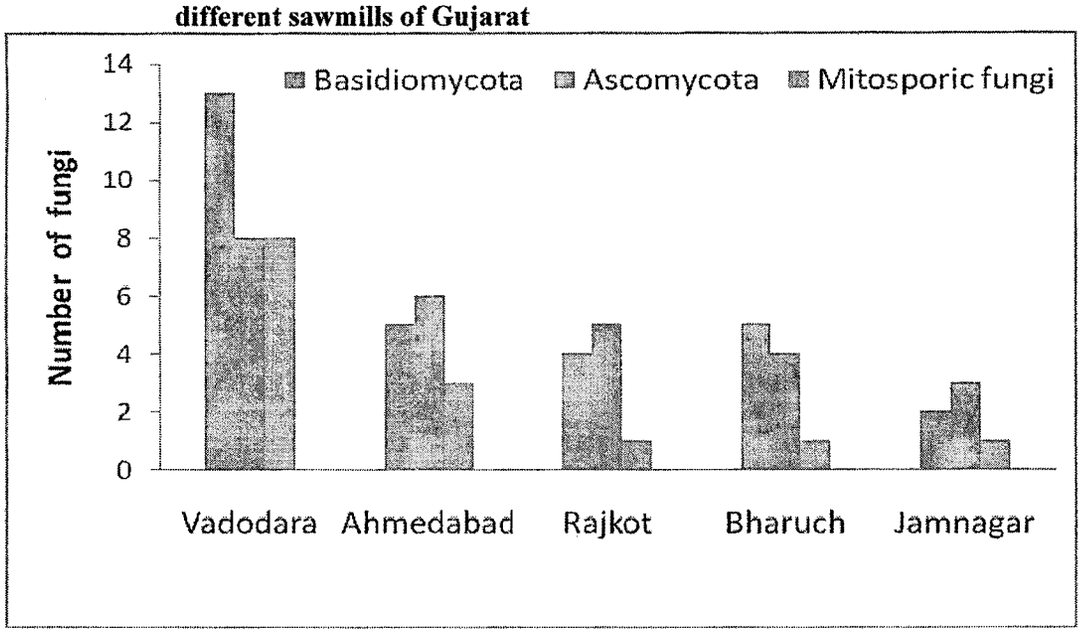


Table 3: Occurrence of different wood decay fungi in sawmills of Gujarat

S.No	Fungi	Group	Family
1	*Aspergillus flavus* Link.	Ascomycota	Trichocomaceae
2	*A. niger* van Tieghem	"	"
3	*Chaetomium globosum* Kunze.	"	Chaetomiaceae
4	*Daldinia concentrica* (Bolton) Cesati and de Notaris	"	Xylariaceae.
5	*D. sacchari* Dargan & Thind	"	"
6	*Hypoxylon rubignosa* Pers. ex. Fr.	"	"
7	*Xylaria feejeensis* (Berk.) Fr.	"	"
8	*X. polymorpha* (Pers.) Grev.	"	"
9	*X. allantoidea* (Berk.) Fr.,	"	"
10	*Agaricus*	Basidiomycota	Agaricaceae
11	*Auricularia aricula* (L.) Underwood	"	Auriculariaceae
12	*Coriolus versicolor* (L.ex Fr.) Quel.	"	Polyporaceae
13	*Daedaleopsis confragosa* (Fr.) Schroet.	"	"
14	*Flavodon flavus* (Klotzsch) Ryv.	"	Steccherinaceae:

15	*Ganoderma applanatum* (Pers. ex. Wallr.) Pat.	"	Ganodermataceae
16	*Ganoderma lucidum* (Fr.) Karsten.	"	"
17	*Lenzites betulina* (Fries.) Donk.	"	Polyporaceae
18	*L. sepiarum* (Wulfen) Fr.	"	"
19	*L. sterioides* (Fr.) Ryv.	"	"
20	*Phellinus badius* (Berk.: Cke) Cunn.	"	Hymenochaetaceae
21	*P. noxius* (Corner) Cunningham	"	"
22	*Trametes hirsutum* (Wulfen) Pilát,	"	Polyporaceae
23	*Sterium* hirsutum (Willd.) Pers.	"	Stereaceae
24	*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc	Mitosporic Fungi	Phyllachoraceae
25	*Fusarium moniliforme* J. Sheldon	"	Hypocreaceae
26	*F. pallidoroseum* (Cooke) Saccardo	"	"
27	*Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.	"	Botryosphaeriaceae.
28	*Nectria cinnabarina* (Tode) Fr.,	"	Nectriaceae
29	*Phomopsis salmalica* A.H. Khan	"	Valsaceae
30	*Trichoderma harzianum* Rifai.	"	Hypocreaceae
31	*T. viride* Pers.	"	"
32	*Trichoderma* sp.	"	"

1.2. Fungi Associated with Forest Trees

Forests cover about 3870 m ha of land, which accounts to 30% of earth (Sedjo 1990). India with a forest area of 76.5 m ha is one of the largest producers of forests products, worth INR 30,000 crores per annum (Oberai 2000). It has been established that wood rotting fungi particularly *Basidiomycetes* members damage forest wood even more than insects, marine animals or bacteria (Rauel and Bamoud, 1985). Fungi play an important role in the forest ecosystems. They are the principal decomposers of dead organic matter, such as dead wood and litter. Lignicolous fungi are described as fungi which develop on living or dead wood (Glava, 1996). Deadwoodology, the ecology of deadwood (Grove, 2002), is a thriving research held with wood-decaying fungi playing a major role in it. The majority of species belong to the phylum *Basidiomycota* (Glava, 1999). Wood-decaying fungi are excellent ecosystem engineers, because they directly modulate the availability of resources other than themselves for several other functional groups (Harley, 1971).

The biodegradation of cellulose and lignified cellulose reaches high levels and is responsible for the return of hundreds of billions of tons of CO_2 annually to the atmosphere and is a major biological component of the terrestrial Carbon Cycle (Hudson 1972). Decaying wood is a short-term sink but a long-term source of organic matter and

nutrients, a habitat of wide array of organisms and after humification it is an important component of forest soil. The massive fungal component is based largely on the role of fungi in two major biological systems: 1. as decay organisms of plant debris; 2. as mycorrhizal partners with trees and other plants (Barrongl 2003). Essentially, modern forestry needs to retain appropriate levels of deadwood in managed forests, ideally in all its forms and density levels, in order to cover the full spectrum of habitat conditions (Samuelsson *et al.,* 1994; Berg *et al.,* 2002; Christensen *et al.,* 2005) for the sake of dependent organisms and in order to achieve sustainability of timber production.

Survey of different forest areas was conducted in four different districts of Gujarat. In Vadodara the survey was undertaken in Sindhrot and Padra areas, In Panchmahal the survey was conducted in Shivrajpur near Pavagadh and Jambughoda Sanctuary in Dahod, Ratanmahal Wild Life Sanctuary area was surveyed and in Narmada district, wadia palace area in Rajpipla was visited to find out association of wood rotting fungi and diseases of forest trees. The fungi found in these 4 districts are listed in Table 4 and 5. The forest areas surveyed in four different districts of Gujarat revealed that 63 wood decaying fungi were found associated with the living trees, wood logs, fallen branches and dead trees. In Vadodara 40 wood decaying fungi were observed, representing *Zygomycota* (3), *Ascomycota* (8) *Basidiomycota* (24), and Mitosporic fungi (5). In Panchmahal 19 wood degrading fungi were observed, representing *Zygomycota* (2), *Ascomycota* (4), *Basidiomycota* (10), and Mitosporic fungi (3). In Dahod 40 wood decaying fungi were observed, representing 4 groups *Zygomycota* (4), *Ascomycota* (8) *Basidiomycota* (19) and Mitosporic fungi (9). In Narmada 42 timber decaying fungi were observed, which represented 4 groups *Zygomycota* (3), *Ascomycota* (7), Mitosporic fungi (7) and *Basidiomycota* (25).

The occurrence and distribution of different Aphyllophoroid Basidiomycetes depends on the type of wood, availability of moisture, temperature, and sun light. The fine scale ecological determinants were investigated and statistically analyzed on woody debris collected from Switzerland and Ukraine (Kuffer *et al.,* 2008), studies have been conducted on *Picea abies* forest (Edman, and Jonsson, 2001), Eucalyptus (Fryar *et al.,* 1999), temperate forest plants (Yamashita, 2010), hazel trees (Norden and Platto 2001). The correlation between species richness of wood decay fungi, stand age, dead wood features have been investigated by Norden and Platto, (2001) in hazel trees in South East Sweden. Distribution of fungal species in deciduous and coniferous trees depends on the type of rot and type of wood present. In the present study different species of wood

rotting fungi were observed in four forest areas of Gujarat i.e. Vadodara, Panchmahal, Dahod and Narmada districts revealed 63 fungi in which 39 members of Aphyllophorales were lignolytic. In a study over 600 species of white rot fungi have been found to be lignolytic after the breakdown of lignin CO_2 may be released in the atmosphere (Dishanth and Rajender, 2006).

Table 4: Fungi found associated with different trees in 4 different districts

S.No	Fungi	Vadodara	Panchmahal	Dahod	Narmda
Zygomycota					
1	*Absidia* van Tieghem	+	+	+	+
2	*Mucor* sp.	+	--	+	+
3	*Rhizopus stolonifer* (Ehrenb.) Vuill	+	+	+	+
4	*Rhizopus* sp.	--	--	+	--
Ascomycota					
5	*Aspergillus* sp.	--	--	+	--
6	*A. awamori* Nakazava.	+		+	+
7	*A. flavus* Link.	+	--	+	+
8	*A. fumigatus* Fresen.	--	--	+	--
9	*A. niger* van Tieghem	+	+	+	+
10	*C. globosum* Kunze	+	--	+	+
11	*D. concentrica* (Bolton) Cesati and de Notaris	+	+	--	+
12	*Helicocephalum* sp.	--	+	+	+
13	*H. rubignosa* Pers. ex Fr.	+	--	+	+
14	*Xylaria polymorpha* (Pers.) Grev.	+	--	--	--
15	*X. feejeensis* (Berk.) Fr.	+	+	--	--
Basidiomycota					
16	*Auricularia auricula* (L.) Underwood	+	--	+	--
17	*A. polytricha* (Montagne) Sacc.	+	--	--	--
18	*Aurificaria indica var. indica*	+	--	--	--
19	*Bondarzewia berkleyii* (Fr.) Sing.	+	+	+	+
20	*Coriolopsis aspera* (Junghuhn) Teng.	--	--	+	+
21	*C. telfari* (Kl.) Ryv.	+	--	--	--
22	*Coriolus versicolor* (L.ex Fr.) Quel.	--	--	+	+
23	*Daedaleopsis confragosa* (Fr.) Schroet.	+	+	+	--
24	*Flavadon flavus* (Klotzsch) Ryv.	+	+	+	+
25	*Fomitopsis cupreorosea* (Berkeley) J. Carranza & Gilbertson	--	--	+	+
26	*Ganoderma aplanatum* (Pers. ex Wallr.) Pat.	+	+	--	--
27	*G. colossum* (Fr.) Bers	+	--	--	--
28	*G. curtisii* (Berk.) Murill	--	--	+	+
29	*G.lucidum* (Fr.) Karsten.	+	+	+	+

30	*Gloeophyllum sepiarium* (Fr.) Karst.	--	--	--	+
31	*Hexagonia apiaria* (Pers.) Fr.	+	+	+	+
32	*H. tennuis* Hooker ex Fries	+	--	+	+
33	*Lenzites betulina* (Fries.) Donk.	+	--	--	--
34	*L. sterioides* (Fr.) Ryv.	+	--	+	+
35	*Microporus affinis var. glabriceps*	--	--	+	--
34	*M. alboater* (Hennings) Kuntze	--	--	+	+
36	*Navisporus floccosa* (Bresadola.) Ryv.	+	+	--	+
37	*Oxyporus ravidus* (Fr.) Bond. and Sing.	--	--	+	--
38	*Phellinus badius* (Berk.: Cke) Cunn.	--	--	--	+
39	*P. caryophyllii* (Racib.) Cunn.	+	--	--	--
40	*P. conchatus* (Pers. : Fr.) Quel.	--	--	--	+
41	*P. gilvus* (Schw., Fr.) Pat.	+	--	+	+
42	*P. hoehnelii* (Bres.) Ryv.	--	--	--	+
43	*P. nilgheriensis* (Mont.) Cunn.	+	--	--	--
44	*P. pachyphloeus* (Pat.) Pat.	+	--	--	--
45	*P. pectinatus* (Kl.) Quel.	--	--	--	+
46	*P. rhabarbarinus* (Berk.) Cunn.	+	--	--	--
47	*P. robustus* (Karst.) Bourd. & Galz.	+	--	--	+
48	*P. setulosus* (Llyod) Imaz.	+	--	--	+
49	*P. shaferi* (Murrill) Ryv.	--	--	--	+
50	*Plyporus violaceo-cinerescens* Petch.	--	+	+	+
51	*P. xanthopus* Fr.	--	+	+	+
52	*Schizophyllum commune* Fr.	+	+	+	+
53	*Trametes gibbosa* (Pers.) Fr.	+	--	--	--
54	*T. lactinea* (Berk.) Pat.	--	--	--	+
Mitosporic Fungi					
55	*Fusarium oxysporum* Schlecht.	+	--	+	--
56	*F. pallidoroseum* (Cooke) Saccardo	--	+	+	+
57	*Cladosporium herbarum* (Pers.) Link.	--	--	+	+
58	*Pestalotiopsis glandicola* (Castagne) Steyaert.	--	--	+	+
59	*Phoma multirostrata* (Mathur et al.) Dorenbosch & Boerema.	+	+	+	+
60	*Theliviopsis* state of *Ceratocystis paradoxa* (Dade) C. Moreau	+	--	+	+
61	*Trichoderma harzianum* Rifai.	+	--	+	+
62	*T. viride* Pers.	+	+	+	+
63	*Trichoderma sp.*	--	--	+	--

+ shows: presence, -- =absence

2. Morphological and Anatomical characters of Basidicarps and Ascocarps

The Vedas (1200BC) may contain the earlier records of fungi particularly the diseases and their control but Linnaeus was probably the first to name a mushroom from India in the 18th centuary. Linnaeus in his "Systema Plantarum" 1753 listed only one genus i.e. Boletus and put all 12 species bearing pores or tubes (Boletaceae and Polyporaceae) under it. *Hexagonia sinensis* Fr. and *Polyporus wightii* Kl. Were probably the earliest records of polypore fungi by Klotzsch (1832) and Berkely (1839) respectively from the collection of Wight. Though we refer occasionally to the articles of Bulliard (Herbier de la France, 1780, 1793), Schaeffer (Fung. Bav. 1780) and Sowerby (Eng. Fung. 1797-1809) but Persoon was first who made an attempt worthy of consideration in a series of papers "Observations Mycologicae" (1795-99) leading to his monumental work "Synopsis Methodica Fungorum" in 1801. All 96 species of poroid fungi three genera were treated under the class Gymnocarpii and order Hymenothecii. The taxonomic study of Indian polypores was initiated by European Scientists towards the middle of the nineteenth centuary. The earliest repots seems to be that of Klotzch (1832,1833). A large number of polypores was reported by Berkeley (1839,1850,1851,1854,1866,1872) based on his study of Dr. Hooker's extensive collection of macrofungi from Sikkim-Himalayas. Other early reports of Indian polypores include those of Montagne (1842), Leveille, (1844), Cook (1876,1881,1891a,b,c), Massee, (1901), Hennings, (1900, 1901), Lloyd, (1898-1925), Butler, (1903, 1909,1918), Theissen (1911), Blatter, (1911) and Murrill, (1924).

Bose, of Carmichael Medical College, Calcutta, was recorded a large number of fungi from Bengal (Bose, 1919-1928) and Assam (Bose, 1937a). From his wide experience with polypores Bose (1944) suggested the use of certain characteristic anatomical features in addition to the characters of basidia and spores for the specific identification of these fungi. The work on polypores of Bengal was continued by Banerjee and co-workers (Agarka and Banerjee, 1933; and Banerjee and Chakravarthy, 1945). Some of their contributions are on polypores of Sikkim- Himalayas (Banerjee 1946, Banerjee and Ghosh 1945) and devising a simple method for producing typical sporophores of *Polystictus sanguineus* (Banerjee and Sinha 1955). Some of the sporadic report on Indain polypores are those of Hanifkhan, (1910), Shersingh, (1924), Mitter and Tandon (1932, 1938), Uppal *et al.*, (1935), Vahid, (1938) and Roy, (1953).

Bagchee and Bakshi from FRI, Dehradun studied extensively the polypores causing diseases of oaks and other economically important forest trees (Bagchee, 1950; Bagchee and Bakshi 1950,1951). Some of the important contributions of Bakshi and coworkers are their new reports of Polypores (Bakshi, 1956,1965) decay of conifers (Bakshi, 1955) Thind and coworkers from Punjab University have reported several Polypores including some new species from western Himalayan and Mussoorie Hills (Thind and Chatrath 1957, 1960). Significant contribution to anatomical studies of several Polypores has been made by Roy, (1968 a, b, 1969) more recently identification of polypores in culture has been undertaken by Bakshi *et al.,* (1969, 1970) and Sen, (1973)

A. Studies on Basidiomycetous Members

1. *Auricularia auricula* (L.) Underwood.

Memoirs of the Torrey Botanical Club 12: 15, 1902

BASIONYM: *Tremella auricula* Linnaeus 1753, *Peziza auricula* L. 1767.

Merulius auricula Roth. 1788. *Exidia auricula-judae* Persoon 1801

The basidiocarps are fleshy, at first peziza-form, then becoming erect and foliaceous, much twisted, ear- shaped, several lobed, sessile, gathered together and attached at a central point, up to 12 cm in height; thin gelatinous, sterile surface curling over hymenium, red-brown when moist. Zona pilosa: Hairs 85-100 µ long, 5-6 µm in diameter, hyaline, without central strand, rounded at tips, not in dense tufts. Zona compacta: 65-75 µm wide, hyphae densely compacted, individual elements not distinguishable. Zona subcompacta supcrioris: 115-130 µm wide, hyphae about 2 µm in diameter, forming a dense network giving the zone a somewhat coarsely granular appearance. Zona intermedia: 285-300 µm wide, hyphae 1.5-2 µm in diameter. Zona subcompacta inferioris: 100-120 µm wide, hyphae about 2.5 µm in diameter. hymenium when moist reddish brown like coffee jelly, smooth, undulating; hymenium when dry almost black, shining, sometimes folded according to manner of drying; Basidiocarps are made up of dikaryotic hyphae having clamp connections. Basidia cylindrical, 3-septate, up to 80 x 8 µm. Basidiospores typical of the genus, oval and curved, 12.5 - I4.68 X 6.12 µm It was found on dead wood of various kinds of woods in different parts of the world (Barrett, 1910). But in present study it was found on trees of *Kleinhuvia hospita, Dalbergia sissoo, Feronia elephantum* and *Pithacellobium dulce.*

Collection examined: Arboratum of Botany department M.S. University Baroda, and Shivrajpur village of Gujarat, collected by N. Praveen Kumar, Accession No: MSU Bot.108, 109, 6-7-2008 and 9-9-2008.

2. *Auricularia polytricha* (Montagne) Sacc.

Atti Ist. Veneto Sci. Lett. Arti, 6 3: 722, 1885,

Basionym: *Exidia polytricha* Montagne1834, *Hirneola polytricha* (Mont.) Fries, 1849. *Hirneola hispidula* Berk. 1874., *Auricula nigra* (Fries) Kuntze, 1891. *Auricula polytricha* (Mont.) Kuntze, 1891., *Auricularia hispidula* (Berk.) Farlow, 1905.

Basidiocarps frequently were having a strongly convex dorsal surface, pileus, largest specimen 5-6 cm. broad, 1-1.5 mm thick. Zona pilosa: Hairs about 450 µm long, hyaline, 5-6.25 µm in diameter, forming dense tufts, with a prominent central strand, tips pointed but frequently broken, appearing truncate when viewed micro- scopically. Zona compacta: 20.56 - 25.89 µm wide, densely compacted, individual hyphae not distinguishable. Zona subcompacta superioris: 75.56 – 85.67 µm wide, hyphae 2-3.12 µm in diameter, oriented mostly perpendicular with the surface. Medulla: About 250 µ wide, hyphae 3.12-6.25 µm in diameter, oriented mostly parallel with the surface. Zona subcompacta inferioris: 90-100 µm wide, hyphae 2-3.12 µm in diameter. Hymenium: 80-90 µm wide; basidia cylindrical, 50-60 X 3.12 -6.25 µm; spores 12.25-15.68 X 5-6.25 µm. It was growing abundantly on dead trunk and branches of *Mangifera indica*, *Polyalthia longifolia* and *Ficus benghalensis*

Collection examined: Botanical Garden, Near D. N. Hall ground Vadodara, Gujarat, collected by Dr. Arun Arya and N. Praveen Kumar, Accession no: MSU Bot.105, 106, 107, 8-7- 2008, 9-9-2008

3. *Aurificaria indica* (Massee) Reid **var. *indica***

Basidiocap annual, stiptiate, attached with 2 – 3 sporophores like a rosette, funnel shaped, thin leathery, pealing off cuticle present in mature sporophores, upper surface is yellow in fresh with black cuticle in the center changed to brown cuticle covering in dry samples. Found on the ground attached to roots of *Leucina leucocephala*, 15.3 – 18.6 cm long, 14 – 18 cm wide, 0.5 – 1 cm thick, margin entire, wavy (Plate I Fig. A). Hymenium golden brown to blackish brown, pores visible to naked eye, angular, 2 - 4 per mm, tubes separated, stalk is centrally present, golden brown to dark brown, 5 - 7.4 cm long, 2.5 – 7 cm width, 1 – 3 cm thick (Plate I Fig. B). Context: brown, black line present, turning black in KOH, Hyphal system monomitic, generative hyphae thin to thick-walled,

septate, branched, rusty brown, 6.3 - 2 µm, basidia pale yellow, clavate, thin walled 16.8 x 3.12 µm. Basidiospores subglobose, hyaline, thin-walled, smooth, 6.1 x 3.12 µm.

Collection examined: Arboratum, Botany Department, MSU, Baroda, (Gujarat); collected by N. Praveen Kumar, Accession no: MSU Bot 80. 8-8-2008

Aurificaria indica var. *indica* differ from *A. indica* by large centrally stiptiate rosette like cluster of pilei up to 20 cm diam. Thin, brown cuticle, concentric zones of different coloures, sulcate ridges, pores 2 - 4 per mm, long stalk up to 8 cm in diameter, context with black line, grayish brown pore layer, fruiting bodies made uo on with thick-walled generative hyphae. Ryvarden and Johansen (1980) reported on dead and living deciduous trees from Asia, Malaya and in Africa found in Kenya

4. *Bondarzewia berkleyii* (Fr.) Bondartsev and Singer

Ann. Mycol. 39: 47, 1941

BASIONYM: *Polyporus berkeleyi* Fries 1851

Sporophore annual, sessile, imbricate, fleshy, watery when fresh, becomes some what rigid on drying, and shrinking, size was 11 x 7.1 x 1 cm. Upper surface light yellow, the surface becomes dark yellow brown due to heavy deposition of spores from fruit bodies situated above when growing in imbricate clusters, glabrous, margin rounded, unequal, thick; context cream colour, soft fleshy, watery when fresh with silky shine with few zonations 2- 3 cm thick; hymenial surface mustared yellow towards margin, pores shallow, angular, unequal, 3 per mm, hyphal system dimitic, basidia persistant, narrow club shaped, 6.32 µm broad; basidiospores yellow, ovoid, slightly thick walled, apiculate 3.25 – 6.31 X 3.12 µm. Earlier it was found on the ground from buried wood or roots, more rarely on the stumps, both in conifer and deciduous forests. In Europe from France and through Central Europe to USSR (Caucasus), North America, Japan, Columbia and Sri Lanka (Ryvarden and Johansen 1980). But in the present study it is found on the roots of living and dead bamboo.

Collection examined: RWLS, Ratanmahal, Arboratum of Botany department Baroda Gujarat. collected by Dr. Arun Arya and N. Praveen Kumar, Accession No: MSU Bot. 110, 6-7-2006 and 7-7-2008

New and Interesting Records of Basidiomycetous Fungi from Ratanmahal Wildlife Sanctuary, Gujarat, India

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Abstract

A survey was undertaken during 2005-2006 to detect the various wood deteriorating fungi in Ratanmahal wildlife sanctuary, situated in southeastern part of the Panchmahal district of Gujarat. We describe on the occurrence of *Lenzites sterioides* (Fr.) Ryv. on *Tectona grandis*, *Hexagonia apiaria* Pers. and *H. tenuis* Hooker ex Fries on twigs of *Mangifera indica*. *Phellinus nilgheriensis* (Mont.) Cunn. was found at the base of living tree *Ailanthus excelsa*, another sporophore of same fungus was found attached to *Anthocephalus cadamba*. *Navisporus floccosa* (Bres.) Ryv. and *Coriolopsis aspera* (Jungh.) Teng was found on branches of *Mitragyna parviflora*. *Trametes gibbosa* (Pers.) Fr. and *T. lactinea* (Berk.) Pat. were found associated with branches of *Tamarindus indica* and *Alangium salvifolium* respectively. *Lenzites sterioides* is 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.

Key words: Interesting records, Basidiomycetous fungi, Ratanmahal wildlife sanctuary, Gujarat

Citation: Arya A, Albert S and Nagadesi P K 2008. New and interesting records of basidiomycetous fungi from Ratanmahal wildlife sanctuary, Gujarat, India. *J Mycol Pl Pathol* 38(2):221-226.

The lignicolous Basidiomycetous members are ranked among the most harmful forest pathogens. From an ecological point of view, however, this fungal group plays an extremely important role as a decomposer in the forest ecosystems. The number of taxa of Basidiomycetes able to utilize components of cell walls of wood as their main source of energy is essentially unknown, but probably several thousand is a good estimate for North America and Europe (Fischer and Wagner 1999). It is interesting to note that history of Aphyllophorales is quite old in India. Important contributors are Bose, Sundararaman and Marudarajan, Bakshi and Thind among others.

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 of mycoflora in Himalayas. He put forth the tissue concept for Indian species of polypore as proposed by Corner. Bakshi (1971) also reported *Polyporus luteo-umbrinus* Romell on sground attached to buried wood or root and dead fallen *Heritiera minor* in Baroda, Gujarat. 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.

Materials and Methods

Topography and material collection. Ratanmahal Wildlife Sanctuary is a relatively small area of 55.65 km^2 consisting of dry deciduous forest. The total existing sanctuary area lies between the river Panam and Orsang. The 11 villages of Ratanmahal forest are situated at the southernmost part of Limkheda taluka of Dahod district of Gujarat state. Ratanmahal lies nearly 35 km south-east from Devgadh Baria, the head quarter of Baria taluka. It is situated between 70° 37' to 74° 11' E Long and between 22° 32' to 22° 35' N Lat.

The forest of the area was part of Kanjeta state. It is bounded by Jabhua district of Madhya Pradesh. on its south-eastern side and Devgadh Baria on north-western side. The climate is sub-tropical arid, which turns damp and humid during monsoon. Rainfall ranges between 957 to 2101 mm. A survey was undertaken in the santuary between Dec 2005 to Oct 2006 and basidiocarps of different fungi were collected and new and interesting records that include a member of Hymenochaetaceae and seven of Polyporaceae are described.

Materials were collected in clean polythene bags from different locations and brought to the laboratory. Basidiomes were studied using macroscopic (eg. size, colour, number of pores/mm, length of tubes) and microscopic (presence or absence of structures, dimensions, vegetative and reproductive characters (Ryvarden 1991). To observe basidia and setae, free hand sections were taken. For the clear observation of setae, trammel setae and setal hyphae, lacto phenol cotton blue was used as staining and mounting medium. Xanthochoric reaction was also tested using potassium hydroxide solution. The various details of specimens were compared with Hymenochaetaceae of India (Sharma 1995), Indian Polyporaceae (Bakshi 1971), CBS Aphyllophorales database, New Zealand Fungi database, and *Species Fungorum*. Certain specimens were sent to the Royal Botanical Garden, Kew for final confirmation. These fungi are kept in fungal collection of Botany Department, MS University of Baroda, India.

Results and Discussion

***Lenzites sterioides* (Fr.) Ryvarden 1972, *Norweg J Bil* 19:232.** Synonyms: *Daedalea sterioides* Fr. 1851 *D. Maculata* Lloyd 1922; *Cerrena sterioides* (Fr.) Murrill 1908; *Striglia sterioides* (Fr.) Kuntze 1891; *Irepex durescens* Cooke 1881.

Annual sporophores found attached to the branches of *Tectona grandis* L. sporophores sessile, fan shaped, 12 x 6 – 7.5 x 0.9 cm; upper surface white to buff, zonate (Fig 1a), context yellowish or buff coloured, soft, corky upto 4 mm broad; hymenial surface pinkish or yellowish. Pore layer 4 - 6 mm, lamellae (forming gills) (Fig. 1b), lamellae regular, close. Basidia clavate basidiospores are hyaline, thin walled, cylindrical to ellipsoidal 3.5 – 5 x 1.8 - 2.2 µm. Fruiting body is dimitic: binding hyphae are hyaline, thick walled, unbranched or rarely branched, aseptate 2-4 µm broad; and generative hyphae are thin walled, hyaline, septate with clamp connection, 1.5- 3µm broad. This fungus has been earlier reported from the Shoolpaneshwar wild life sanctuary (Arya 2004).

Collection examined. Ratanmahal wildlife sanctuary, (Gujarat); collected by Mr. N. Praveen Kumar; Accession No: MSU Bot. 51; 7-10-2006.

***Hexagonia tenuis* Hooker ex Fries 1836, *Epicrisis Systematis Mycologici* : 497.**

Synonyms: *Boletus tenuis* Hooker ex Kuntz 1822; *Daedaleopsis tenuis* (Hook ex Fr.) Lmazeki

Basidiocarps (Fig.1c), circular present on dried twigs of *Mangifera indica*. Sporophores resupinate, semicircular to circular, context dark brown, less than 1mm thick, hymenial surface reddish to black, pores hexagonal sometimes round,1- 4 per mm; basidia hyaline, clavate. Basidiospores cylindrical, 8.8 x 2.2 - 4.4 µm in size. Hyphal system trimitic.

Collection examined. Ratanmahal wildlife sanctuary (Gujarat); collected by Prof. Arun Arya; Accession No: MSU Bot. 57; 6-12-2005

***Hexagonia apiaria* (Pers.) Fr. 1838, *Epicrisis Systematis Mycologici* : 498.** Basyonym: *Polyporus apiarius* Persoon 1827.

Fruit body annual, solitary, sessile with smooth margins, 2.8 x 1.1 cm and 4 mm thick, corky. Pileus circular, light brown to dark cinnamon coloured. Pores hexagonal (Fig. 1d), larger in the centre. Hyphal system trimitic, generative hyphae hyaline, septate and with clamp connections, 1.5 - 2.2 µm wide. Binding hyphae thick walled, skeletal hyphae dominating in the basidiocarp, yellowish to pale rusty brown, unbranched. Basidia clavate, cystidiod hyphae projecting into hymenium. Basidiospores not seen. Found on fallen branches of *M. indica*.

Collection examined. Ratanmahal wildlife sanctuary and Arboratum of Botany Department, Baroda (Gujarat); Collected by Prof. Arun Arya and Mr. N. Praveen Kumar; Accession No: MSU Bot. 56 and 58; 6-12-2005 and 7-10-2006.

The fungus has been reported from fallen angiospermous trunk of *Tectona grandis* from the forest of Tirunelveli district, Tamil Nadu and Mundanthurai Sanctuary (Ryvarden and Johansen, 1980). According to Bakshi (1971) the basidiospores were not found in this species while Ryvarden and Johansen (1980) reported occurrence of basidiospores on this member of Aphyllophorale.

***Phellinus nilgheriensis* (Mont.) Cunn. 1965, *Bull New Zealand Dept Sci Industr Res* 164: 226.**

Sporophores of *P. nilgheriensis* (Fig. 1e) were found attached to the base of large trunk of *Ailanthus excelsa*. The fruiting body is perennial, large 46.5 long x 30.5 cm broad with 5 cm thickness, sessile, yellow to light brown in colour. Top of context was smooth. Hyphal structure was dimitic. Tube layer 5 – 10 mm deep, minute pores are present on lower surface. Basidiospores are 4 – 7 µm, ovoid in shape.

Collection examined. Arboratum of Botany Department, Baroda, (Gujarat); Collected by Mr. N. Praveen Kumar; Accession No: MSU Bot. 61; 25-11-2006.

Figures 1. a = Basidiocarp of *Lenzites sterioides* showing rings (x 0.4); b = ventral view of *Lenzites* showing formation of gills (x 0.4); c = basidiocarps of *Hexagonia tenuis* (x 0.17); d = sporophores of *H. apiaria* showing hexagonal pores (x 0.75); e = sporophore of *Phellinus nilgheriensis* (x 0.15); f = circular yellow coloured sporophore of *P. nilgheriensis* (x 0.2)

Another sporophore of *P. nilgheriensis* was found attached to the stem of living tree of *Anthocephalus cadamba*. Perennial fruiting body was large, almost circular structure, 38 – 45 cm in diameter 5 – 7 cm thick, the body was attached to stem with a short stalk. The fruiting body was yellow in colour (Fig. 1f). and light in weight.

Collection examined. Arboratum of Botany Department, Baroda, (Gujarat); Collected by Prof. Arun Arya; Accession No: MSU Bot. 62; 25-12-2006

Phellinus is a member of Hymenochaetaceae of Aphyllophorales. This wood rotting fungus has been known as *Polyporus* (Donk 1960) and *Fomes* (Lowe 1957) in earlier texts. Sharma (1995) remarked that *P. nilgheriensis* occurs on dead angiospermic wood, causing a white stringy rot, a rare species found from tropical to temperate forests. Sporophores are woody, perennial, and dimitic. Tubes are lined with hymenial layer. The fungus causes roting of a number of commercial plants such as rubber, tea and coffee in tropical countries (Hodges 1984; Nandris et al 1987). Reid and Dickson (1980) reported *Phellinus robustus* (P. Karst) Bourd and Galz. growing on *Quercus robur* in Britain. Blanchette (1980) studied wood decomposition by *Phellinus (Fomes) pini*. Nicole et al (1995) reported ultrastructure and cytochemical investigation of *P. noxius* in wood chips of *Betula papyrifera*. They reported that *P. noxius* lacks rhizomorphs and thus spreads as ectotrophic mycelium across root contacts.

A large number of *Phellinus* spp. are reported on different trees in various parts of the country. *P. ribis* was present on *Quercus incana, Jakaranda mimosa* and *Lantana* spp. and *P. robusta* on *Abies* sp. (Thind and Rattan 1971). Roy (1979) found *P. durissimus* growing on the base of *Swietenia mohogani, Casuarina equisetifolia* and *Mimusops elengi*. Thind and Dhanda (1980) found sporophore of *P. cereus* on *Dalbergia sissoo*.

***Trametes gibbosa* (Pers.) Fr. 1838, *Epicrisis Systematis Mycologici*:492.** Synonym: *Merulius gibbosus* (Pers.) Fr.

Basidiocarp is annual, tough, white rotting, shelf or hoof shaped (Fig. 2a, 16.9 x 9.4 x 0.9 cm thick, concentrically zoned, smooth polypore. Pores small (Fig. 2b), fusiform simple cystidia present, spores ellipsoid to cylindrical, colourless, 7 – 9 µm in length.

Collection examined. Botanical Garden The M S University of Baroda, (Gujarat); Collected by Prof. Arun Arya; Accession No: MSU Bot. 76; 7- 3-2005.

***Navisporus floccosa* (Bresadola) Ryv. 1980, *Preliminary Polypore Flora of East Africa*, Fungiflora Oslo, Norway, p 636.** Basionyms: *Trametes floccosa* Bres 1896; *Fomes introstuppeus* Hennings 1892; *Polyporus farinosus* Lloyd

The sporophore (Fig. 2c) are annual, sessile, light in weight, 10 – 15 cm x 5 – 8 cm x 0.5 – 1 cm context yellow to light brown, pore irregular 2 – 4 per mm, sporophores are dimitic, soft in texture, hyphae are amyloid, spores are ellipsoid.

Collection examined. Kavadia colony, Narmada District and Arboratum of Botany Department, Baroda (Gujarat); Collected by N. Praveen Kumar and Prof. Arun Arya; Accession No: MSU Bot. 66 and 67; 5-9-2005 and 25-11- 2006.

The fruiting bodies were sent to Royal Botanical Gardens, Kew, England and are identified as *Navisporus floccosa* (Bres.) Ryv. This is new report for India (Bilgrami et al 1979, 1981 and Jamaluddin et al 2004). Earlier the fungus is reported from east Africa (Ryvarden 1980).

***Coriolopsis aspera* (Junghuhn) Teng. 1963, Chung-Kuo Ti Chen-Chun : 759.** Basionym : *Trametes aspera Polyporus asper* Jungh

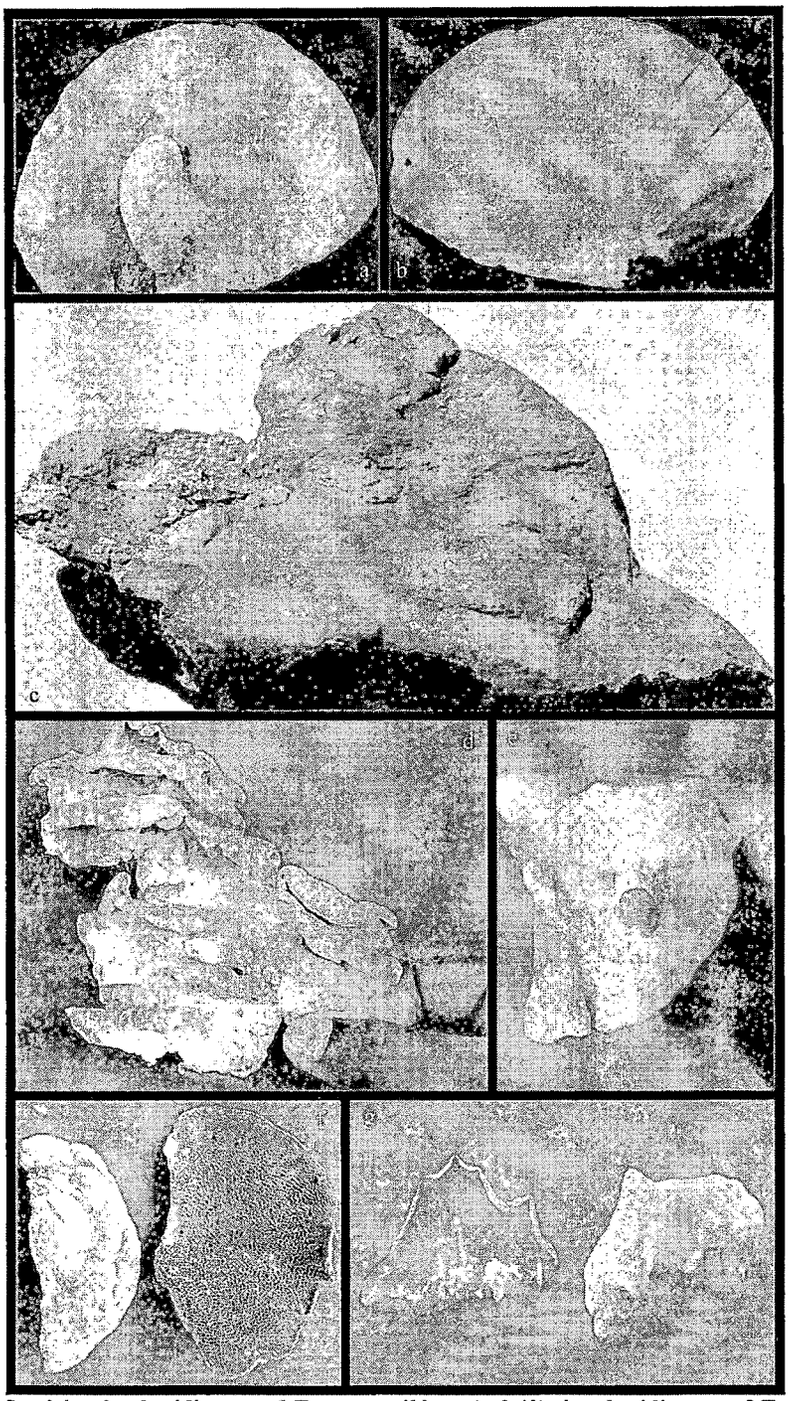
Fruit body (Fig. 3d) annual, imbricate forming a cluster on branches of *Mitragyna parviflora* (Fig. 2d). Basidiocarp 6cm long and 5.5 cm wide 0.5 cm thick. Yellowish to brown in colour, upper pileus hairy, margin rounded but not completely smooth, it is deflexed. Pore surface darker than context, pore round 3-4 per mm, fungal tissue turning black in KOH. Body shows very light coloured growth zones (Fig. 2e). Hyphal system trimitic, binding hyphae irregular in outline, strongly branched. Spores cylindrical 9 – 10 x 3 - 4.4 µm, hyaline, thin walled and non-amyloid.

Collection examined. Ratanmahal wildlife sanctuary, (Gujarat); Collected by Prof. Arun Arya; Accession No: MSU Bot. 71; 6-12-2005.

According to Tomsovsky et al (2006) the genus *Coriolopsis* is different from *Trametes* based on nuclear ribosomal DNA studies.

***Trametes lactinea* (Berk.) Pat.1900, *Essai tax Hym* : 92.** Synonyms: *Trametes levis* Berk. *T. moritziana* Lev.; *T. hololenca* Cooke; *T. mulleri* Berk

The fungus produces white rot in hard woods. Sporophores are sessile, applanate, simple or imbricate, semicircular, hard, 5.8 x 4.5 cm. the thickness of sporocarp is 0.6 - 0.8 cm.



Figures:2. a = Semicircular basidiocarp of *Trametes gibbosa* (x 0.43); b = basidiocarp of *Trametes* showing pore surface (x 0.43); c = sporophore of *Navisporus floccose* (x 0.3); d = basidiocarps of *Coriolopsis aspera* in cluster (x 0.81); e = single basidiocarp showing zonation on dorsal surface of *Coriolopsis* (x 0.92); f = dorsal and ventral views of *Trametes lactinea* (x 0.94); g = basidiocarps of *C. aspera* (x 0.54)

Surface is matted tomentose (Fig. 2f), context yellow or ochraceous, pores rounded 2 - 3 per mm. basidiospores 4 - 7.5 x 2.2 – 3 µm. The basidiocarps are trimitic.

Collection examined. Kavadia colony Narmada District (Gujarat); Collected by Prof. Arun Arya; Accession No: MSU Bot. 77; 5-9- 2006.

The fungus is common on dead hard woods in the plains of India (Bakshi, 1971). It causes white stringy rot.

Basidiomycetous fungi are important component of a forest ecosystem. More studies are required to locate these higher fungi and suggest measures to preserve this valuable gift of nature from extinction. More systematic researches will lead to understanding and identifing the potential of fungal biodiversity, to provide novel substances that can be used to treat the maladies of humans, animals and crop plants

Acknowledgements:

The authors are thankful to the Head, Department of Botany, The M S University of Baroda for laboratory facilities, and to Dr. D N Pegler from Royal Botanical Garden, Kew, U. K. for conforming the identity of the fungi. This study was funded by DST, New Delhi.

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Accepted: Jul 21, 2008

6. *Coriolopsis gallica* (Fries) Ryv.

Norweg. J. Bot. 19: 230. 1972.

BASIONYM: *Boletus favus* Bulliard 1789

Basidiocarp well developed, up to 18 cm wide and 11 cm deep but usually about 9 cm wide; convex; semicircular, bracket-shaped; sometimes fused laterally with other caps; brown; densely hairy, becoming glabrous on the margin. Pore surface gray-brown; with angular, 4- to 6-sided pores 1-3 mm wide, becoming elongated and jagged (gill-like) with age; tubes 15 mm deep, often paler than the pore surface. Hyphal system trimitic, generative hyphae septate, thin-walled, hyaline, clamped, wider lumen, branched 6.25 µ in diameter; binding hyphae thick-walled, golden, tortuous, branched 3.12 µ in diameter; and skeletal hyphae thick-walled, golden, aseptate, nbranched, narrow lumen, 6.25 µ in diameter. Cystidia absent; in KOH dark gray to black on flesh fading slowly back to the original color. Basidiospores extremely variable in size, even within a single mushroom; (8-) 9.33-16.1 (-21) x 3.12-6.25 µ; smooth; cylindrical.

Collection examined. Ratanmahal Wild Life Sanctuary, (Gujarat); Collected by N. Praveen Kumar; Accession No: MSU Bot. 72; 5-7-2006.

7. *Coriolopsis telfari* (Kl.) Ryv.

Norw J. Bot. 19: 230, 1972.

Synonym: *Polyporus telfarii* Kl., 1833 *Trametes cristata* Cke. 1886,

Hexagonia dybowskii Pat. 1892 *Trametes wildermaii* Bres 1911

Sporophore sessile, effuso-reflexed, imbricate, leathery on drying, 9 x 4 x 0.2 cm resupinate for several cm, upper surface yellowish brown to cinnamon, glabrous; context brownish yellow, fibrous, 1 mm thick; hymenial surface yellowish brown,, pores angular, thickwalled, 2 – 3 per mm, pore tubes less than 1 mm long; hyphal system trimitic, skeletal hyphae light yellowish, thick walled, usually with narrow lumen, 3.12 – 6.25 µ in diameter; binding hyphae yellowish, thickwalled, narrow lumen, branched 1.6 – 3.12 µ in diameter, generative hyphae hyaline, thinwalled, branched septate with clamp connections 3.12 µ in diameter; basidia clavate, 4.5 – 6.25 µm, badidospores hyaline, cylindrical, sometimes shortly curved, 9.33 - 12.4 X 3.12 – 4.5 µm.

Collection examined : Sawmill, Vadodara, Gujarat, collected by N. Praveen Kumar, **Accession no**: MSU Bot.115, 4-4-2007.

8. *Coriolus versicolor* (L. ex Fr.) Quel.

Enchiridion Fungorum: 175, 1886.

BASIONYM: *Boletus versicolor* L. 1753, *Tametes versicolor* (Fr.) Pilat.

Polyporus versicolor Fr. 1821, *Polyporus nummularius* Pers. 1827

Sporophores annual, pileate narrowly attached, sessile, solitary, usually imbricate, fan shaped, coriaceous when fresh, rigid when dry. 2.5 X 3-12 X 0.1-0.5 cm. upper surface dimidiate, flabelliform, grayish, tomentose, velvety, concentrically zonate with multicoloured zones, The zones are of very different colour: yellowish, brownish, greyish, reddish to blackish. Margin thin, acute, entire or weakly incised and lobed, often wavy and paler than the rest of the upper surface. Lower surface cinamomum yellow, often glancing, Pores angular, 3-5 per mm, tubes single-layered, up to 2 mm long, Sterile margin 0.5-3 mm broad, Context white, homogeneous and fibrous, 0.5-2.5 mm thick. Hyphal system trimitic, Basidiospores hyaline, cylindrical, smooth, thin walled 4-5 x1.5 µm in diameter.

It was found on dead and living deciduous trees, seldom on conifers. Cosmopolitan in distribution, it is common in South-Eastern Africa (Ryvarden and Johansen 1980). Bakshi, (1971) reported on living Indian oaks in Chakrata, India. It is also found on dead hardwoods and conifers in Narkanda, Bashahr (Himachal Pradesh); Mussoorie, Chakrata (Uttaranchal) Darjeeling (West Bengal). During present study it was found associated with trees of *Buchnania lanzan.*

Collection examined. Ratanmahal Wildlife Sanctuary, (Gujarat); Collected by N. Praveen Kumar; Accession No: MSU Bot. 73; 5-7-2006.

9. *Daedaleopsis confragosa* (Fr.) Schroet.

Krypt.-Fl. Schlesien 3-1(4): 493, 1888.

BASIONYM: *Boletus confragosus* Bolton 1791

Sporophores of was sessile, reniform, dimidiate, attached by short base, single, corky , almost a circular,12 x 10 x 3 cm, upper surface dark yellow strongly concentrically closely zoned, glabrous, context brown fibrous 1 cm thick, hymenial surface cinnamon yellow daedaloid reaching to lamellae , 1-2 pores per mm pore tube 1 cm long, hyphal system dimitic. It was found on wood from Calcutta and Shillong in India, Southern Europe, and America (Bakshi, 1971). But in the present survey it was found on trunk of African Mahagoni.

Collection examined: Sawmill in Vadodara Gujarat, collected by N. Praveen Kumar, Accession no: MSU Bot. 120, 6-7-2007.

10. *Flavadon flavus* (Klotzsch) Ryv.

Refer Padhiar *et al.*, (2009) (Appendix I)

11. *Fomitopsis cupreorosea* (Berk.) J. Carr. & Gilbert.,

Mycotaxon 25(2): 476, 1986.

BASIONYM: Polyporus cupreoroseus Berk. 1856, *Trametes cupreorosea* Lloyd 1920, *Microporus cupreoroseus* (Berk.) Kun., 1898,

Basidiocarp annual, solitary and imbricate, sessile or with a contracted base, dimidiate and broadly attached, with a resupinate hymenial surface frequently present, coriaceous to woody when dry; Pileus applanate, 5.0 – 9 x 2.6 - 5.3 x 0.3 cm (Plate I Fig. C), resupinate part up to 0.8 -1.5 x 0.5 cm; upper surface grayish brown, first velvety, fibrillose, becoming glabrous when old, with a weak silky dull shine, weakly sulcate, radiate-striate, strongly zonate in variable shades, old specimens dotted with protuberances or warts close to the base; margin sharp, acute, thin, entire; Pore surface cinnamon brown, with a sterile margin up to 1.5 mm; pores round, angular or subdaedaleoid, 1 - 3 per mm (Plate I Fig. D), dissepiments thick, entire; tubes concolorous with the pore surface, indistinctly stratified, up to 7 mm long, old tubes stuffed with white mycelium; Context light brown, sometimes with a dark zone separating the tubes from context, dense, fibrous, up to 0.3 cm thick, with a black in KOH. Hyphal system trimitic; generative hyphae with clamps, occasionally branched, 3.15 µm in diam; skeletal hyphae yellowish brown, thick-walled, nonseptate, 6.13 µm in diam; binding hyphae moderately branched, thick-walled 3.0-4.0 µm in diam. Cystidia or other sterile hymenial structures absent. Basidia clavate, 4 - sterigmate, 19.0 x 6.5 µm, with a basal clamp. Basidiospores cylindrical, hyaline, smooth-walled, 5.0 - 7.0 x 2.5 - 3.5 µm, negative in Melzer's reagent. Found on the fallen branches of *Terminalia crenulata.*

Collection examined: Ratanmahal Wildlife Sanctuary, Gujarat, Collected by N. Praveen Kumar; Accession no: MSU Bot. 85; 7-12-2006, Rot: white fibrous rot.

Carranza-Morse *et al.,* (1986) reported it on hard wood trees, very common on burnt wood from Mexico, Central and South America; Caribbean Islands causing Brown cubical rot

12. *Ganoderma applanatum* (Per.) Pat.

Bull. Soc. Mycol. France 5: 67, 1889.

BASIONYM:*Boletus applanatus* Pers. 1799, *Polyporus applanatus* (Pers.) Wallr., 1833, *Fomes applanatus* (Pers.) Gillet, 1878, *Placodes applanatus* (Pers.) Quel., 1886, *Phaeoporus applanatus* (Pers.) Schroet., 1888,*Ganoderma megaloma* (Lev.) Bres., 1912.

Sporophore perennial, sessile, applanate, single, corky soon becoming hard and woody in dry condition, 12 - 18 X 8 - 11 X 2 - 4 cm some times very large, upper surface brown, zoned, uneven, crusty; context light brown, interspersed with white lint material, fibrous, with silky shine, 2-3.5 cm thick. Hymeneal surface white when fresh turning light brown on drying, pores round, 4-5 per mm, pore wall thick, pore tube distinct from context, generally with a distinct white region bordering pore surface stratified, Cuticle hard, less than 0,5 mm thick, context is 2 mm broad, hyphal system trimitic, Basidiospores brown, broadly ellipsoid, thickwalled with outer wall smooth, inner wall echinulate, truncate 6.25- 9.33 (10) X 5.1 – 7.8 (8) µm. Found on *Santalum album, Polyathia longifolia,* and *Pithacelobium dulce.*

Collection examined: Arboretum, Botany department, Biochemistry department, Faculty of Science gate, Vadodara, Gujarat. Collected by N. Praveen Kumar, Accession no: MSU Bot. 94,127,128; 5-6-2005, 6-7-2007, 7-7-2008.

It is a wound parasite on a great variety of hardwoods species and attack both heartwood and sapwood. The attacked trees are liable to snap on the stem at the region of decay (Bakshi, 1971).

13. *Ganoderma colossum* (Fr.) Bers.

Fungi Malay. no 425, 1918,

BASIONYM: *Polyporus colossus* Fr. 1851. *Polyporus hollandii* Mass. 1901

Sporphore annual, sessile, applanate, semicircular, corky, soft when fresh, light in weight 25 x 18 x 6 cm, upper surface glabrous, semiglossy, yellow, laccate, cuticle present, cracks up under drying and is often destroyed, margin of lighter colour than the basal part, hymenial surface white when fresh, pale brown when dry, pores round 2-3 per mm, tubes concolorous with pore surface, pale brown, up to 3 cm deep, context soft and punky when fresh, cream, corck, up to 6 cm, Hyphal system di-trimitic, generative hyphae hyaline, thin-walled and with clamps, 3.12 - 5.56 µm wide, in the subhymenium the hyphae are moderately branched, in the context partly intertwined in dense clusters with very short cells and strong branching, it is these dense masses of generative hyphae that give the context its characteristic consistency so different from the other *Ganoderma* sp, skeletal hyphae pale yellow to hyaline, solid, 2.4 - 4.9 (5) µm wide, richly present in the context. Cystidiols present at hyphal end, ventricose, hyaline organs, up to 30 µm long, difficult to find unless the specimen has been properly dried. Basidia spherical with a short narrowed base, 4-sterigmate, 20-30 x 13-17 µm, Basidiospores echinulate, truncate to ovoid, yellow 14.23 -18.9 x 8.67 -12.65 µm. It was found on angiosperms of many

kinds. Pantropical, but not seen from East Africa (Ryvarden and Johansen, 1980). It is found on dead trunk of *Polyalthia longifolia* growing in Faculty of Science, MSU campus, Vadodara

Collection examined: Biochemistry Department, Vadodara, Gujarat; collected by N. Praveen Kumar, Accession no: MSU Bot. 90, 8-9-2007.

14. *Ganoderma curtisii* (Berk.) Murill 1908, N. Am. Fl. 9: 120

BASIONYM: *Polyporus curtisii* Berkeley, Hook. 1849

Basidiocap annual, stipe lateral, aplanate, covered with cuticle, pileus rusty brown to grayish brown, golden brown zones (Plate II Fig. A), glabrous, undulate, usually slightly laccate, 10.8 x 7.5 x 06 -1cm thick, margin was blunt to entire, wavy, rusty brown. Hymenium rusty brown to grayish brown with yellow line of zone, 0.4 cm thick, pores circular (Plate II Fig. C), 3 – 5 per mm, tubes separate (Plate II Fig. B). Context brown, 0.3 cm thick, black line absent, Hyphal system dimitic, Cuticle was made up of golden yellow, thick-walled with 3.12 µm diameter, bottle shaped, 25 – 46.8 x 9.3 µm. Basidia light yellow, clamped at base, clavate, 21.8 x 3.12 µm , 2 sterigmata. Basidiospores brown, truncate, ellipsoidal to oveal, 8.5 – 11.4 x 5.7 – 6.2 µm (Plate II Fig. D, E). Steyaert, (1980) reported this polypore fungus on dead wood, especially dead stumps in summer and fall, from North California USA. But in present study it was found at the base of living tree of *Emblica officinalis* and found on trunk of *Peltophorum ferrugineum.*

Collection examined: Ratanmahal Wildlife Sanctuary, sawmill of station road, Vadodara, (Gujarat), collected by N. Praveen Kumar, Accession no. MSU Bot. 84, 91. 7-12-2006 and 4-4-2007.

15. *Ganoderma lucidum* (Fr.) Karsten.

Rev. Mycol. (Toulouse) 3(9): 17, 1881.

BASIONYM: *Boletus rugosus* Jacquin1774, *Boletus lucidus* W. Curtis, 1781

Ganoderma tsugae Murrill, 1902. *Ganoderma mongolicum* Pilat, 1940.

The sporophores perennial, stiptiate corky becoming woody later, 14-16 x 10-12 x 1-3 cm. many grow up to 30cm. stalk lateral, varnished and encrusted, up to 10 cm long and 0.5 to 4.5 cm thick, upper surface shiny with laccate crust, reddish brown, smooth. Cutis thin, a fraction of 1 mm, Cutis of hymenioderm type composed of anticline inflated extremities of hyaline context, hyphae swollen by melanoid substances leaving usually a central lumen; the melanoid substances are easily saponifed by KOH; Context brown, 2-10 mm thick, pores small, brown, 90-250 µ diameter, pore tubes 6-7

mm long, hyphal system timitic; Basidiospores brown, thick walled, minutely verrucose, truncate at base, 9.33 – 11.25 x 5 - 6.25 µm. It causes white rot. A large number of sporophores were collected from the base of *Feronia elephantum* from arboratum and *Emblica officinalis* from Rathanmahal.

G. lucidum (Ling chi in Chinese and Linjue in Thai) is popularly called as Reishi mushroom. Tha Latin word *lucidum* means shiny or brilliant and refers to the warnished surface of reishi cap, which is reddish orange to black.

Collection examined: Arboratum of Botany department and Ratanmahal Wildlife Scantuary, Gujarat; collected by N. Praveen Kumar, Accession no. MSU Bot. 92, 93, 7-12-2006 and 4-7-2007

16. *Gloeophyllum sepiarium* (Fr.) Karst.

Bidr. Känn. Finl. Nat. Folk 37: 79, 1882.

BASIONYM: *Agaricus sepiarius* Wulfen 1786,

Lenzites saepiaria Wulf. ex Fries 1836-1838

Sprophore sessile, reflexed, developing close to substrate, solitary, corky, 5.6 X 5.4 X 2 cm; upper surface sepia cloloured, margin pale, glabrous, weakly zoned. Hymenial surface snuff brown pores usually lamella, lamellae irregular with edges mainutely serrated, 12-14 per cm, 3-5 mm broad. Context snuff brown, corky 2-5 mm thick; basidia cylindric, 12-16 x 6.25 µm; basidiospores hyaline, oblong ellipsoid, 9.3 x 5.12 µm; cystidia hyaline, slightly thick walled embedded in hymenium; hyphal system dimitic, Causes brown-rot on *Picea glauca* and *P. mariana*, single records on *Larix laricina* and *Salix* sp. Saprophytic on stumps and decorticated, seldom on corticated, fallen trees, preferring open forests (Niemelä, 1985). But in the present study it was found on the living tree of *Peltophorum ferrugineum* causing brown rot.

Collection examined: Wadia Palace, Rajpipla, Narmada (Gujarat); collected by N. Praveen kumar, Accession no: MSU Bot. 125, 22-4-2007

20. *Lenzites betulina* (L.) Fries

Epicrisis Systematis Mycologici: 405, 1838.

BASIONYM: *Agaricus betulinus* Linnaeus 1753, *Daedalea betulina* L. ex Fries 1821,

Basidiome sessile, attached by a broad base, dimitiate corky,usually 8 x 4 x1.5 cm. upper surface lignt brown, closely concentrically zonate, zones usually with varying shades of brown, soft matted tomentosed to finely hirsute, context brown, fibrous, up to 1 mm thick; hymenial surface brown, lamellate, lamellae wavy, uniform; hyphal system trimitic, generative hyphae light brown, thickwalled with narrow lumen un branched,

3.12- 6.25 µ in diameter, skeletal hyphae, hyaline, thick walled, much branched 3.12 µ in diameter binding hyphae hyaline, thinwalled, branched, septate, 3.13 µ in diameter; cystidia hyaline, thickwalled, sharp pointed unincrusted 12-26 X 3.12 – 6.25 µm, basidiospores hyaline, short cylindric, 6.35 x 3.12 µm. it causing white rot in broad-leaved and coniferous trees. It was found usually on hardwood logs of Indian oaks, maple, horse chestnut, alder, birch also on conifers like spuree, deodar fir and chir in temprate regions of the Himalayas (Bhakshi, 1971). But in the present study it was found on the teak wood

Collection examined: Sawmills in Vadodara, Gujarat; collected by N. Praveen Kumar, Accession no: MSU Bot. 130, 6-7-2007

21. *Microporellus violaceocinerascens* (Petch) David & Rajch.

Mycotaxon 22: 303, 1985.

Synonime: *Polyporus violaceocinerascens* Petch. 1916. *Daedalea iocephala* Ryv., 1983

Sporphore annual, stipitate, circular, almost funnel shaped with depression at the center, up to 10 cm diameter and about 5mm thick, stalk central or concentric, usually bulbous at the base, concolorous with pileus, covered with violet hairs about 2mm thick. Upper surface vinaceous brown, minutely tomentosed, zonate, margin thin, even context brownish, tough, 0.5 mm thick; hymenial surface whitish, pores large, angular, 1-2 per mm, pore tube yellowish brown, 1 mm long, Basidiospores hyaline ellipsoid, apiculate, l-guttulate 6 – 8 X 4.5 – 6 µ. Cystidia: hyaline, fusiform, encrusted at the tip 6.25 – 12.45 µm. generative hyphae thickwalled, collapsed, branched, simple septate, 3.12 – 6.25 µm in diameter. It was found on dead stump of *Pongamia glabra*, *Dalbergia* sp. (Bakshi, 1971). It was known from Sri Lanka, India, Pakistan, and Indonesia. It was first time reported in China by Yang, (2000). In the present study it was found on *Terminalia arjuna, T. chebula,* and *T. crenulata.*

Collection examined: Ratanmahal Wildlife Scantuary, Gujarat; collected by N. Praveen Kumar, Accession no: MSU Bot. 144, 6 - 6-2006

22. *Microporus affinis* var. *glabriceps*

Sporophore annual, solitary, spathulate, short stipe, attached laterally, semicircular, dimidiate, flat and depressed in the area around the stipe. 2.7 - 5 x 1.4 – 3.3 x 0.3, (Plate I Fig E) margin thin and usually flat. Pileus glabrous with slightly raised sulcate zone of dark brown crust of 20 - 40 µm, upper surface reddish brown to almost black, Stalk lateral, 2. 5 cm long 0.5 cm width, velutinate throughout, round to slightly flattened, glabrous, blackish brown crust present. Hymenium grayish yellow, margin 1 -

3 mm wide and pure white, pores round, very minute, 7 per mm, tubes light cream, up to 1 mm deep (Plate I Fig. F). Context pure white and dense, up to 3 mm thick, Hyphal system trimitic and dextrinoid, Spores ellipsoid, hyaline and thin-walled, non-amyloid and 6.3 x 2.5 µm. Found on the fallen branches of *T. crenulata*

Collection examined: Ratanmahal Wildlife Sanctuary, (Gujarat); collected by N. Praveen Kumar, Accession no MSU Bot. 96, 7-2-20.

It differs from *Microporus affinis* by absence of tomentose, black distinct crust over a white context, small sized sporophore, absence of black line, spores with large size of 6.3 x 2.5 µm ellipsoidal. It resembles to *M. xanthopus* but it differs by having, a stalk is lateral and velutinate throughout, spores are slightly shorter.

23. *Microporus alboater* (Henn.) Kun.

Revisio generum plantarum 3: 494, 1898.

BASIONYM: *Polyporus alboater* Hennings, 1895

Basidiocarp annual, solitary or in small groups, centrally or rarely laterally stipitate, pileus infundibuliform, circular, even in laterally stipitate specimens where the lobes often meet around the point of attachment, leaving only a narrow opening from the stipe to the margin, pileus 4 – 7 cm in diameter, 1 – 2 mm thick, coriaceous and papery-thin along the margin, margin even and wavy. Pileus smooth, zonate with numerous concentric zones of reddish brown, upper surface deep brown to almost black (Plate II Fig. F), very short and dark skeletal hyphae from the cuticle but these are not visible to the naked eye, glabrous at maturity, nonxanthochoric. Stipe up to 4 cm long, light brown to more dirty brown with age, 2 - 3 mm in diameter, in young specimens hirsute and covered with grayish hairs, but with age these wear away from the upper part. Hymenium first light cream with a narrow white sterile margin (Plate II Fig. G), with age it darkens and is often discoloured with dark spots, pores very small and entire, 8 per mm, tubes very short, up to 1 mm (Plate II Fig. H). Context was pure white at stipe and pileus, up to 1 mm thick. Hyphal system trimitic, Basidiospores were dextrinoid, subglobose to ellipsoid, hyaline to pale yellow, thin-walled, 6.7 x 3.1 µm (Plate II Fig. I, J.). Found on fallen branches of *T. arjuna, T. bellerica.*and *T. crenulata*

Collection examined: Ratanmahal Wildlife Scantuary, Shoolpaneshwar Wildlife Sanctuary, Didiapada (Gujarat) collected by N. Praveen Kumar, Accession No: MSU Bot. 80 and 81, 82, 7-12-2006, 30-1-2007. 23-9-2008. Rot: white fibrous rot.

Ryvarden and Johansen, (1980) reported on deciduous wood. It is a rare central-African species and specimens only seen from the Cameroons and Zaire and it may be that it is restricted to the central African rain forest.

24. *Microporus xanthopus* (Fr.) Kunt.

Rév. gen. Pl. 3:494, 1898.

Synonyme: *Polyporus xanthopus* Fr., (1821) *Trametes xanthopus* (Fr.) comb. nov, 1989

Sporphore centrally stipitate, single, funnel shaped, petal like flexible, corky, circular, 3-6cm in diameter, stipe yellow, or yellowish brown, indistinctly pubescent to glabrous, smooth or occasionally lightly furrowed to upper variegated, 0.7-3.5 cm long, 3-4 mm thick, base expanding. Upper surface yellowish red, chestnut to dark maroon, margin lighter, concentrically zoned with shades of colour, glabrous, slightly radiately cracking, shiny, context white, fibrous, up to 1-2mm thick. Hymenial surface with shades of pink to brown, soned, margin lighter, thin, sterile, pores regular, more or less round, 6-8 per mm, pore tubes light pink, up to 0.2 mm long. Basidia clavate, 9-13 X 3 – 4 µ, Basidiospores hyaline, ovate to cylendric, 4 - 6 X 1.4 - 2.5 µ. It was reported on the dead wood of Sal and variety of other hearwoods (Bakshi 1971). It was also found on dry deciduous wood, often in open habitats like savanna, riverbeds and on trunks left after lumbering. It was very common throughout the tropics in the Old World, from Western Africa to the Pacific Area (Ryvarden and Johansen 1980). But in the present study basidiocarps were found on *T. arjuna, T. crinulata* and *Diospyrus melanoxylon*

Collection examined : Ratanmahal Wildlife Sanctuary, Gujarat; collected by N. Praveen Kumar, Dr. Arun Arya, Accession No: MSU Bot. 147,148, 7-12-2006

26. *Oxyporus ravidus* (Fr.) Bond. and Sing.

Sporophore annual, sessile, effuso-reflexed, pilei laterally fused, Attached by a broad base, flexible when fresh, rigid when dry 12 x 10 x 2.5 cm, upper surface cream, uneven, with distinct crust and cracking, azonate, glabrous, margin thinning out, incurved when dry, context white often with dark brown line separating tomentum from context, up to 1 cm thick; hymenial surface white, pores angular to nearly lamellar, 1-3 per mm, usually extend up to the margin, pore tubes 2-5 mm long. hyphal system dimitic, Hymenium composed of basidia and cystida; basidia clavate 6.25 – 9 µ in diameter, basidiospores hyaline, thinwalled, oblong elliptical, guttulate 6.25 – 9.33 X 3.12- 5.12 µm, cystidia abundant, embaded, hyaline fusiform, rarely cylindrical, 3.12 – 6.25 µ in diameter. It was found on stumps and logs of conifers and rarly on hardwoods

and very common in the West Himalayas (Bakshi 1971). But in the present study it is found attached to the living tree of the *T. arjuna.*

Collection examined: Ratanmahal Wildlife Sanctuary, Gujarat; collected by N. Praveen Kumar, **Accession no:** MSU Bot. 125, 7-2-2007

27. *Phellinus adamantinus* (Berk.) Ryv.

Norweg. J. Bot. 19: 234, 1972.

BASIONYM: *Polyporus adamantinus* Berk. 1854,

Pyrrhoderma adamantinum (Berk.) Imazeki, 1966,

Basidiacarps perennial, pileate, solitary, applanate, semicircular to usually dimidiate up to 12 cm wide, 8 cm broad, 2 cm thick at base, woody hard; pileus glabrous, dark brown to grayish black, with a thick crust, uneven with rather wide, rounded and sulcate zones, cracking radially and concentrically with age; margin thick, obtuse, pale yellowish brown; pore surface grayish to deep brown tubes indistinctily stratified, dark grayish brown; pores small, 7-9 per mm; context up to 5 mm thick, dark yellowish brown, shiny with a luster, limited on upper surfaces by a black line, hard and rigid on drying with radial white strands in the lower part. Hyphal system dimitic; generative hyphae simple septate, 1-3.12 µm wide, skeletal hyphae thick walled, pale yewllow to rusty brown, 3.12-6.35 µm wide; hymenial setae none, spores globose to drop shape, thin walled to slightly thick walled hyaline to pale yellow, 5.12 - 6.25 µm in diameter.

it was found on dead and living deciduous woods, causing a white rot; cosmopolitan species occurring from tropical to temperate forests (Sharma 1995). It was also found on deciduous wood and widespread in South-Eastern Asia, India, Thailand, Indonesia and New Guinea (Ryvarden and Johansen 1980). In the present study it is found on living trees of *P. ferrugineum* causing white rot. Bakshi (1971) described the East Himalayan collection of this polypore fungus as *Fomes adamantinus.*

Collection examined: Wadia Palace Rajpipla, Gujrat; collected by N. Praveen Kumar Accession no: MSU Bot. 131, 22 - 4-2007

28. *Phellinus badius* (Cooke) Cunn.

Bull. New Zealand Dept. Sci. Industr. Res. 164: 233, 1965.

BASIONYM: *Polyporus badius* Berkeley 1841 *Fomes badius* Berk. ex Cke., 1885.

Basidiocarp perennial, seesile, hoof shaped to ungulate easily detachable from host, 10 x 8 x 2 cm, head woody; pilear surface yellowish brown when yound brownish when maturity. Glabrous, weakly zoned, rimose, crust up to 0,2mm thick margin obtuse, sterie pore surface dark brown glancing tubes ferruginous brown, paler than pore surface,

stratifieddistinctly, 3mm deep in each layer pores 7 per mm angular, pore wall thick. Context brown lustrous, corky when fresh hard on drying 1 – 5 mm thick faintly zoned, granular core of dull yellowish brown mycelium with patches of white mycelium and dark ressih brown hard glossy granles scattered throughout, hyphal system dimitic. Hymenial setae absent or very rarely present in older specimen. Basidia broadly clavate 14X 7 µm, Basidiospores broadly ellipsoidal thickwalled 6.3 x 5.1 µm golden brown.

It causes a white rot of heartwood of living *Acacia* and mesquite in the Sonoran Desert, rarely on other associated hardwoods like *Acacia greggii, Chilopsis linearis, Prosopis juliflora*, (Gilbertson 1979). A common and serious parasite on *Acacia catechu* less oftenly also found on *Acacia Arabica, Albizzia* sp. and other living leguminaceous trees, causing a white rot (Sharma 1995). But in the present study it was found associated with living tree of *P. ferrugineum.*

Collection examined: Wadia Palace Rajpipla, Gujrat; collected by N. Praveen Kumar, Accession no: MSU Bot. 101, 22 - 4-2007.

29. *Phellinus caryophyllii* (Racib.) Cunn.

Bull. New Zealand Dept. Sci. Industr. Res. 164: 238, 1965.

BASIONYM: *Trametes caryophylli* Racib. 1900. *Fomes caryophylli* (Racib.) Bres. 1912

Basidiocarp annual, effused- reflexed, semicircular, broadly attached, 17.5 cm long, 16.5 cm wide, 1 cm thick at base, woody heard; pilear surface rusty brown, velvety, soon glabrous with distinct black crust, narrow zones in sharp edges. Pore surface rusty brown, with sterile reddish brown border; tubes dark brown, distinctly stratified, 4 mm deep in each layer pores round, 6 per mm. Context concolotous with tubes, limited on the upper surface by a black crust, 2 mm thick. Hyphal system dimitic; hymenial setae noone; basidia subclavate, 15.6 x 6.25 µm; basidiospores pale yellow, globose, 3.5 x 2.1 µm in diameter. It was a common and serious heartrot parasite on deciduous trees in tropical forests causing a white rot (Sharma 1995). It was found associated with deciduous wood, in South-Eastern Asia and Buitenzorg, Java (Ryvarden and Johansen 1980). In the present study it was found on living trees of *P. ferrugineum* causing white rot.

Collection examined: Wadia Palace Rajpipla, Gujrat; collected by N. Praveen Kumar, Accession no: MSU Bot. 132, 22 - 4-2007

30. *Phellinus conchatus* (Pers. : Fr.) Quel

Enchiridion Fungorum: 173, 1886.

BASIONYM: *Boletus conchatus* Pers.1 795, *Polyporus conchatus* Pers. ex Fr. 1821.

Basidiocarps perennial, sessile, imbricate, semicircular, convex, pileus up to10.5 cm broad, 6 cm wide and 2 cm thick at base, woody heard; upper surface yellowish brown, tomentose, with age becoming black, glabrous with a distinct thick crust in narrow, sharo sulcate zones, finely cracked radially; margin rounded, wide, yellowish brown. Pore surface dark brown; pores 6-8 per mm; tubes yellowish, indistinctly straitified. Context golden brown, up to 3 mm thick with one black layer. Hyphal system dimitic. hymenial setae abundant, 21.84 x 9.3 µm, ventricose, dark reddish brown, misshaped with irregular; tramal setae present, up to 40 µm long and 6.25 µm wide, straight. Basidia 12.4 x 3.12 µm, clavate; Basidiospores abundant, pale yellow, 6.25 x 3.12 µm, globose, uniguttulate.

It was causing uniform white rot of dead wood of several hardwood like *Acer macrophyllum, Alnus incana, Betula papyrifera, Populus trichocarpa, Salix bebbiana,* and *S. lasiandra* (Gilbertson 1979). It was frequently present on the dead branches of standing and living trees of *Salix* and *Pyrus*; less common on dead woods of *Mangifera indica, Cotoneaster becillaris, Mallotus philippinensis, Quercus incana* and rarely on the species of *Viburnum, Populus*, and *Betula* causing white rot and killing the branches and ultimately entire tree (Sharma 1995). In the present study it is found on the living trees of *P. ferrugineum* causing white rot

Collection examined: Wadia Palace Rajpipla, Gujrat; collected by N. Praveen Kumar, Accession no: MSU Bot. 133, 22 - 4-2007

31. *Phellinus extensus* (Lev.) Pat.

Essai taxonomique: 97,1900.

BASIONYM: *Polyporus extensus* Léveillé, 1846

Basidiocarps perennial, usually solitary, sessile, pileate, broadly attached, dimidiate, conchate to applanate, up to 5 cm wide, 6 cm broad and 1 cm thick at base; pilear surface reddish brown to reddish black, velvety reddish brown; pore surface dark redissh brown; tubes up to 5 mm deep, indistinctly stratified; pores round and small, 7-10 per mm, dissepiments rather thick; context dark to reddish brown, limited on upper side by a thick black line, fibrous, shiny, up to 4 mm thick. Hyphal system dimitic; hymenial setae scattered to abundantly present, strongly ventricose, thick walled, dark brown, 15 – 20 x 5-9 µm, tips straight and acute, basidia broadly clavate, 9.5-12.5 x 5 -7 µm, hyaline spores globose, pale brown, slightly thick walled, 3.5-4 µm in diameter.

It was found on dead angiospermous wood, also on living *Pinus* and *Quercus* species; causing white pocket rot a rare species in tropical and sub tropical forests of

India (Sharma 1995). It was also found on dead angiosperms from the West Indies, Tanga, Prov. in Tanzania and Mpanga forest in Uganda, India (Ryvarden and Johansen, 1980). But in the present study it was found on living tree of *P. ferrugineum* causing white rot

Collection examined: Wadia Palace Rajpipla, Gujrat; collected by N. Praveen Kumar, Accession no: MSU Bot. 134, 22 - 4-2007

32. *Phelinus gilvus* (Schw., Fr.) Pat.

Essai taxonomique: 97, 1900,

BASIONYM: *Boletus gilvus* Schweinitz 1822, *Polyporus gilvus* Schw. 1822.

Fomes gilvus (Schw.) C.G. Lloyd; Thind and Chatrath, 1957.

Basidiocarp annual, imbricate, sessile, coriaceous, hard and brittle on drying, pileus dimidiate upto 11 cm broad, 8.5 cm wide and 1.2 cm thick, applanate, Upper surface golden brown, lighter towards margin, azonate, finely velutinate, finelt and densly warted with irregular protruberances; margin thin, acute, even, Pore surface dark purplish brown, fertile up to the margin, glancing when fresh (Plate VI Fig. C), tubes reddish brown, single layered, 2-4 mm deep, pores round and regular, 5-6 per mm, context palr reddish brown 5 mm thick zonate, fibrous, with a thin cuticle on upper side in older specimens, hyphal system dimitic, hymenial setae abundant 24.8- 36.5 (40.5) x 6.25 – 9.33 µm subulate, sharp, thickwalled, dark reddish brown in KOH; basidia clavate, 6.25-12.35 X 4.23- 6.25 µm; basidiospores ellipsoid, hyaline, thinwalled, more or less flatend at one end, 6.5 x 4.25µm.

It was found on living and dead angiospermous wood of many genera more common on *Quercus, Eucalyptus, Cornus, Acacia, Shorea, Cassia and Dalbergia.* Usually not found in coniferous wood. Causing a uniform white rot of dead woods and heart rot of living trees, widely distributed in the tropical and warmer temperate forests. (Sharma 1995). It was causing a uniform white rot of dead wood of hardwoods and a heart rot of living trees like *Alnus, Betula, Fraxinus, Juglans, Phoenix, Platanus, Populus, Prosopis, Prunus, Quercus,* and *Salix* (Gilbertson, 1979). In the present study it was found on *T. bellerica.*

Collection examined : Ratanmahal Wildlife Sanctuary, Gujarat, Collected by N. Praveen Kumar, Accession no: MSU Bot. 135, 6- 12-2005

33. *Phellinus hoehnelii* (Bres.) Ryv.

A preliminary polypore flora of East Africa: 173, 1980

BASIONYM: *Fomes hoehnelii* Bresadola 1912

Basidiocarps perennial, pileate, subapplanate, sessile, broadly attached, woody hard on drying, 25 cm long, 12cm wide, 2 cm thick, pilear surface glabrous, encrusted, rusty brown; margin obtuse, thick, entire finely velutinate. Pore surface golden brown; pores 3-5 per mm; tubes up to 1 cm deep, dark brown. Context bright yellowish, delimited on upper surface by a black, 2-7 mm thick crust. Hyphal system dimitic; setal hyphae present in context, frequent, ferruginous, thick walled up to 400 µm long, 6.32 µm wide, projecting obliquely into the hymenium and the tubes; hymenial seatae 30 x 6.5 µm, golden brown, ventricose; basidia 16 X 9.35 µm, clavate; spores abundant, subglobose, golden brown, slightly thick walled, 5.65x 3.12 µm (Plate I Fig. H). It was found on angiosperms, known from The Philippines and Indonesia (Ryvarden and Johansen 1980). It was also found on living *Terminalia* causing white rot, a rare species found in the tropical forests of kerala and Arunachal Pradesh only (Sharma, 1995). In the present study it was found associated with living tree of *P. ferrugineum* causing white rot

Collection examined: Wadia Palace Rajpipla, Gujarat; collected by N. Praveen Kumar, Accession no: MSU Bot. 135, 22 - 4-2007

34. *Phellinus linteus* (Berk & Curt.) Teng.

Chung-kuo Ti Chen-chun: 762, 1963

BASIONYM: *Polyporus linteus* Berkeley & M.A. Curtis, 1858

Basidiocarps perennial, solitary to imbricate, sessile, broadly to narrowly attached, rigid and woody hard when dry; pileus dimidiate to semicirculae, applanate to ungulate, up to 15 cm wide, 18 cm broad and 4 cm thick near the base; upper surface first matted tommentose, dark reddish brown to dark chestnut brown, concentrically zoned and sulcate, later glabrous and grey to black from the covered with mosses from the base; margin acute to rounded, velutinate, entire usually paler than the rest of the upper surface; pore surface yellowish to reddish brown; tubes slightly paler than the context, distinctly stratified, up to 12 mm deep, separated by a thin context later, sterile margin up to 2 mm broad; pores round to weakly angular, 5 -7 per mm; Context golden to dark brown, fibrous shiny up to 10 mm thick. Hyphal system dimitic; hymenial setae ventricose, adundant, 25-32 (35) x 6.25 -12.33 µm, thick walled, dark brown; basidia 9.33 -12.5 X 6.25 -9.33 µm, broadly clavate; Basidiospores ovoid to subglobose, pale golden yellow to rusty brown, slightly thick walled, 4.5 - 5.12 (5.5) X 3.12 - 6.25 µm. It was found on dead and living branches of hard wood trees especially belonging to the genera *Quercus, Cassia, Lonicera, Pyrus, Prunus, Rhus, Albizzia, Corylus* etc. causing white pocket rot; widely spread in tropical to temperate forests (Sharma, 1995). It was

also found on angiosperms in pantropical, tropical America, In Africa specimens studied from Ethiopia, Tanzania, Kenya and Zaire and India (Ryvarden and Johansen, 1980). In the present study it was found on living tree of *P. ferrugineum* causing white pocket rot

Collection examined: Wadia Palace Rajpipla, Gujarat, collected by N. Praveen Kumar, Accession no: MSU Bot. 136, 22 - 4-2007

35. *Phellinus nilgheriensis* (Mont.) Cunn.

Bull. New Zealand Dept. Sci. Industr. Res. 164: 226, 1965.

BASIONYM: *Polyporus nilgheriensis* Montagne, 1842

Basidiocarps perennial, solitary, sessile, applanate, semicircular, up to 13 cm broad, 7 cm wide and 3 cm thick at the base, woody hard, glabrous, blackish brown at the base, with a thick black crust becoming thicker with age, margin round, pore surface dark reddish brown, tubes dark brown, stratified, 2 cm thick near the base; pores round 8-9 per mm; context golden brown, fibrous with several black lines ar the upper surface, hyphal system dimitic, hymenial setae none, basidia clavate 9.33 – 12.5 x 6.25 µm, basidiospores subglobose, rusty brown, 6.25 x 5.24 µm. It was found on dead angispermic wood causing white stringy rot a rare species found from tropical to temperate forests (Sharma 1995). It was also found on deciduous wood in Cuba, Tanzania and India (Ryvarden and Johansen 1980). But in the present study it was found on living tree of *P. ferrugineum* causing white pocket rot.

Collection examined: Ratanmahal Wildlife Sanctuary, Gujarat; collected by N. Praveen Kumar, Accession no: MSU Bot. 137, 6 - 12-2005

36. *Phelinus noxious* (Corner) G. Cunningham

Bull. New Zealand Dept. Sci. Industr. Res. 164: 221, 1965.

BASIONYM: *Fomes noxius* Corner 1932

Basidiocarps perennial, imbricate, effused-reflexed, woody hard, light in weight when dry, 12 cm wide 6 cm broad, 5 cm thick near the base, upper surface black, glabrous, with a hard crust, azonate, frequently nodulose at the center, margin , obtuse entire, yellowish brown; pore surfacereddish brown, tubes distinctly layered, 2 mm deep in each layer, pores small 6-8 per mm, sangular, moderately thickwalled entire, darker than the context; context 2 cm thick at base, zoned, pale brown, radially fibrous; hyphal system dimitic, setal hyphae present, 6.25 – 15.6 µm wide, up to 425 µm long, rare and narrow in context, frequently projecting into the lumen of tubes, dark reddish brown, tips obtuse; hymenial setae absent, Basidia hyaline, 6.25 -9.33 x 3.12 -6.25 µm, Basidiospores hyaline, subglobose, smmoth, thinwalled, 3.12 – 4.89 (5) X 3.12 µm. It

was found on dead angiospermous woods, also found as parasite on species of *Poinciana, Cinchona, Acacia, Coffea* and *Thea*; causing a white rot, a common tropical to subtropical species especially in Eastern Himalayas, Plains of Eastresn and Southern India (Sharma, 1995). It seems to be an important parasite on angiosperms, more on gymnosperms and found rarely in tropical America, Dominican Republic, Venezuela and Brazil (Ryvarden, 2004). But in the present study it was found on *A. arabica* causing white rot

Collection examined: Sawmill in Vadodara, Gujarat; collected by N. Praveen Kumar, Accession no: MSU Bot. 138, 6 - 6-2006

37. *Phellinus pachyphloeus* (Pat.) Pat.

Essai taxonomique: 97, 1900

BASIONYM: *Polyporus pachyphloeus* Patouillard1889

Basidiocarps perennial, solitary, woody, light in weight on drying, applanate, broadly attached, sessile; pileus 20 cm broad, 16 cm wide and 10 cm thick; upper surface tomentose, reddidh brown, thick heard crust,rugulose in wide concentric zones; irregularly cracking; margin obtuse, entire, persistently velutinate. Pore surface grayish brown; tubes distinctly stratified, usually concolorous with context, up to 5 mm thick in each layer; pores small, invisible to naked eye, 8 pores per mm. Context yellowish brown, with white mycelial strands, woody, 5 cm thick, limited black crust at upper surface. Hyphal system dimitic, setal hyphae present in context, dark brown, up to 200 µm long and 15 µm wide; tips pointed, thick walled, frequently projecting into the lumen of the tubes. Hymenial setae present, projecting above the hymenium, thick walled, golden brown, ventricose, 28.5 x 18.32 µm. Basidia clavate, 4 sterigmate, hyaline, 10.23 x 3.12 µm. Spores globose, thin walled, rusty brown, 4.5 x3.12 µm, nonamyloid, usually absent in dried herbarium specimens. It has been reported both from living and dead trees, angiosperms and gymnosperms, and from a fern root. It was widespread in Southern Asia, Australia, Africa, Kenya, Tanzania and Malawi (Ryvarden and Johansen 1980). It was also found on dead and living tree trunks and branches of Angiospermous trees like *Ficus* and *Mangifera* and less frequently on species of *Anogeissus, Terminalia, Cassia, Bruguiera, Acer Rhizophora, Albizzia, Shorea* etc. never collected on conifers; causing a white stringy rot of sap wood and heart wood, commom species in the plains of India (Sharma, 1995). But in the present study it was found on living tree of *P. ferrugineum* causing white stringy rot

Collection examined: Wadia Palace Rajpipla, Gujarat; collected by N. Praveen Kumar, Accession no: MSU Bot. 139, 22 - 4-2007

38. *Phellinus pectinatus* (Kl.) Quel.

Enchiridion Fungorum: 173, 1886,

BASIONYM: *Polyporus pectinatus* Klot. 1833,

Fomes pectinatus (Kl.) Cke; Thind and Chatrath, 1957

Basidiocarps perennial, pileate, applanate, frequently imbricated with several pilei from a common base, up to 22 cm wide, 15 cm broad and 3.5 cm thick near the base, woody hard and heavy when dry; upper surface compressible tomentum when young, yellowish brown, with age a black surface is exposed from base, finely sulcate with a thin black crust; margin entire, usually paler than the basal part of the pileus. Pore surface golden brown, glancing on turning to incident light, tubes distinctly stratified, 2 to 0.5 mm thick pores thiny, invisible to the naked eye, 6-8 pores per mm. Thin context present between tube layer, context duplex in younger specimens, the lower part very dense and dark reddish brown, 0.1 cm thick, the upper part more loose consistency than the lower part, distinct black line separating the upper tomentum and lower denser part. Hyphal system dimitic, hymenial setae absent, Basidia 8 X 6.35 µm, spores abundantly present, globose, hyaline, often collapsed 3.12 X 2.5 µm in size (Plate I Fig. G). It was found on deciduous wood in East Africa from Ethiopia south to Malawi (Ryvarden and Johansen 1980). It was also found on living trees belonging to genera *Murraya, Carissa, Pyrus, Prunus, Eugenia*; causing white stringy rot. It is a common tropical species extending up to warmer temperate zones (Sharma, 1995). But in the present study it was found on living tree of *P. ferrugineum* causing white stringy rot

Collection examined: Wadia Palace Rajpipla, Gujarat; collected by N. Praveen Kumar, Accession no: MSU Bot. 140, 22 - 4-2007

39. *Phellinus robustus* (Karst.) Bourd. & Galz.

Bull. Soc. Mycol. France 41: 188, 1925

BASIONYM: *Fomes robustus* P. Karsten 1889

Basidiocarp perennial, effused-reflexed or sessile, first cushion like then ungulate to applanate, 11cm long 7.8 cm broad and 3 cm thick upper surface rusty brown to almost black firt finely tomentosed, glabrous dull crusty, margin rounded, glabrous black fertile below. Pore surface grayish brown, tubes distinctly stratified, 3 mm deep with thin layer of context in between pores. Pores small, circular, 5 per mm. Context rusty brown shiny distinctly stratified woody hard, 3mm thick Hyphal system dimitic, hymenial setae

rare to scattered, ventricose, thick walled reddish brown 26.5 X 6.3 µm tip acute. Cystidiole hyaline, narrowly clavate, ventricose with tip elongated up to 100 µm and projecting in tubes. Basidia clavate hyaline 12.5 X 6.3 µm spores globose often apiculate, hyaline smooth 6.3X 5.2 µm in diameter (Plate I Fig. I). Strongly dextrinoid. It causing White rot of heartwood of living hardwoods. On oaks in the Southwest the basidiocarps develop near the base of living trees and was associated with a butt and root rot in *Prunus, Quercus* (Gilbertson, 1979). It was found most common on living trees of *Abies pindow, Picea smithiana* and less frequently on species of *Quercus, Salix, Acer, Juglans, Aesculus* and *Taxus*; causing heart rot of living trees, very rarely on stumps/ dead rotting wood (Sharma, 1995). But in present study it was found on the living trees of *P. ferrugineum* and dead tree trunk of *Polyalthia longifolia.*

Collewction examined : Wadia Palace Rajpipla, Near the Botany Department, Vadodara, Gujarat; collected by N. Praveen Kumar, Dr. Arun Arya, Accession no: MSU Bot. 102, 103, 22-4- 2007, and 7-8-2007.

40. *Phellinus rhabarbarinus* (Berk.) Cunn.

Bull. New Zealand Dept. Sci. Industr. Res. 164: 229, 1965.

BASIONYM: *Polyporus rhabarbarinus* Berk. 1839 *Fomes rhabarbarinus* Berk. 1970

Basidiocarps perennial, solitary, applanate, attached by a broad lateral base, elongated, 25 cm long, 28.5 cm wide, 3 cm thick at base, woody hard when dry; pileus glabrous, concentrically sulcate and zonate, dark brown to black, with black crust; margin entire, matted tomentosed, lighter than the pileus. Pore surface, dark reddish brown, tubes concolorous with pore surface, 4 mm deep in each layes indistinctly stratified, pores round,small, 7 per mm. Context yellowish brown, fibrous, 5 mm thick at base. Hymenial setae abundant, rusty brown, ventricose, straight, thick walled 37.33 x 12.48 µm; basidia broadly clavate, hyaline, 12.48 x 6.25 µm in size; basidiospores hyaline, subglobose, 3.12x 2.5 µm in diameter. It was found on dead angiosperms, and it was probably collected in Brazil (Ryvarden and Johansen 1980). It was also found on dead hardwoods; causing white rot, a rare tropical to subtropical species (Sharma 1995). But in the present study it was found on living tree of *Peltophrum ferrugineum* causing white stringy rot.

Collection examined: Wadia Palace Rajpipla, Gujarat; collected by N. Praveen Kumar, Accession no: MSU Bot. 141, 22 - 4-2007

41. *Phellinus setulosus* (Llyod) Imaz.

Bull. Tokyo Sci. Mus. 6: 104, 1943.

BASIONYM: *Fomes setulosus* Lloyd 1915

Basidiocarps perennial, imbricate, sessile, broadly attached, woody heard, applanate, ungulate, 24 cm long, 18 cm wide, 2 cm thick near the base; upper surface reddish brown slowly becoming black, finely tomentosed, slowly glabrous, concentrically zoned, sulcate, without crust; margin yellwosih brown, thin, entire, velutinate. Pore surface dark brown; tubes stratified, 3 mm deep; pores 7 per mm. Context reddish brown, lacking distinct cuticle above, fibrous, up to 5 mm thick. Hyphal system dimitic, hymenial setae abundant, ventricose, often strongly swollen at base, apex straight, dark brown, thick walled with narrow lumen, 21.38 x 9.36 µm; basidia 15.6 x 6.25 µm; basidiospores pale yellow, globose, thin walled, 5.23 x 4.23 µm in diameter. It was found on dead wood in Africa like Kenya, Tanzania and Rwanda (Ryvarden and Johansen 1980). It was also found on dead angiospermous woods; causing white pocket rot; a rare species in the tropical forests (Sharma, 1995). But in the present study it is found on living tree of *P. ferrugineum.*

Collection examined: Wadia Palace Rajpipla, Gujarat; collected by N. Praveen Kumar, Accession no: MSU Bot. 142, 22 - 4-2007

42. *Phellinus shaferi* (Murrill) Ryv.

Norweg. J. Bot. 19: 235, 1972,

BASIONYM: *Fuscoporella shaferi* Murr. 1907,

Poria shaferi (Murr.) Sacc. & Trott. 1912.

Basidiocarp perennial, resupinate, effused, attached to on upper surface facing hymenial layer, golden brown (Plate II Fig. L), glabrous, 5 x 2.8 x 0.5 cm, margin is thin. Hymenium reddish brown with grayish tinge (Plate II Fig. K), pores angular to incised, 0.4 cm thick, 5 - 8 pores per mm (Plate II Fig. O), tubes concolorous with pore surface, up to 2 mm deep. Context present up to 1 mm thick, reddish-brown and fibrous. Setae present dark brown, acute tip, straight 12 - 20 x 4 - 8 µm (Plate II Fig.M, N). Hyphal system dimitic, skeletal hyphae golden brown, wider lumen, aseptate, unbranched, 3.12 µm generative hyphae pale yellow thin-walled branched, septate, 2 µm. Basidia not found. Basidiospores globose, pale yellow, thin-walled, 3.5 - 4.5 µm in diameter (Plate II Fig. P, Q). It was known only from Montserrat in the West-Indies (Ryvarden and Johansen, 1980). It was collected on Fergus Mountain, Montserrat, West Indies, on a

decorticated trunk (Murrill, 1907). But in the present study it was found on living tree of *Aegle marmelos.*

Collection examined: Near D.N Hall, Vadodara (Gujarat). Collected by Dr. Arun Arya, Accession no: MSU Bot.100, 15-3-2007

43. *Phelinus xerenticus* (Berk.) Pegler

Kew Bull. 21: 44, 1967.

BASIONYM: *Polyporus xeranticus* Berkeley 1854 *Polyporus cereus* Berk. 1854

Basidiocarps annual to perennial, effused-reflexed to sessile, solitary to imbricate, semicircular to elongated, often laterally confluent, up to 9 cm broad, 8 cm wide 5 -10 mm thick, coriaceous and flexible; upper surface first brightyellow then cinnamon and finally chesrnut brown, occasionally sulcate with faint concentric zones, tomentose, soon smooth with a cottony consistency, pore surface first bright ochraceous to yellow; tubes concolorous with pore surface, pores 4-5 per mm, distinctly stratified, thin layers of context: present in between tube layers, thin, dense, bright yellow, up to 5 mm thick, hyphal system dimitic, hymenial setae numerous, projecting, acuminate, rusty brown, 40-70 (90) X 5-11 µ, emerging out of hymenial layer, basidia 6.25 -9.33 X 3.12-6.25 µm, claviform; spores ellipsoid, 3.12 – 6.25 X 2.5 – 4 µm hyaline. Pegler found it on *Quercus* as sole host, but collections of *Abies* and were found in Nepal, Mongolia, USSR, China and Japan. It was also recorded from other Asian countries with native *Quercus* sp. (Ryvarden and Johansen, 1980). It was found on the bases of dead trees/ stumps of Oaks, causing white pocket rot with firm brown areas; one of the commonest Hymenochaetaceae in the temperate forests of Himalayas (Sharma, 1995). In the present study it was found on living tree of *P. ferrugineum* causing white pocket rot.

Collection examined: Wadia Palace Rajpipla, Gujarat, collected by N. Praveen Kumar, Accession no: MSU Bot. 143, 22 - 4-2007

44. *Schizophyllum commune* Fr.

Refer Padhiyar *et al.*, (2009) (Appendis I)

Collection examined: Arboratum of Botany department, Jivaraj sawmill in Vadodara Gujarat; collected by N. Praveen Kumar Accession No: MSU Bot. 145, 146; 7- 6- 2007 and 5-6-2008.

B. Studies on Ascomycetous fungi

47. *Daldinia concentrica* (Bolton) Cesati and de Notaris

Comment. Soc. Crittog. Ital. 1: 197. 1863.

Synanime: *Sphaeria concentrica* Bolton,1789; *Sphaeria fraxinea* Sibth., 1794.

The Ascocarps are ball like rounded or hemispherical initially brown and dense, stroma spherical, turbinate, sessile, aggregated, smooth, perithecial mounds, 2-10 cm diam x 3 - 4 cm high; violet-black in colour, with KOH-extractable pigments dark purple. Section of stroma shows concentric rings. The tissue between perithecia grayish brown, woody; the tissue below the perithecial layer composed of alternating zones, the darker zones dark brown, woody, 0.2-0.6 mm thick, the lighter zones brown, pithy, persistent, 0.6-1 mm thick.On the upper side cup shaped perithecia are present. Perithecium tubular, 0.3-0.5 mm diam x 1-2 mm high, shows attachment of asci with 8 inequilateral ascospores. Ostioles slightly papillate. Asci 212.35-250 µm total length x 9.33-12.25 µm broad, the spore-bearing part 75-90 µm long, the stipe 130-170 µm long, with apical ring bluing in Melzer's iodine reagent, discoid, 0.5-1 µm high x 3.12- 4.5 µm broad. Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 12.45-18.7 x 6.25-9.33 µm, smooth; epispore smooth. Found on *Feronia elephantum, Dalbergia sisoo* from arboretum, *Tamarindus indica* of Botanical garden and *Azadirachta indica* from sawmill in Vadodara.

Collection examined: Arboratum of Botany department, Botanical garden and saw mill in Vadodara; collected by Dr. Arun Arya, N. Praveen Kumar, Acccession No: MSU Bot 149.150, 6-6-2007, 7-7-2008

48. *Xylaria polymorpha* (Pers.) Grev.

The fungus produces erect finger like ascostorma, 3-10 cm tall; up to 2.5 cm across; tough; shaped like a finger but occasionally flattened; usually with a rounded tip; at first coated with a pale dust of conidia (asexual spores), soon blackish with a pale tip and eventually black overall; surface becoming minutely pimpled and wrinkled with maturity. The stroma has outer black coloured ectostroma and white endostroma. The perithecia are embedded in periphery. Each ascus present in periphery produces black coloured inequilateral ascospores. Asci cylindrical, 75 - 84 X 4.4 µm thick. Ascospores 6.6 – 11 X 3.3 - 4.4µm (Average 8.8 X 4.4 µm), Saprobic on decaying hardwood stumps and logs, usually at or near the base; sometimes appearing terrestrial but actually attached to buried wood; growing alone or, more commonly in clusters; causing a soft rot of the wood; appearing in spring and not decaying until late summer or fall. It was found on *A. arabica* from saw mills in vadodara and growing on *Hibiscus rosa sinensis* in botanical garden.

Collection examined: Saw mill in Vadodara, Botanical garden of Botany department vadodara, Gujarat; Collected by N. Praveen Kumar, Acccession No: MSU Bot 151,152, 5-5-2006, 4-6-2007

49. *Hypoxylon rubiginosum* Pers. ex. Fr.

Summa Veg. Scand. II, p. 384. 1849.

Synanime: *Sphaeria rubiginosa* Pers., 1796; *Hypoxylon stereoides* Fr., 1849.

Stromata effused-pulvinate, sometimes pulvinate, plane with perithecial mounds, 0.3-12 cm x 0.3-5 cm broad x 0.5-1.2 (-1.5) mm thick; surface grayish sepia; brown granules immediately beneath surface and between perithecia, with KOH-extractable pigments rust; the tissue below the perithecial layer usually inconspicuous; Perithecia, 0.2-0.5 mm diam x 0.3-0.6 mm high; Ostioles lower than the stromatal surface. Asci 110-180 µm total length x 6 -9 µm broad, the spore-bearing parts 6.3-9.3 mm long, the stipes 2.5-8.0 mm long, with apical ring lightly bluing to bluing in Melzer's iodine reagent, discoid, 0.8-1.5 µm high x 2-3 µm broad. Ascospores dark brown, unicellular, ellipsoid-inequilateral, (8-) 9.33-12.25 x 4 - 6 µm, It is found on *Tectona grandis.*

Collection examined: Ratanmahal Wildlife Sanctuary, Gujarat; Collected by N. Praveen Kumar, Acccession no: MSU Bot 153, 6-12-2005

Table 5: Occurrence of different wood degrading fungi in Ratanmahal Wildlife Sanctuary

Ascomycota:	
Xylariaceae:	*Daldinia concentrica* (Bolt. ex Fr.) Cesati & de Notaris
	Hypoxylon rubiginosum Pers. ex. Fr.
Basidiomycota:	
Auriculariaceae :	*Auricularia auricula* (L.) Underwood
Aphyllophorales	
Ganodermataceae :	*Ganoderma applanatum* (Pers. ex. Wallr.) Pat.
	Ganoderma lucidum (Leyss.) Karst.
Schizophyllaceae:	*Schizophyllum commune* Fr.
Hymenochaetales	
Hymenochaetaceae:	*Phellinus gillvus* (Schw., Fr.) Pat.
Schizoporaceae:	*Hyphodontia comtopsis* Burdsall and Nakasone
Lachnocladiaceae:	*Vararia pallescens* (Schw.) Rogers and Jacks.
Polyporaceae:	*Hexagonia apiaria* Pers. ,
	Hexagonia tenuis Hooker ex Fries
	Coriolopsis gallica (Fries) Ryv.,
	Coriolopsis aspera (Jungh.) Teng.
	Coriolus versicolor L. ex Fries.,
	Daedalea adamani Berk.
	Daedalea quericina f. *resupinata* Hennings
	Lenzites sterioides (Fr.) Ryv.

	Polyporus xanthopus Fr.
	Polyporus violaceo- cinerescens Petch.
Fomitopsisaceae:	*Fomitopsis pinicola* (Swartz) P. Karsten.
	Fomitopsis rosea (Alb. and Schwein.) P. Karst.
Steccherinaceae:	*Flavodon flavus* (Klotzsch) Ryv.

The list of southern Indian fungi compiled by Rangaswami and coworkers (1970) included 44 polyporoid species belonging to 13 genera of which only 5 are from Kerala. More recently Natarajan and Kolandavelu (1985) described some resupinate aphyllophorales from Tamilnadu region and this includes the poroid members *Inonotus Polymorphus* (Rostk.) Pilat, *Phelinus alladii* (Bers.) Ryv. *P. umbrinellus* (Bers.) Ryv. and *P. purpureo-gilvus* (Petch) Ryv. The most recent work on Polyporaceae of India is by Roy and De (1996) which gives a record of only six polypores from Kerala. 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 fallen *Heritiera minor* in Baroda, Gujarat. 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. In their compilation of fungi of India, Butler and Bisby (1931) listed 293 polyporoid species in 16 genera. A monograph on Indian Polyporaceae on the trees and timbers by Bakshi (1971) describes 355 species belonging to 15 genera.

In the present study 46 Aphyllophorales (Basidiomycota) and 3 Ascomycota members were collected and studied for their morphological and anatomical characters. In which new and interesting record of Basidiomycetous fungus was *Lenzites sterioides,* which was recorded for the first time on *T. grandis* and second time from the country (Arya *et al.,* 2008). *Navisporus floccosa, Coriolopsis aspera, Ganoderma curtisii, Microporus alboater,* and *Phellinus shaferi* were reported for the first time from India. Certain new varieties of fungi reported included *Microporus affinis* var. *glabriceps* and *Aurificaria indica* var. *indica.* The new host records for the fungi were *Phellinus badius, P. caryophyllii, P. conchatus, P. hoehnelii, P. pachyphloeus, P. pectinatus, P. rhabarbarinus* and *P. robustus* on *Peltophorum ferrugineum* (Decne.) Benth. Fungus *Irpex hydnoides* was found for the first time on *Tamarindus indica* L.

Detailed Life Cycles

Navisporus floccosa (Bres.) Ryv. causing Heart rot

Heart rot fungi are specialized organisms that attack the heartwood of living trees. They colonize the central portion of tree and begin the decomposition process of wood which ultimately leads to death of trees. Infection occurs when basidiospores are released from the hymenial surface. They accumulate in the butt region of the host host tree. Favourable moisture and temperature conditions permit the spores to germinate. The fungus grows slowly into the vulnerable wood tissues of the trunk. The Butt rot fungus initially enters into the roots to produce decay and then spreads into the butt portion of tree. It produces predominantly or solely heart rot, invading only the tree's central column of physiologically inactive (nonliving) heartwood. It spreads more in a vertical direction than in a horizontal plane from the place of infection zone.the fungus produced hollow in the fallen tree trunk.

The first symptoms indicative of fungal infection are discoloration of heartwood. It becomes dark brown, sapwood becomes brown. Incipient decay in heartwood is difficult to detect but wood colour is intermediate between that of sound and decayed wood, i.e. it is usually of darker colour than normal heartwood. The cross section of infected *Ailanthus excelsa* stem shows a barrier zone line indicating that almost all of the heartwood is infected and only a little portion of sound wood is left (Plate VIII Fig. E). The dark brown zone indicates that the wood is in advanced stage of decay with only remnants of fibers in hole.

Fungus degrades both cellulose and lignin, finally leaving behind a yellowish-white, spongy mass. With advancement of decay the wood tissues are accumulated with bundles of hyphae, whereas remnants of fibers are present in the center. As the decay proceeds to the top of the tree, the fruiting bodies develop at butt region. During July 2007 (Monsoon months) due to the high wind velocity the weak tree were up rooted. Fungus severely decayed heartwood and produced the fruiting bodies outside the trunk. The small pices of wood samples when placed on the PDA medium, pure culture of the fungus was obtained which indicated that the fungus was in active state.

Ganoderma rot

Ganoderma lucidum (Fr.) Karsten is a white rot fungus that produces numerous enzymes that allows it to degrade wood tissues, primarily lignin and cellulose. As the fungus destroys the wood internally, the xylem (water-conducting tissue) will eventually

be affected. It spreads primarily by the spores produced in the basidiocarp (conk). The basidiospores become incorporated into the soil, germinate in presence of moisture and then grow over the *Cassia* roots. The fungus can also spread through root grafting from infected to healthy roots. *G. lucidum* first infects main roots, gradually, the fungus spreads to the secondary or tertiary roots. The white mycelium progresses upwards towards the crown of trunk, where the fungus develops strands or ribbons called rhizomorphs. These structures produce fan-shaped fruiting bodies called brackets or semicircular conks at or near the base of the tree in rainy season. These brackets are stalked. Infected trees show the wilting of the foliage followed by death of the tree. Affected shoot buds of *C. fistula* exhibited a yellowing and sparseness of leaves, defoliation and progressive dieback of limbs, particularly at the top of the tree. The fungus colonized and degraded the trunk tissue closest to the soil line first, expands in diameter at the base and moves upto the center or near-center of the trunk. Therefore, the disease progression pattern within the trunk is best described as cone-shaped, i.e. widest at the soil line and narrowing to a pinpoint upwards. With time, the infected roots and trunk would become spongy and disintegrate.

Spores of *G. lucidum* are released from the brackets or the conks, which may vary in size from 6.25 - 9.35µm in size. These semicircular fruiting bodies produced millions of spores that are released over a five- to six-month period. Spores can be released under a variety of environmental conditions. When the soil is dry, the brackets derive the moisture from the tree to enable the continuation of spore release. By the time the tree shows visual symptoms of infection, it is usually too late to control fungal invasion and damage. If the fungus is restricted to the heartwood, only the structural integrity of the tree is impaired. Otherwise, trees with heart rot may remain productive for many years. The *G. applanatum* also causes the butt rot. Where it produces numerous brackets (Conks) in case of severe infection. As infection increases in upward direction the mycelial cords come out of the bark and forms bracket shaped fruiting bodies throughout the trunk region. *Ganoderma* root rot in an *Acacia arboretum* was studied by Harsh *et al.,* (1993). Screening resistance of *Dalbergia sissoo* clones against *Ganoderma lucidum* root rot disease in field conditions was tried by Harsh et al., (2010). In the present study the *Ganoderma* root-rot was identified and studied the detailed didease cycle on *Cassia fistula.* The root rot disease was caused by *Ganoderma lucidum* on roots of *Cassia.*

Phellinus hoehnelii (Bres.) Ryv. causing stem rot

The first outward indication of stem decay is presence of fruiting bodies (sporocarps) on the outside of the stem. The fruiting bodies are formed after the fungus has been present for some time and has caused a significant decay. Basidiospores released from pore layer of basidiocarps during wet periods cannot penetrate stem, but gain entry to trees by colonizing exposed dead wood and branch stubs, open knots, and wounds. Spores germinate and develop into vegetative structures capable of extracting nutrients from wood. The fungus grows down a branch stub or into a wound surface to reach the inner wood and digest the various wood components resulting into wood decay. Decay columns slowly expand, in such cases shrinking of sapwood areas results and this result into physiological stress and slower the growth of trees. The fungus decayed wood inside the stem, it also grew out along branch traces and killed the cambial inside branches. The softened inner wood also created habitat suitable for excavation of cavity nests by birds.

The symptoms produced in heart wood decay were discoloration of the decayed wood. The cambium produces the barrier zone against the decay wood. The columns extend in both directions i.e. towards the shoot and root to form a hole inside the decayed wood. Inside the hole, fungal hyphae forms loose network of mycelium which appears in form of white patches. *P. hoehnelii* causes white rot and creates decayed wood that often tends to be lighter in colour, with a stringy texture or a pocketed appearance. In case of severe infection *P. hoehnelii* produces more number of sporophores at butt region and throughout the trunk region. Fruiting bodies may develop on the bole for many years as the decay column expands. A large number of old tree of *P. ferrugineum* showed disease caused by *P. hoehnelii* (Bres.) Ryv. and different species of *Phellinus*.

Three different wood decay fungi causing wood rotting diseases were studied. The *Navisporus floccosa* heart rot was recorded in living trees of *Ailanthus excelsa* Roxb. for the first time. The details of *Phellinus hoehnelii* stem rot in living trees is studied for the first time.

3. Cultural Characters of Certain Timber Degrading Basidiomycetes

3.1. Isolation of timber degrading fungi

The fungi associated with the wood samples were isolated. 80 fungal cultures were obtained from 18 different plant species (Table 6). The growth of fungi was much better in Malt extract medium. The results showed that many fungal species were not

host specific and affected most of the wood species tested. Frequently isolated molds were *Aspergillus* spp., *Trichoderma* spp., and *Rhizopus* spp., and frequently wood decay fungi were belonging to the phylum *Basidiomycota.* In nature, the percentage occurrence of *Trichoderma* spp. was 20.5, for *Rhizopus* spp. 14.1 and it was 14.1 for members of Basidiomycota.

Around 10 wood decay fungi were isolated on Malt extract agar medium. They were two isolates of *Lenzites strioides, Phellinus nilgheriensis, Navispora floccosa, Ganoderma lucidum, Hexagonia apiaria, Schizophyllum commune, Trametes* sp., *Flavodon flavus* and two other members of Basidiomycetes, *which* cause white rot in different woods. The grey mold was caused by *Absidia* sp., *Rhizopus stolonifer* and member of Mucarales, Brown mold was caused by *Aspergillus awamorii,* Green mold was caused by *A. flavus, A. fumigatus, Trichoderma viride* and *T. harzianum.* Black mold was caused by *A. niger, Aspergillus* sp. *Chaetomium globosum, Chaetomium* sp. and *Cladosporium herbarum,* and *Phoma multirostrata* and Pink mold was caused by *fusarium oxysporum* and *F. pallidoroseum.*

Table 6: Isolation of wood inhabiting fungi from Plants growing in Ratanmahal Wildlife Sanctuary on PDA and Malt Extract Agar medium.

Plant	Isolate	Fungal taxon	PDA	MAE
Holarrhena antidysenterica (Apocynaceae)	RS1a	*Trichoderma viride*	+	--
	RS1b	*Aspergillus niger*	+	--
	RS1c	*A. fumigatus*	+	--
	RS1d	*Absidia* sp.	+	--
	RS1e	*Fusarium pallidoroseum*	+	--
	RS1f	*Rhizopus stolonifer*	+	--
Tectona grandis (Verbenaceae)	RS2a	*Aspergillus awamorii*	+	--
	RS2b	*Lenzites sterioides2*	--	+
	RS2c	*Trichoderma harzianum*	+	--
	RS2d	*A. flavus*	+	--
	RS2e	*Phellinus* sp.	--	+
	RS2f	*Rhizopus stolonifer*	+	--
Alangium salvifolium (Alangiaceae)	RS3a	*Schizophyllum commune*	--	+
	RS3b	*Lenzites sterioides* 1	--	+
	RS3c	*Chaetomium globosum*	+	--
	RS3d	*Cladosporium herbarum*	+	--
	RS3e	*Trichoderma harzianum*	+	--
	RS3f	*Rhizopus* sp.	+	--
Madhuca indica	RS4a	Basidiomycetes	--	+

(Sapotaceae)				
	RS4b	*Trametes* sp.	--	+
	RS4c	*Phoma multirostrata*	+	--
	RS4d	*Black mycelium*	+	--
	RS4e	*Theliviopsis state of Ceratocystis paradoxa*	+	--
	RS4f	*Theliviopsis* state of *Ceratocystis paradoxa*	+	--
	RS4g	*Pestalotiopsis* sp.	+	--
Terminalia crenulata (Combretaceae)	RS5a	*Aspergillus fumigatus*	+	--
	RS5b	*Flavodon flavus*	--	+
	RS5c	*Trichoderma viride*	+	--
	RS5d	Hyphomycetes	+	--
	RS5e	*Helicocephalum* sp.	+	--
	RS5f	*Fusarium oxysporum*	+	--
	RS5g	Black mycelium	+	--
	RS5h	*Rhizopus stolonifer*	+	--
Mitragyna parviflora (Rubiaceae)	RS6a	*Trichoderma viride*	+	--
	RS6b	*Fusarium oxysporum*	+	--
	RS6c	Hyphomycetes	+	--
	RS6d	*Rhizopus stolonifer*	+	--
Lannea coromendalica (Anacardiaceae)	RS7a	*Trichoderma harzianum*	+	--
	RS7b	Mucor *racemosus*	+	--
	RS7c	*Aspergillus niger*	+	--
	RS7d	Black mycelium	+	--
	RS7e	*Rhizopus stolonifer*	+	--
Holoptelia integrifolia (Ulmaceae)	RS8a	*Phoma multirostrata*	+	--
	RS8b	*Ganoderma lucidum*	--	+
	RS8c	*Trichoderma viride*	+	--
	RS8d	*Rhizopus stolonifer*	+	--
Diospyros melanoxylon (Ebenaceae)	RS9a	*Aspergillus awamori*	+	--
	RS9b	Black mycelium	+	--
	RS9c	*Aspergillus sp*	+	--
	RS9d	Black mycelium	+	--
	RS9e	*Pestalotiopsis* sp.	+	--
Terminalia bellerica (Combretaceae)	RS10a	*Phellinus nilgheriensis*	--	+
Garuga pinnata (Burseraceae)	RS13a	*Trichoderma viride*	+	--
	RS13b	*Trichoderma* sp.	+	--
	RS13c	*Chaetomium globosum*	+	--
	RS13d	*Rhizopus stolonifer*	+	--
Bambusa arundinacea (Bambusaceae)	RS14a	*Bondarzewia berkleyii*	+	--

	RS14b	*Pestalotiopsis* sp.	+	--
	RS14c	*Aspergillus niger*	+	--
	RS14d	*Trichoderma harzianum*	+	--
	RS14e	*Rhizopus* sp.	+	--
Emblica officinalis (Euphorbiaceae)	RS15a	*Phoma multirostrata*	+	--
	RS15b	*Theliviopsis* state of *Ceratocystis paradoxa*	+	--
	RS15c	*Trichoderma viride*	+	--
	RS15d	*Rhizopus stolonifer*	+	--
Dalbergia sissoo (Fabaceae)	RS16a	*Trichoderma harzianum*	+	--
	RS16b	*Aspergillus flavus*	+	--
	RS16c	*Phoma multirostrata*	+	--
Cassia fistula (Caesalpiniaceae)	RS17a	*Trichoderma viride*	+	--
	RS17b	*Navispora floccosa*	--	+
	RS17c	*Trichoderma harzianum*	+	--
	RS17d	*Hexagonia apiaria*	--	+
	RS17e	*Rhizopus stolonifer*	+	--
Mangifera indica (Anacardiaceae)	RS18a	*Aspergillus flavus*	+	--
	RS18b	*Trichoderma harzianum*	+	--
	RS18c	*A. niger*	+	--
	RS18d	*Helicosephalum* sp.	+	--
Cordia dichotoma (Boraginaceae)	RS19a	*Absidia* sp.	+	--
Uknown wood	UKa	*Trichoderma* viride	+	--
	UKb	*Absidia* sp.	+	--

+ isolated , -- no growth was observed

3.2. Cultural characters of certain wood rotting fungi

1. *Coriolus versicolor*

On the Malt extract agar plates with tannic acid it showed a positive reaction for oxidases and Laccase, catalase/tyrosinase negative, peroxidase positive, In KOH it turns brown, Growth characters: Growth rapid, plates covered in two weeks >70mm. Advancing zone even, densed, hyaline, appressed for short distance in advance of aerial' mycelium. Mat white, raised cottony-woolly, otherwise felty, mycelium grown up sides of Petri dish and across cover after three weeks. Reverse bleached. Odor absent. Growth of colony 1.5 cm. diameter on gallic acid agar and 2. 5-3.0 cm. diameter on tannic acid agar.

Hyphal characters: Advancing zone: hyphae hyaline, nodose-septate, with clamp, 3.12 -6.25 μ diameter. Aerial mycelium: (a) hyphae as in advancing zone; (b) fiber

hyphae numerous, thick-walled, lumina discernible only at bases of branches, frequently branched, 2.0-3.12 µm diameter, curving and interwoven; (c) chlamydospores fairly numerous, some times arthroconidium like, usually found lying free in preparations for microscopie examination, thin-walled, 4.5 - 9.33 x 3.12 – 6.25 µm. Submerged mycelium: hyphae as in advancing zone without clamps. Cosmopolitan.

2. *Flavodon flavus*

Refer Padhiyar *et al.*,(2009) (Appendix I)

3. *Ganoderma lucidum*

On Malt extract agar medium with tannic acid it showed positive reaction to oxidase and strong positive reaction to laccase, and Strong positive reaction to peroxidase. Negative reaction to catalse/tyrosinase. In KOH it turns to rusty brown. Growth characters: Growth rapid, plates covered in one weeks and covers 70 mm in one weak. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white, azonate, with color subsequently masked by overgrowth of whitish 'bloom', at first slightly raised, cottony, then appressed. Reverse unchanged for two to three weeks, then finally bleached. No odor. On tannic acid agar growth of colony was 2.0-4.0 cm in diameter in seven days.

Hyphal characters. Advancing zone: hyphae hyaline, nodose-septate, with clamps, 3.12 - 5.5 (6.0) µm diameter. Aerial mycelium, thin tough skin that peels from agar (a) hyphae as in advancing zone, with frequent branches and numerous small projections; (b) fiberous hyphae very numerous, with walls thick and refractive, lumina narrow or apparently lacking, except in main hyphae, frequently branched, the ends long, slender, curving and interwoven, 1.0 - 3.12 µm diameter; (c) cuticular cells thin-walled, produced by inflation of nodose-septate hyphae, at first with contents staining in phloxine, then empty, closely compacted and interwoven with fiber hyphae and staghorn hyphae to form pseudoparenchymatous layer, which may remain hyaline or become brown; (d) chlamydospores numerous, walls slightly thickened, terminal and intercalary, broadly ovoid to elongate, 12.25 - 21.65 x 6.25 - 12.5 µm. Submerged mycelium: (a) nodose-septate hyphae without clamps and (b) chlamydospores as described above.

4. *Hexagonia apiaria*

On Malt extract agar medium with tannic acid it showed positive reaction to oxidase and strong positive reaction to laccase and positive reaction to peroxidase. Negative reaction to catalse/tyrosinase. In KOH it becomes brown. Growth characters: Growth rapid coverd petri plate in one week, Advancing Zone, even, densed, hyaline,

areal mycelium is apprised. Mat white, Plumose, azonate, later changes to cinamin brown. Reverse bleached. Odor absent.

Hyphal characters: Advancing zone: hyphae hyaline, septate, with clamp, 3.12 µm in diameter. Arial mycelium (a) hyphae as in advancing zone; (b) fiberous hyphae numerous, thick-walled, narrow lumen, branched, 3.12- 6.25 µm diameter, curving and interwoven; (c) chlamydospores few, smooth, thin-walled, 3.12 – 6.25 x 3.12 µm.

5. *Lenzites betulina*

On Malt extract agar medium contaning tannic acid showing strong positive reaction to oxidases, and laccase, positive reaction to peroxidase negative reaction to catalase/tyrosinase. In KOH it becomes pale brown. Growth characters: Growth moderately rapid, covers 25-40mm in two weeks. Whole plate covered in four weeks. Advancing zone even, hyaline distant, and appressed in narrow zone and slightly raised aerial mycelium extending to limit of growth. Mat white, the newest growth slightly raised, woolly, becoming felty to lacunose, often with balls of mycelium, Basidiocarps typically not formed, becoming patchy, very tough, cream coloures in older cultures, all peeling readily from agar, the mycelium frequently grown up sides and down between lid and base of Petri dish. Reverse bleached. Odor: absent. Colony growth on tannic acid agar is 2.5- 4.5 cm. in diameter in seven days.

Hyphal characters. Advancing zone: hyphae hyaline, nodose-septate, 3.2-5.5 µm diameter. Aerial mycelium: (a) hyphae as in advancing zone; (b) fiberous hyphae very numerous, thickwalled and refractive, narrow lumen, much branched, 3.12- 6.25 µm diameter. Submerged mycelium: (a) nodose-septate hyphae without clamps and (b) fiber hyphae as in aerial mycelium. It was cosmopolitan in distribution.

6. *Lenzites sterioides*

On Malt extract agar medium containing tannic acid showing strong positive reaction to oxidases, and positive reaction to laccase and peroxidase. Negative reaction to catalase/tyrosinase. In KOH it becomes pale brown. Growth characters: Growth rapid, covers petri plate with in two – four weeks. Advancing Zone, wavey some times and mostly even, densed, hyaline, areal mycelium is raised for some distant and appressed as it becomes old. Mat white, cottony-wooly, becomes locally floccose, azonate, later changes to creamy white, thin layer of mycelium balls pealed from the agar. Reverse unchanges. Odor absent.

Hyphal characters: Advancing zone: hyphae hyaline, mostly septate, with clamp, 1.2 - 3.12 µm in diameter. Arial mycelium (a) hyphae as in advancing zone, thinwalled,

branched, wider lumen with 6.25 µm in diameter; (b) fiberous hyphae thick-walled, narrow lumen, branched, 3.12 - 6.25 µm diameter; (c) chlamydospores numerous, ellipsoidal, smooth, thin-walled, 6.25- 12.52 x 3.12-6.25 µm. Submerged mycelium: hyphae as in advancing zone without clamps. Mostly on Angiospermous wood.

7. *Navisporus floccosa*

On Malt extract agar medium with tannic acid it showed positive reaction to oxidase, laccase and peroxidase. Negative reaction to catalase/tyrosinase. In KOH it becomes pale brown. Growth characters: Growth rapid, covers petri plate in three week, covers >70mm in two weeks, Advancing zone wavy, densed, hyaline, areal mycelium is raises always. Mat white, cottony-wolly, azonate, later changes to cream colour. Reverse bleached. Odor absent.

Hyphal characters: Advancing zone: hyphae pale yellow, septate, with clamp, 3.12 – 6.25 µ in diameter. Arial mycelium (a) hyphae as in advancing zone; (b) fiberous hyphae numerous, yellowish in colour, thick-walled, narrow to wider lumen , branched, aseptate, 6.25 - 8.23 µm diameter; (c) chlamydospores few, elipsoidal, thin-walled, 3.12 – 6.25 x 3.12 µm.

8. *Schizophyllum commune*

Refer Padhiyar *et al.*, 2009 (Appendix I)

9. *Sterium hirsutum*

On Malt extract agar medium with tannic acid it showed negative reaction to oxidase, laccase and peroxidase and positive reaction to catalase/tyrosinase. In KOH it becomes pale yellow to red. Growth characters: Growth very rapid, covers petri plate in two week, covers >70mm in one week, Advancing zone even, densed, hyaline, areal mycelium is raises for some distance and becomes apprised as culture becomes old. Mat creamy, plumose, azonate, later changes to creamish yellow. Reverse bleached. Odor absent.

Hyphal characters: Advancing zone: hyphae pale yellow to hyaline, septate, multiple clamp, branched, 3.12 – 12.56 µm in diameter. Arial mycelium (a) hyphae as in advancing zone; (b) fiberous hyphae numerous, yellowish in colour, thick-walled, narrow to wider lumen, branched, aseptate, 3.125 - 9.33 µm diameter; (c) chlamydospores absent. Submerged mycelium: hyphae as in advancing zone without clamps. On various Angiospermous woods, cosmopolitan.

10. *Trametes pini*

On Malt extract agar medium with tannic acid it showed positive reaction to oxidase, and laccase and negative reaction to peroxidase and catalase/tyrosinase. In KOH it becomes rusty brown. Growth characters: Growth rapid, covers petri plate in three weeks. Advancing zone even, densed, white, raised aerial mycelium extending to limit of growth later it appressed. Mat white, pale yellow in old culture raised, azonate, cottony woolly to woolly, tufted to form a rough surface. Reverse less colored appears brown, later it was darker. Odor was not felt.

Hyphal characters. Advancing zone: hyphae hyaline, with simple septa, having few clamps 3.12-5.5 µm diameter. Aerial mycelium: (a) hyphae as in advancing zone; (b) hyphae with slightly thicker walls, with contents yellow, brown, septate, branched, 3.12-6.25 µm diameter, characteristically with scattered dark brown cells in hyaline or pale hyphae, frequently helicoid; (c) expansions on hyphae up to 8.5 µm diameter chlamydospores in a terminal or intercalary position, usually with walls brown and thickened; (d) setae numerous in some isolates, rare, slender, pointed, with walls thick and dark brown, 30.0-67.0 x 6.5-9.33 µm.

4) Molecular characters of certain Timber Degrading Fungi

It is evident from the table 7 that wood rotting fungi were collected from different areas of Gujarat. Initially 18 RAPD primers (20 primers Kit E, IDT USA; 160 primers, kit- J, K, L, N, O, P, Q, R, Operon technologies Inc., USA) were screened and out of which 15 primers responded with minimum 6 loci (bands) were included in the study. In the further screening 10 primers which gave fingerprints with good resolution and band reproducibility were used in the final analysis to characterize *L. sterioides, L. betulina, L. exima, Flavodon flavus* strain 1, *F. flavus* strain 2, *G. lucidum, G. applanatum, Phelinus robustus* , *S. commune, Pluerotus* sp., and *S. hirsutum.* For AFLP DNA fingerprinting initially 24 combinations of *EcoR*I and *Mse*I with three nucleotide selective primers were screened, and out of which 12 primer combinations that gave sharp fingerprints in which 4 primer combinations with maximum number of loci with good resolution were selected for molecular analysis of above described genera. In total 166 RAPD loci and 590 AFLP loci were generated and used to study molecular divergence and to deduce genetic relation among the genera.

4.1. RAPD analysis:

Use of ten RAPD primers has produced totally 166 markers out of which 165 markers were found to be polymorphic. Markers obtained for each primer varied from 10 (OPL-5) to 22 (OPL-18) (Plate III Fig. A). On an average each primer produced 16.6 markers out of which 16.5 were polymorphic markers. Primer OPO-19 and OPN-6 has produced 1 common markers in all the species studied which is the highest number with any primer used in this study. 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). The Genetic similarity (GS) between *L. sterioides* and *L. betulina* is 0.693, the genetic similarity between *L. betulina* and *L. exima* is 0.596, the genetic similarity between *L. exima* and *F. flavus* strain 1 is 0.789, the genetic similarity between *F. Flavus* strain 1 and *F. flavus* strain 2 is 0.584, the genetic similarity between *F. flavus* strain 2 and *G. lucidum* is 0.56, the genetic similarity between *G. lucidum* and *G. applanatum* is 0.627, the genetic similarity between *G. applanatum* and *P. robustus* is 0.59, the genetic similarity between *P. robustus* and *S. commune* is 0.566, the genetic similarity between *S. commune* and *Pleurotus* sp. is 0.777, the genetic similarity between *Pleurotus* sp. and *S. hirsutum* is 0.548. On the contrary intrageneric GS was found 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 among all genus studied. In the pair wise comparison the mean PP was 98.87 (Table 8 & 9, Histogram 2).

4.2. AFLP analysis:

Using 4 combinations of AFLP selective primers 590 markers were generated out of which 586 makers were found to be polymorphic. On an average each primer has produced 49.16 markers with 48.88 polymorphic markers. Markers obtained for each primer varied from 153 (EAGG/MCTG) to 135 (EAGG/MCAT) (Plate IV Fig. A). Over all PP among seven genera was found to be 99.32. The GS found in between *L. sterioides* and *L. betulina* is 0.597, the genetic similarity between *L. betulina* and *L. exima* is 0.825, the genetic similarity between *L. exima* and *F. flavus* strain 1 is 0.717, the genetic similarity between *F. flavus* strain 1 and *F. flavus* strain 2 is 0.564, the genetic similarity between *F. flavus* strain 2 and *G. lucidum* is 0.514, the genetic

similarity between *S. commune* and *Pleurotus* sp. is 0.88. 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 showed 100% polymorphism among 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 (Table 10 & 11, Histogram 3).

The dendrogram generated according to Jaccard [1908] from the binary data of RAPD and AFLP. Based on the 166 RAPD fragments, dendrogram was prepared to determine the relationship between 7 genera of wood rotting fungi Aphyllophorales. The 7genera were clearly divided into two groups I and II. The II group includes the *F. flavus* strain 2 separated from all wood rotting fungi studied. The main group I is sub grouped into A and B. the sub group A includes the *L. sterioides* and *L. betulina* with approximately 60% similarity. The sub group B includes the *P. robustus* as sub group B1 with in the same sub group. The other wood rotting fungi form the sub group B2 with the same sub group of B. the *L. exima* and *F. flavus* strain 1 showed 100% similarity in RAPD dendrogram. The *Pleurotus* sp. and *S. commune* showed approximately 90% genetic similarity. The *G. Lucidum* showed around 70% similarity with *L. exima* and *F. flavus* strain 1. The *G. applanatum* is forming into a separate group whereas the *G. lucidum* is showing some similarity with *G. applanatum* forming as separate cluster (Plate III Fig B).

Based on the 590 AFLP fragments, dendrogram was prepared on the basis of similarity between the 7 genera of wood rotting fungi Aphyllophorales. The 7 genera were clearly divided into two groups I and II. The group I contains *S. hirsutum* formed as separate group. The group II is sub grouped into A and B. The B group contains *G. applanatum* formed as separate group when compared to genetic similarity of wood rotting fungi under the study. The A. group is sub grouped into A1 and A2 with in the same sub group A. from the *G. lucidum* cluster only the remaining wood rotting fungi studied show similarity and forming sub group A2. *L. betulina* and *L. exima* showed 100% genetic similarity. The *S. commune* and *Pleurotus* sp. showed around 80% similarity and the *F. flavus* strain 2 shows around 60% genetic similarity with the *S. commune* and *Pleurotus* sp. (Plate IV Fig. B).

Table 7: Details of the geographic locations of collected fungal samples used in the present study

Serial number	Sample code	District provenance of collection (Gujarat)	Latitude	Longitude
1	L. S	Panchmahal	22° 46' 46" N	73° 39' 49" E.
2	Len A	Vadodara	25° 25' N	76° 70' E
3	Len a	Narmada	21.24° to 22° N	72.4° to 73.15° E
4	FFA	Vadodara	25° 25' N	76° 70' E
5	FF P	Panchmahal	22° 46' 46" N	73° 39' 49" E.
6	Gano p	Panchmahal	22° 46' 46" N	73° 39' 49" E.
7	Gano A	Vadodara	25° 25' N	76° 70' E
8	Phe	Narmada	21.24° to 22° N	72.4° to 73.15° E
9	Schizo	Vadodara	25° 25' N	76° 70' E
10	Pluer	Ahmedabad	23° 03' N	72° 40' E
11	Steri	Vadodara	25° 25' N	76° 70' E

Table 8: RAPD primers showing the percentage of polymorphism/similarity in different timber rotting fungi.

Primer Name	No. of makres	Percentage of polymophism	percentage of similarity
OPL-1	16	100	0
OPL-5	10	100	0
OPO-7	15	100	0
OPL-14	21	100	0
OPL-18	22	100	0
OPN-10	16	100	0
OPL-4	12	100	0
OPO-19	16	93.75	6.25
OPQ-15	18	100	0
OPN-6	20	95	5
	Mean	98.87	1.12

Table 9: RAPD Genetic similarity of different wood rotting fungi

	2	3	4	5	6	7	8	9	10	11
1	0.693									
2	0.651	0.596								
3	0.633	0.590	0.789							
4	0.530	0.560	0.578	0.584						
5	0.620	0.614	0.693	0.747	0.560					
6	0.620	0.627	0.729	0.675	0.572	0.627				
7	0.560	0.518	0.633	0.602	0.524	0.687	0.590			
8	0.584	0.554	0.645	0.699	0.548	0.639	0.651	0.566		
9	0.590	0.584	0.651	0.657	0.602	0.608	0.669	0.620	0.777	
10	0.560	0.578	0.620	0.627	0.548	0.639	0.602	0.566	0.614	0.548

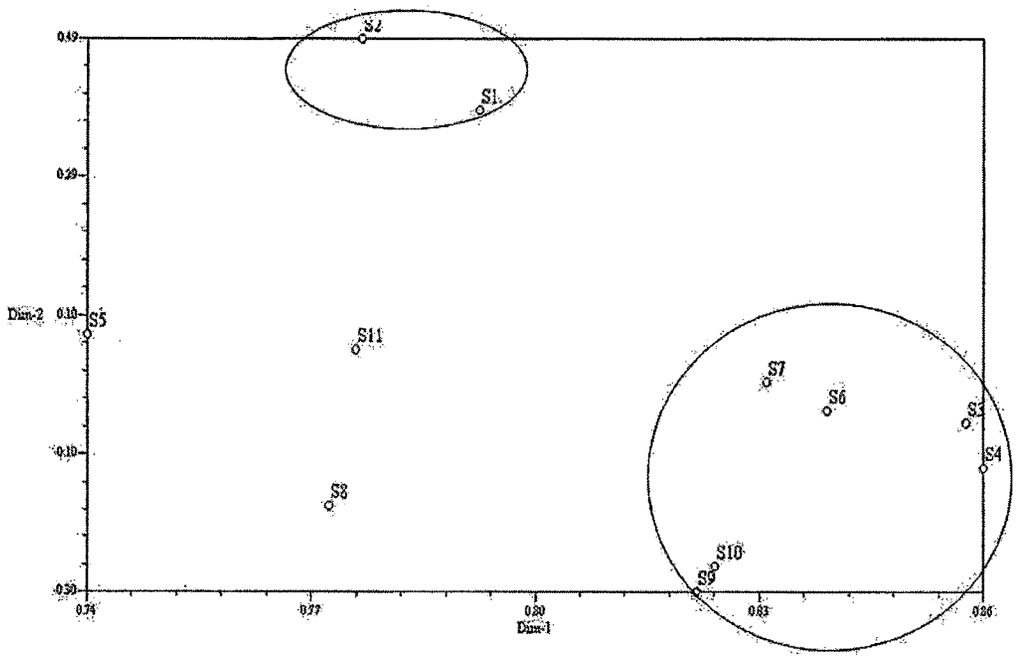
Table 10: AFLP selective primers showing the percentage of polymorphism/ similarity in different timber rotting fungi.

Primer Name	No. of makres	Percentage of polymophism	percentage of similarity
P48(EACG/MCTT)	151	99.33	0.67
P52(EAGC/MCAT)	151	100	0
P60(EAGG/MCAT)	135	98.52	1.48
P63(EAGG/MCTG)	153	99.34	0.66
	Mean	99.2975	0.7025

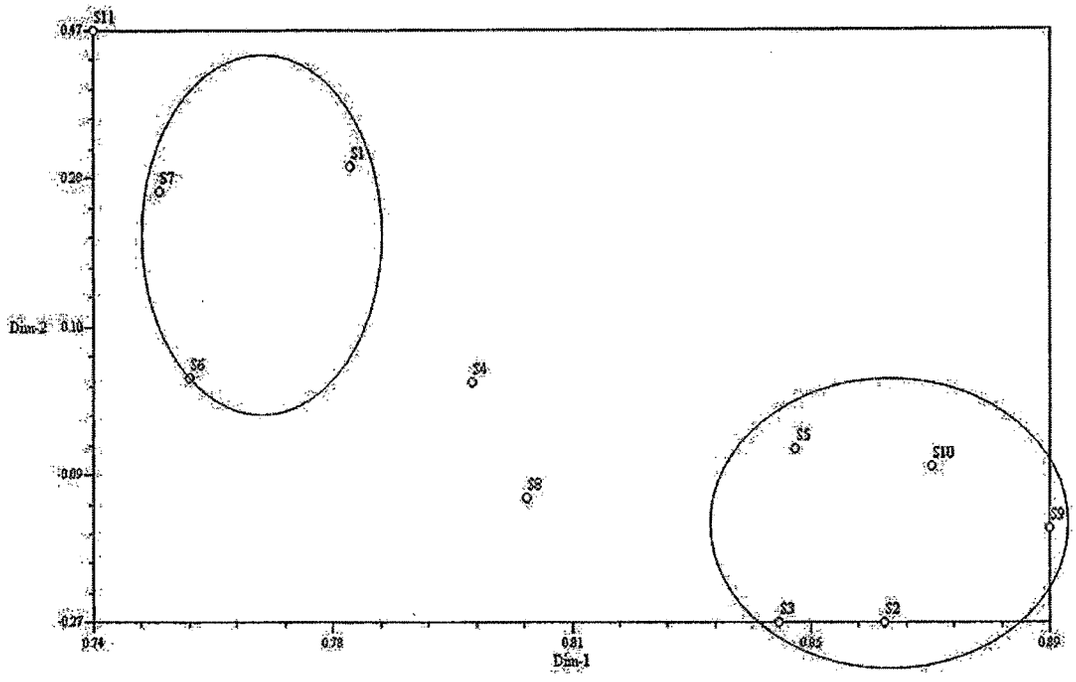
Table 11: AFLP Genetic similarity of different wood rotting fungi.

	2	3	4	5	6	7	8	9	10	11
1	0.597									
2	0.585	0.825								
3	0.542	0.725	0.717							
4	0.542	0.623	0.625	0.564						
5	0.532	0.590	0.612	0.556	0.514					
6	0.569	0.732	0.758	0.664	0.572	0.637				
7	0.561	0.666	0.685	0.598	0.570	0.608	0.646			
8	0.573	0.678	0.724	0.627	0.576	0.593	0.658	0.639		
9	0.581	0.697	0.756	0.649	0.598	0.612	0.693	0.620	0.880	
10	0.568	0.632	0.637	0.578	0.576	0.541	0.656	0.569	0.608	0.614

Histogram 2: RAPD Percentage of cumulative analysis of different wood rotting fungi



Histogram 3: AFLP Percentage of cumulative analysis of different wood rotting fungi



The term DNA fingerprint too often is used improperly to refer to any complex pattern of DNA bands on a gel. Before a banding pattern can be classified correctly as a DNA fingerprint, a thorough statistical analysis is needed to validate its unique properties. The analysis generally is simple, based on using frequencies of individual bands within populations to calculate the probability that two randomly chosen individuals will share the same band pattern. DNA fingerprints based on both RFLPs and RAPDs have been widely used in fungi (Rosewich and McDonald, 1994). Their primary use is to identify clones or clonal lineages with a high degree of confidence. Their utility in population genetics can be extended beyond identification of clones by conducting genetic analysis to determine the molecular diversity and genetic relationships among the individual using amplicons in the DNA fingerprint. DNA fingerprints undoubtedly will be as useful for soilborne fungi and successfully used for identification characterization as they have been for other fungi (McDonald, 1997).

Amplified fragment length polymorphisms (AFLPs) offer a potentially powerful tool to detect polymorphic DNA sequences in fungi. AFLPs share many characteristics with RAPDs. They are dominant and usually only have two alleles per locus. AFLPs

have an advantage over RAPDs because more loci are screened in a single experiment and the longer primers make it more likely that an AFLP will be reproducible. AFLPs are likely to be useful for DNA fingerprinting applications because a large number of loci can be screened in one reaction. The disadvantages of AFLPs are that they require more technical expertise than RAPDs (ligations, restriction enzyme digestions, and two step amplification and analysis through long length sequencing polyacrylamide gels), and they suffer the same analytical limitations as RAPDs (McDonald, 1997). The large area to flourish is fungal plant pathology, because previous limits imposed by fastidious culture requirements (often limited to hosts) difficult genetics owing to a refractory sexual phase, the inability to obtain mutants and long life cycles have been bypassed with direct molecular study, including genomic analysis and intraspecific comparisons by RAPD technique (Valent and Chumley, 1991; Kistler and Miao, 1992).

The five RAPD primes (CRL-1, 2,7,11,34) produced reproducible and consistent banding patterns. For each primer, the RAPD banding patterns generated could differentiate the *Ganoderma* isolates from different hosts (Zakaria *et al.* 2009). In the present study also the RAPD primes produced reproducible and consistent banding patterns and also a single primer it self showed variation in the banding patterns in the wood rotting fungi under study to separate from each other. Within the same species small variations of banding patterns were observed as reported earlier (Zakaria *et al.*, 2009). In the present study also same results were observed when same genera were used for RAPD analysis. The Banding pattern of RAPD showed variations in some of the *G. boninese* isolates from oil plam (Zakaria *et al.*, 2009). In the present study the RAPD analysis differentiated the different genera into groups based on the variations in banding patterns.

To develop a method for the discrimination of basidiomycetes species and strains with vegetative mycelia, DNAs isolated from the mycelia of *Coprinus* and *Tricholoma* strains, were subjected to random amplified polymorphic DNA (RAPD) analysis. Seven *Coprinus* species could be distinguished, clearly showing species-specific DNA patterns in the RAPD analysis (Yasuhiro and Yanagi, 1999). In the present study also the 3 species of *Lenzites* clearly showing species specific DNA pattern. One species of unknown *Coprinus* strain was identified as *C. cinereus* by this method. Six strains of *C. cinereus* and 4 of *C. angulatus* could also be distinguished by the presence of strain specific RAPD fragments. Five members of the *Tricholoma* family, *T. matsutake* and related 4 species, also showed species – specific DNA patterns in the RAPD analysis.

The discrimination of *Tricholoma* species was confirmed by cluster analysis based on the 192 RAPD fragments (Yasuhiro and Yanagi, 1999). In the present study the five member of polyporaceae family, 3 species of *Lenzites*, 2 species of *Ganoderma*, 2 strains of *F. flavus*, also showed species specific DNA patterns in the RAPD analysis. This discrimination of polyporaceae members was confirmed by dendrogram generated based on the genetic similarity of 166 RAPD fragments. The 5 species could be clearly divided into 5 groups in complete agreement with the taxonomic classification. RAPD analysis of mycelial DNA is a suitable method for distinguishing basidiomycetes species and strains (Yasuhiro and Yanagi, 1999). Very few studies were made towards this aspect using the AFLP and in the present study we could get very consistent results and most of the observations made following result of RAPD.

The present part of work, Molecular characters of certain Timber Degrading Fungi using RAPD and AFLP is the novel study where the results were compared and contrasted among the two most used and reported to be very competitive techniques. As reported earlier in higher plant kingdom, the present study conducted concludes that both the techniques gave good consistent conclusions in comparison. However, AFLP showed better resolution and more diversity, will have better application in intras pecies diversity the characterization studies and also have huge potentials in identification of different and economically important isolates. The present study will pave way further exploitation of the molecular marker techniques for identification, characterization, diversity analysis, to deduce the phylogenetic relations among the new group of fungi and exploitation of these markers for the improvement of industrially important fungal strains.

5) Physiological Studies on Certain Timber Degrading Fungi

5.1. Selection of Suitable Culture Medium

The growth requirements of only a small number of wood rotting fungi have been studied in media of known composition (Hacskaylo *et al.*, 1954). Further most previous studies of wood destroying and related Basidiomycetes members have been carried out in stationary culture. At least quantitative and qualitative difference in growth and metabolic activity are to be expected between stationary and shaken cultures (Foster, 1949). Carbon, nitrogen, sulphur and phosphorus are indispensable for all living beings, vitamins and trace elements are also used by a majority of fungi. It is therefore, necessary to provide all these essential elements, in utilizable form, in a medium for their

successful growth. The knowledge achieved through these nutritional studies has resulted into a number of natural, semi synthetic and synthetic culture media. However, "there is no universal natural substrate or artificial medium upon which all fungi will grow," (Lilly and Barnett, 1951). Even closely related forms may differ considerably in their nutritional requirements qualitatively as well as quantitatively. This selective nature of different fungal organisms has compelled the investigators to perform some preliminary studies and design suitable culture media. A suitable culture medium supporting good growth, sporulation and secretion of enzymes of fungi will be of immense value not only for conducting detailed nutritional studies but will also be helpful in maintaining their stock culture (Arya, 1982).

It is evident from table 12 that with increase in incubation period the growth of all white rot fungi i.e. *L. sterioides, T. pini, H. apiaria,* and *N. floccosa* increased in all the medium tested. *L. sterioides* showed maximum growth in Czapek Dox medium and least growth in Modified malt extract agar medium. *T. pini* and *N. floccosa* showed maximum growth in Czapek Dox medium and least growth in Modified Malt extract medium. *H. apiaria* showed highest growth in Czapek Dox medium and least growth in Basal medium (Histogram 4).

There is general agreement that the majority of Hymenomycetes are partially or totally deficient only for vitamine thiamine; also growth in malt extract, casein hydrolysate and other complex organic mixtures, generally is better than in a synthetic medium containing simple nitrogen compounds and supplemented with vitamins (Lilly and Barnett, 1951). In case of certain wood rotting fungi growth has been obtained in synthetic medium equal in amount to that obtained in malt extract (Robbins, 1950; and Yusef, 1953). Studies revealed Czapek Dox medium produced that maximum growth of the all white rot fungi.

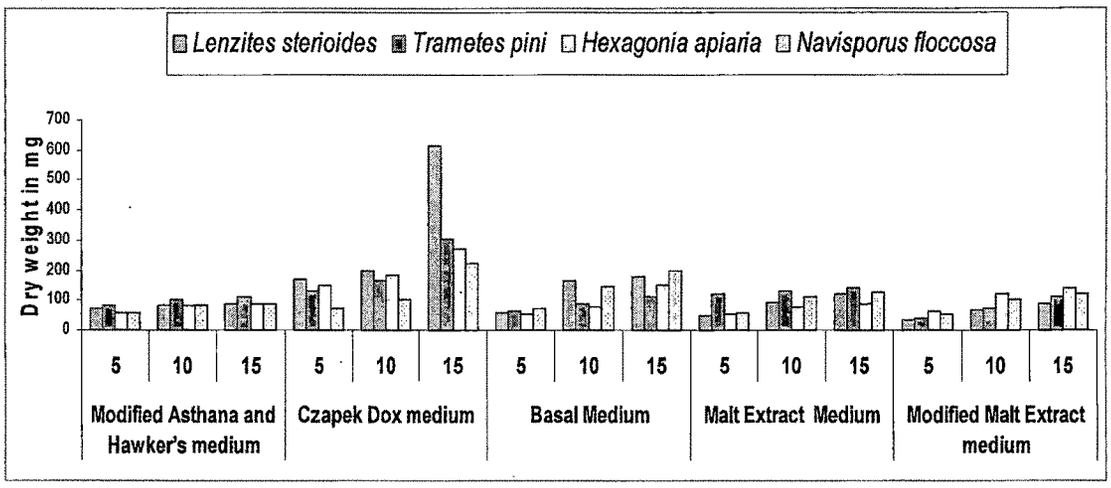
Among 19 mushrooms tested on three different media, *A. cylindracea, C. maculate* and *G. lucidum* could be served as good sources of exobiopolymer production in MCM medium. The mycelial growth of various muchrooms was in the order of *C. militaris*> *A. polytricha* > *P. ostreatus*> *T. suaveolenes* (Kim *et al.,* 2002). In the present study the best medium for the growth of all wood rotting fungi was Czapek Dox medium and it will be used for the further studies. The mycelial growth was recorded in the order *L. strerioides*> *T. pini,* >*H. apiaria,* > *N. floccosa.*

Table 12: Effect of Different media on growth of four wood decay fungi

		Lenzites sterioides		*Trametes pini*		*Hexagonia apiaria*		*Navisporus floccosa*	
Mediumused	**Days**	**Dry wt* (mg)**	**Final pH**	**Dry wt* (mg)**	**Final pH**	**Dry wt* (mg)**	**Final pH**	**Dry wt* (mg)**	**Final pH**
Modified Asthana and Hawker's medium 'A'	5	73±1.0	7.50	80±1.5	7.36	60±1.8	8.25	57±1.0	8.52
	10	80±1.0	7.60	100±1.6	6.25	80±2.5	8.15	80±1.6	9.46
	15	85±1.5	8.22	112±2.0	6.52	89±1.1	8.52	89±1.7	19.12
Czapek Dox's medium	5	170± 1.5	6.15	130±1.2	4.15	148±3.1	5.35	70±2.3	6.13
	10	197±2.5	6.36	162±1.5	3.50	185±2.6	5.10	100±2.5	5.93
	15	614±1	5.93	306±3.1	3.31	270±1.9	4.98	220±2.8	5.45
Basal Medium (Hacskaylo et al., 1954)	5	56±1.7	5.7	65±2.9	6.50	55±1.2	5.13	71±1.5	6.2
	10	166±1.1	6.1	86±1.5	6.90	78±1.8	4.75	144±1.1	6.0
	15	178±1.5	4.5	110±2.5	7.12	150±2.3	4.52	199±1.5	5.5
Malt Extract Medium	5	50±1.2	5.04	120±1.6	3.65	55±2.5	6.30	60±1.2	4.25
	10	90±1.0	4.32	130±1.3	2.66	77±2.6	5.70	109±1.6	3.86
	15	120±1.7	3.98	140±1.9	2.00	89±1.4	5.15	125±1.8	3.31
Modified Malt Extract medium	5	35±1.2	5.12	40±2.8	3.24	62±2.5	4.32	52±1.9	6.76
	10	66±0.6	5.53	74±1.2	2.95	120±1.1	4.56	102±2.5	7.23
	15	85±1.2	5.96	112±1.5	2.53	140±2.5	4.89	120±2.8	7.54

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

Histogram 4: Selection of suitable medium for growth of four wood rotting fungi



5.2. Influence of different Hydrogen ion concentration

It is evident from the table 13 that with the increase in pH the growth increased up to the pH 4 in case of *L. sterioides*. The maximum growth was observed at pH 4 (290 mg) and lowest growth was observed at pH 2 (35 mg).

T. pini showed that with the increase in pH the growth also increased up to the pH 4.5 (340 mg) and lowest growth was (80mg) observed at pH 2, 9 and 10 respectively. The final pH values varied from 3 to 9.5.

H. apiaria showed that as the pH increased the growth also increased up to the pH 4 and decreased later. The maximum growth was observed at pH 4 and lowest growth was observed at pH 10. The final pH values varied from 3 to 9.

N. floccosa showed that with the increase in pH the growth also increased up to the pH 5 and decreased later on. The maximum growth was observed at pH 5 and lowest growth was observed at pH 2. The final pH values varied from 3 to 9 (Histogram 5).

Acid was invariably produced in the malt extract solution and maximum acidity (as pH) was attained in two weeks or less: by white rot fungi in 10 days and by the brown rot species in 14 days. But in the present study the maximum acidity was 3-4 for the all white rot fungi in 15 days. Similarly the lowest pH values (1.5-1.7) were produced by *P. palustris, L. lepideus, P. radiculosa* and *T. malicola* (Kim *et al.,* 2002). Contrary to these in the present study the lowest pH value 3.03 was showed by *N. floccosa.* With most organisms the terminal pH (at the time of maximum mycelial weight) was virtually the same as the maximum pH recorded, that is there was little evidence for the reversal of the reaction, with age, from acid towards alkaline. But in the present study the final pH values ranged between 3 and 9.5 i.e. changing from acidic to alkalinity in white rot

fungi. The initial pH of the fermentation broth of *G. lucidum* slowly decreased, whereas the initial pH of the fermentation broth of *A. cylindracea* and *C. maculate* interestingly increased up to 6.67 and 7.37 respectively at the end of fermentation (Kim *et al.*, 2002).

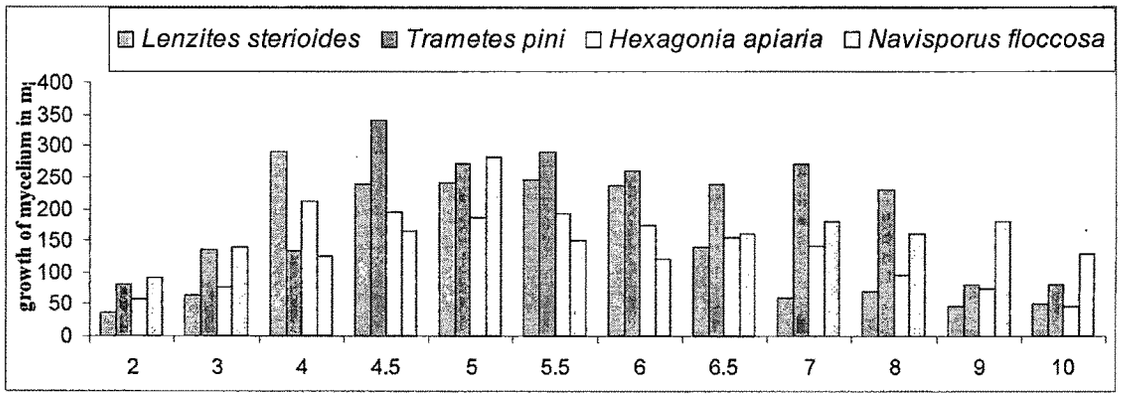
The growth of the wood-rotting Basidiomycete *Coriolus hirsutus* was more at high acidity of the medium, although the optimum level of pH was 5.6–5.8 (Emelyanova, 2005). But in the present study the optimum pH for the growth of wood rotting Basidiomycetes fungi was pH 4 for the *L. sterioides* and *H. apiaria*, 4.5 for the *T. pini* and 5 for the *N. floccosa*. *Auricularia polytricha* (Mont) Saccardo, was found growing on decaying logs of wood in large numbers within the marshy riverine ecosystem area of Wilberforce Island, Bayelsa State, Nigeria. The sporophores of this edible mushroom were cultured and biomass production in submerged liquid medium was assessed. Very good biomass was produced at pH 6.5 after 10 days of incubation (Jonathan *et al.*, 2009)

Table 13: Effect of Different pH on growth of four wood decay fungi

		Lenzites sterioides		*Trametes pini*		*Hexagonia apiaria*		*Navisporus floccosa*	
Treatment No	**Initial pH**	**Dry wt* (mg)**	**Final pH**	**Dry wt* (mg)**	**Final pH**	**Dry wt* (mg)**	**Final pH**	**Dry wt* (mg)**	**Final pH**
1	2	35±1.5	3.39	80±1.2	3.56	58±1.5	3.04	90±2.5	3.03
2	3	63±2.8	3.60	135±1.8	3.84	77±1.8	3.34	140±1.2	3.24
3	4	290±2.5	6.54	133±2.3	4.63	212±1.2	4.55	125±2.8	3.83
4	4.5	240±2.8	4.69	340±3.0	6.17	195±2.3	4.78	165±1.8	4.66
5	5	242±1.0	5.56	270±3.5	6.52	186±2.5	5.23	281±1.7	4.85
6	5.5	245±1.7	6.45	290±2.5	6.32	192±1.7	5.86	150±2.3	5.26
7	6	236±1.4	6.39	260±2.1	6.27	173±3.2	6.23	120±3.2	5.17
8	6.5	140±2.6	6.00	240±1.4	6.41	154±2.4	6.43	160±2.5	5.05
9	7	60±3.1	6.35	270±1.8	6.40	142±1.9	6.12	180±1.2	6.12
10	8	70±1.8	7.75	230±1.7	6.50	96±1.4	6.50	160±4.0	6.08
11	9	46±2.6	8.04	80±1.3	8.55	74±2.5	8.55	180±2.8	6.00
12	10	51±1.2	9.05	80±1.3	9.85	46±2.2	9.12	130±3.4	9.26

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

Histogram 5: Effect of pH on the growth of different wood rotting fungi



5.3. Influence of different temperatures

It is evident from the table 14 that the *L. sterioides* showed increase in growth up to 20°C and decreased later on as the temperature increased. The maximum growth was observed at 20°C and lowest at 40°C. The final pH of the medium varied from 3.7 to 4.7.

T. pini showed increase in growth up to 25°C and decreased later on as the temperature increased. The maximum growth was observed at 25° C and lowest growth was observed at 5°C. The final pH of the medium was ranged in between 4.32 to 4.85 as the temperature increased.

H. apiaria showed increase in growth up to 20°C and decreased later on as the temperature increased. The maximum growth was observed at 20°C and lowest growth was observed at 40°C. The final pH of the medium varied between 4.23 and 4.98.

N. floccosa showed increase in growth up to 25°C and decrease later on as the temperature increased. The maximum growth was observed at 25°C and lowest growth was observed at 5°C. The final pH of the medium ranged between 4.45 and 6.75 as the temperatures increased. At maximum growth of *N. floccosa* the final pH values was 5.63 (Histogram 6).

Maximum specific growth rate of *Coriolus hirsutus* was recorded at high temp. of 30–36°C (Emelyanova, 2005). White rot fungi were collected from Chirinda and Chimanimani hardwood forests in Zimbabwe and were studied with respect to growth and temperature optima. Temperature optima were found to vary (between 25–37°C) amongst the isolates (Tekere *et al.*, 2001). But in the present study the optimum temperature for the growth of *L. sterioides* and *H. apiaria* was 20°C and for the *T. pini* and *N. floccosa,* it was 25°C

Radial growth of twelve species of wood-rotting basidiomycetes was examined over a range of temperatures on malt agar: optimum growth rate occurred at between 20 and 30°C (Boddy, 1983). Variability in growth rate of several different isolates of five of the species was assessed at 10° and 20°. A significant difference in growth rates of different isolates was found in four species at 20°C (Boddy, 1983).

In the present study the growth of the wood rotting Basidiomycetes i.e *L. sterioides, T. pini, H. apiaria* and *N. floccosa* was examined over a range of temperature on Czapek Dox medium and it was observed that the significant growth of the fungi occurred between 20°C and 30°C. In the present study wide range of temperature tolerance was shown by *T. pini* and N. *floccose*. Their maximum growth was observed at

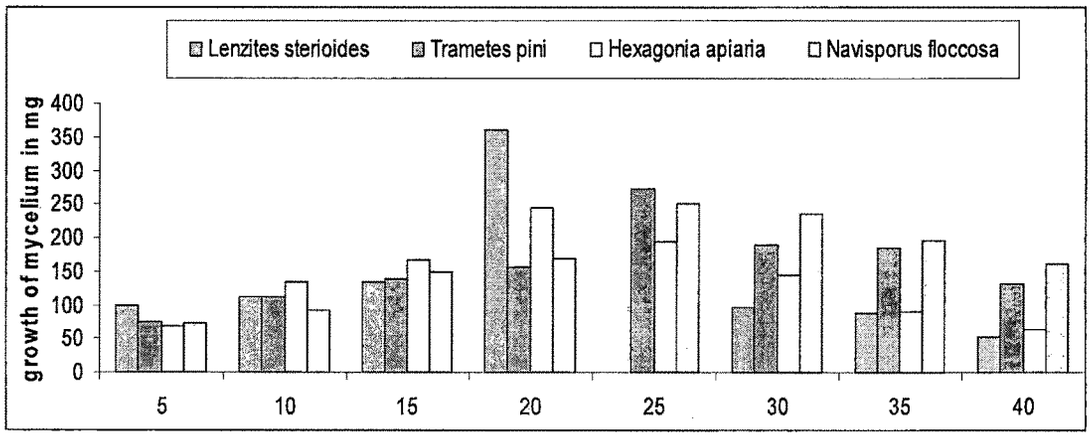
25°C. *A. polytricha* could produce mycelial biomass within the temperature range of 10 and 40°C. The maximum biomass was obtained at 25°C after 15 days of incubation. It could be seen clearly that the minimum, optimum and maximum temperatures for biomass production of this fungus were 10, 25 and 40°C respectively. Similar observations were made by Gbolagade *et al.*, (2006) for *Pleurotus florida.*

Table 14: Effect of Different Temperatures on growth of four wood decay fungi

Treat. No	Temp in °C	*Lenzites sterioides*		*Trametes pini*		*Hexagonia apiaria*		*Navisporus floccosa*	
		Dry wt* (mg)	Final pH	Dry wt* (mg)	Final pH	Dry wt* (mg)	Final pH	Dry wt* (mg)	Final pH
1	5	100±2.6	3.73	74±1.8	4.32	68±1.2	4.23	73±1.3	4.45
2	10	113±2.4	3.85	111±1.4	4.42	134±1.0	4.46	92±1.5	5.05
3	15	135±1.6	3.80	139±1.8	4.66	168±2.5	4.59	150±1.8	5.24
4	20	361±1.8	3.70	155±2.5	4.80	243±1.7	4.70	170±2.5	5.53
5	25	141±1.2	3.67	273±2.2	4.50	194±1.8	4.83	250±3.5	5.63
6	30	96±1.0	3.68	190±3.0	4.65	145±2.8	4.50	235±4.2	6.50
7	35	87±1.5	3.62	184±1.2	4.55	91±2.5	4.39	195±2.6	6.90
8	40	53±1.7	4.76	132±1.5	4.85	64±1.9	4.98	160±1.2	6.75

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

Histogram 6: Effect of different temperatures on growth of four wood rotting fungi



5.4. Effect of different carbon sources

Friel and Mcloughlin, (2000) reported that mycelial growth of *Agraicus bisporus* was enhanced by malt extract a key component in PMP medium. Among the different carbon sources, mannitol and sorbitol stimulated the best mycelial growth of 110.15 and 100.45 mg/30 cm3, respectively in *S. commune* isolates (Adejoye *et al.*, 2007). While studying *S. commune* found glucose as best source followed by fructose, xylose and mannose. Sugar alcohols like manitol also produced good growth of *S. commune* (Adejoye *et al.*,

2007). Sugar alcohols and polysaccharides get hydrolyzed to monosaccharide before they will enter into respective pathways (Mahier and Cordes, 1971). Bealing, (1953) observed that invertase preparations catalyzed the transfer of fructofuranosyl groups not only to water, but also to various alcohols and sugars by transglucosidation glucose and manintol have been reported as good substrates for vegetative growth (Hammond, 1978). It was found that Most taxa degraded cellulose and starch via the synthesis of cellulases and amylase, respectively (Thormann *et al.,* 2002). In order to study the effect of fungi on different wood block, it was thought desirable to use teak sawdust as one of the carbon substance.

In the medium containing D – arabinose as carbon source the wood rotting member *H. apiaria* showed the maximum growth of mycelium, whereas, least growth was observed in case of *L. sterioides* and *T. pini* (Table 15). In the medium containing D-xylose as carbon source the maximum growth was shown by *H. apiaria* and *L. sterioides,* whereas, lowest mycelial growth was shown by *T. pini.* In the medium containing sucrose as the carbon source the maximum growth of mycelium was observed incase of *L. sterioides* whereas, lowest growth of mycelium was observed in *N. floccosa.* The final pH of the medium varied from 3.31 to 5.93. In the medium containing malt extract as carbon source the maximum growth of mycelium was observed in *L. sterioides,* whereas, lowest growth was shown by *N. floccosa.* In the medium containing teak wood sawdust as carbon source the maximum growth of mycelium was observed in case of *T. pini* whereas, the lowest growth of mycelium was shown by *N. floccosa.* The final pH of the medium varied from 2.54 to 5.24.

The maximum mycelial dry weight of *L. sterioides* was obtained (614 mg) when sucrose was used as carbon sources, whereas, the lowest growth was 56 mg when D - arabinose was used as sole carbon source. The maximum dry weight of *T. pini* was 423 mg when teak sawdust was used as carbon sources whereas D-xylose produced lowest dry weight. The maximum growth of *H. apiaria* was obtained on malt extract, Malt extract as a complex source was found suitable for 2 strains of *Stereum hirsutum* (Jonathan *et al.,* 2009) and the lowest on D-arabinose. The maximum growth of *N. floccosa* was obtained on the malt extract as a carbon source while it was lowest on the D-arabinose.

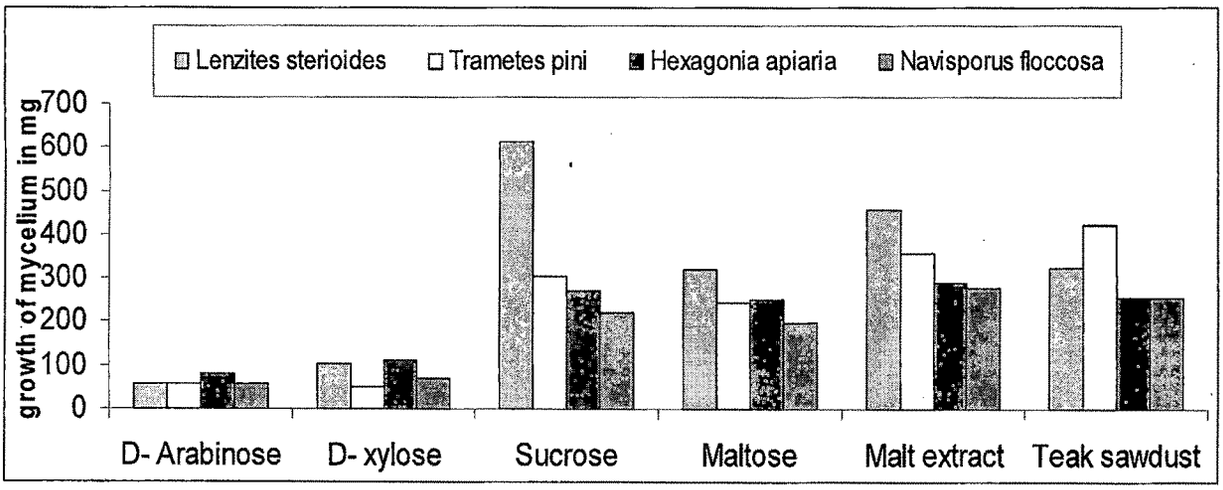
In the present study after reaching to the maximum mycelial growth of wood rotting fungi, the Hyphae undergoing autolytic degradation and best mycelial growth in *L. strioides, T. pini, H. apiaria* and *N. floccosa* were observed in case of sucrose, teak sawdust, and malt extract were used as carbon source in the medium (Histogram 7).

Table 15: Effect of Different Carbon source on mycelial dry weight and change in pH of four wood decay fungi

		Lenzites sterioides		*Trametes pini*		*Hexagonia apiaria*		*Navisporus floccosa*	
Treat. No	**Carbon source**	Dry wt*	Final pH	Dry wt*	Final pH	Dry wt*	Final pH	Dry wt*	Final pH
1	**D- Arabinose**	56±2.8	3.80	56±1.8	3.96	78±1.8	3.98	58±1.4	4.43
2	**D- xylose**	101±2.5	3.80	49±1.5	3.98	110±1.5	3.59	69±2.6	4.80
3	**Sucrose**	614±1.8	5.93	306±1.2	3.31	270±2.6	4.98	220±2.8	5.45
4	**Maltose**	321±1.5	4.26	243±1.8	2.92	250±2.8	5.52	198±3.4	5.85
5	**Malt extract**	456±1.0	6.24	356±1.4	2.13	289±2.4	4.63	276±3.8	5.34
6	**Teak sawdust**	325±2.8	4.67	423±2.5	2.54	256±1.7	4.21	253±2.3	5.24

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

Histogram 7: Effect of different carbon sources on growth of four fungi



5.5. Utilization of various sugars by four wood degrading fungi

5.5.1. Utilization of monosaccharides

Research findings have revealed that disaccharides get hydrolyzed to monosaccharides before they enter into different pathways. The range of carbon source utilized for mycelial growth of different fungi is very wide. Monosaccharides, disaccharides and polysaccharides can be used as suitable carbon sources. Monosacharides (such as fructose, glucose etc.) or maltose among the disaccharide are the most suitable carbon sources for *A. auricula* (Luo, 1993). Apart from occurring freely in various parts of the plants, these sugars are also present as component units of oligosaccharides and different polysaccharides. Monosaccharide also takes part in the synthesis of reserve

carbohydrates of fungal mycelium and as such, various fungal polysaccharides are there which consist of monosaccharide units like glucose, mannose and galactose etc.

In the present study daily chromatographic analysis was undertaken to determine the presence of various sugars in the culture medium. The results of mycelial growth, drift in pH and utilization of monosaccharides by different wood rotting fungi under study have been summarized in Tabel 16.

D-Xylose (Rf 0.62)

This aldopentose occurs in nature in the form of xylans and a constituent of polysaccharides of cell wall i.e., in hemicellulose and plant gums. It is evident from the table 16 that D- xylose was present in the culture filtrates of all the four test fungi up to 15 days of incubation. The growth rate increased in first 5 days and later on decreased. The fungal growth rate was increased up to first 5 days and later on decreased up to 10 days how ever a slight increase was observed up to 15 days in *L. sterioides* and *H. apiaria*. The final pH of the medium was acidic in all thr four fungi.

D – arabinose (Rf 0.70)

This aldopentose occurs in nature in the form of arabans as common constituents of plant polysaccharides and various gums especially gum arabica. Chromatographic analysis of the medium showed that none of the wood rotting fungi under the study could assimilate this sugar completely with in 15 days of incubation. The growth rate was increased in first 5 days in *L. sterioides* and *N. floccosa,* and decreasing later days of incubation. The growth rate is constantly going in *H. apiaria*. The growth rate was increased in first 5 days, later decreased up to 10 days and then increase up to 15 days in *T. pini*. The growth of mycelium was increasing as the incubation period is increasing. The final pH of the medium was acidic in all the four fungi.

Both isomers of arabinose were employed in this nutritional investigation of *Calvatia* species but neither of them promoted satisfactory growth. According to Lilly and Barnett, (1956), from the distribution of the isomers of arabinose in organisms, it would be expected that L-arabinose would be utilized readily by more fungi than D-arabinose. In the case of *Calvatia* species, two of the strains, 1019 B and 766, grew better on L-arabinose, and two strains, 1018 F and 1020, were superior on D - arabinose (Sedlmayr *et al.,* 1961). In the present study the D - arbinose showed 56mg, 78mg and 58mg of mycellial growth for *L. sterioides*, and *T. pini, H apiaraia,* and *N. floccosa* respectively. A study has been made of the carbon nutrition of *Coriolopsis occidentalis* using carbohydrates in liquid growth medium.

5.5.2. Utilization of oligosaccharides

The oligosaccharides are complex sugars composed of two or more monosacharides units linked together by glycosidic bonds. They occur freely in nature or as the units of polysaccharides. These water soluble compounds yield monosaccharide components on hydrolysis.

The simple carbohydrates tested, the oligosaccharides supported growth best followed by xylose (Fawole, 1973). In the present study the oligosaccharides showed better growth than the monosaccharides.The present studies were conducted in order to ascertain the pathway of utilization of different oligosaccharides and probable effect of their hydrolytic products on the growth of the wood rotting fungi under study. The rate if assimilation of the component sugars by the four organisms was detected chromatographically. The details of the results have been summarizes in table 17.

Disaccharides

Sucrose (Rf 0.43)

This disaccharide is of common occurrence in plants. A large number of workers have shown that most of the fungi are able to hydrolyse sucrose into glucose and fructose and thus it is assimilated through a hydrolytic pathway. Table 16 indicates that all the wood rotting fungi under the study utilized sucrose after hydrolysis, which indicates that they were capable of producing sucrase or Trans –fructosidase enzyme in sufficient amount. It yields maximum mycelial yield of wood rotting fungi i.e., *L. sterioides, T. pini, H. apiaria* and *N. floccosa* after 15 d. As the incubation period is increased the growth of the wood rotting fungi also increased. It is evident from table 16 that 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.* This shows that *Hexagonia* was able to utilize this disaccharide with much slower rate.

Maltose (Rf 0.40)

It does not usually occur in the free form in chlorophyllus plants but this disaccharide is obtained as an intermediate product during the digestion of strarch to glucose. It consists of two glucose units which are held together by œ – 1, 4 glucoside linkages. Maltose is utilized by a majority of fungi through a hydrolytic pathway. It yields two molecules of glucose when hydolysis is accomplished by the enzyme œ – glucosidase.

Maltose was utilized by the present fungi through a hydrolytic pathway. Its presence was detected upto 6, 15, 10, 15 days respectively in *L. sterioides, T. pini, H. apiaria,* and *N. floccosa.* Its hydrolytic products were detected in wood rotting fungi i.e., *L. sterioides, T.*

pini, H. apiaria, and *N. floccosa*. Between 6 and 12, 4 and 15, 6 and 10, and 6 and 15 days respectively. The dry weight of all the wood rotting organisms was maximum on this sugar after15th days. The better yield may be due to hydrolysis of maltose by α – glucosidase enzyme which yields two glucose units. The glucose units were used efficiently by all the wood rotting organisms. The better growth of mycelium is also due to the slow and steady growth of all wood rotting fungi. The final pH of the medium was slightly acidic in case of *L. sterioides*. The pH of the medium was acidic in case of *T. pini*. The final pH of the medium was slightly acidic in case of *H. apiaria*. The pH of the medium shifted towards neutral side in case of *N. floccosa*.

Out of 21 carbon sources, *Lobivia lateritia* strains exhibited maximum mycelial growth on maltose followed by raffinose, starch and lactose with variable preference of different strains (Jana and Purkayastha, 1987). Swartz, (1933) was the only one who reported on the carbon requirement of *Calvatia* species. He found maltose the best sugar for producing mycelium of *Calvatia saccata, C. caelata* and *C. gigantean.* In the present study the maltose showed good growth of mycelium in wood rotting fungi i.e., *L. sterioides, T. pini, H apiaraia*, and *N. floccosa* respectively.

The mycelial growth of *Cystoderma amianthinum* was checked in the media supplemented with 11 different carbon sources. Fructose was found best screened as carbon source for the mycelial growth of *C. amianthinum* (Shim *et al.*, 2005). But in the present study It was completely utilized in 13 days. Submerged culture of Nigerial mushroom *Pleurotus florida* grow well on glucose containing medium (Gbolagade *et al.*, 2006). In the present study the wood rotting fungi under study showed fair growth of mycelium when maltose was broken down to two glucose molecules, it was utilized with in 4 to15 days of incubation. Chi *et al.*, (1996) reported that mycelial growth of *Phellinus linteus* dissimilar among monosaccharides. In the present study also the growth of mycelium depend on the nature of the wood rotting fungi under study.

Sucrose was not a good carbon source for *Calvatia* species. It could be that the organisms produced the hydrolyzing enzyme very slowly or in a small quantity, as both components of this disaccharide (glucose and fructose) produced a satisfactory growth when used separately. Apparently the glucose to fructose linkage was not easily broken (Sedlmayr *et al.*, 1961). In the present study the sucrose yielded the glucose and fructose which were utilized in 8 to 10 days by wood rotting fungi under study.

Table 16: Utilization of Different sugars by wood decay fungi

		Lenzites striatulus				*Trametes pini*				*Hexagonia apiaria*				*Navisporus floccosus*			
Mono-saccharides	Days	Dry wt± (mg)	Rate of growth	Final pH	Presence (days)	Dry wt± (mg)	Rate of growth	Final pH	Presence (days)	Dry wt± (mg)	Rate of growth	Final pH	Presence (days)	Dry wt± (mg)	Rate of growth	Final pH	Presence (days)
D-arabinose	5	31±1.3	37	3.50		22±1.8	22	4.00		38±2.6	28	4.21		25±1.0	25	4.50	
	10	49±1.8	11	3.82		35±1.5	13	4.03		54±2.8	26	4.24		42±1.5	17	4.45	
	15	56±1.5	12	3.80	15	56±1.2	21	3.96	15	78±3.2	24	3.98	15	58±2.5	16	4.43	15
D-xylose	5	38±2.5	38	3.50		29±3.3	29	3.50		46±3.5	46	3.85		30±2.3	30	4.20	
	10	45±1.9	13	3.84		38±2.5	9	3.93		67±2.8	21	3.76		51±2.5	21	4.39	
	15	101±1.0	56	3.80	15	49±1.3	11	3.98	15	110±1.5	43	3.59	15	69±2.8	18	4.80	15

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

Table 17: Utilization of Sucrose and Maltose sugars by different wood decay fungi

	Average dry wt. (mg)*			Final pH			Presence of sugars (days)		
organism	**5**	**10**	**15**	**5**	**10**	**15**	**Sucrose / Maltose**	**Glucose "**	**Fructose**
Lenzites sterioides	170±1.8	197±2.8	614±1.8	6.15	6.36	5.93	8	1-5	1-10
Trametes pini	130±1.0	162±2.2	306±1.0	4.15	3.50	3.31	10	2-8	2-11
Hexagonia apiaria	148±2.5	185±1.8	270±1.5	5.35	5.10	4.98	12	1-8	1-14
Navisporus floccosa	70±3.5	100±3.6	220±1.2	6.13	5.93	5.45	10	1-8	1-12
Lenzites sterioides	150±3.0	187±2.7	321±2.5	4.30	4.70	4.26	6	6 -12	--
Trametes pini	120±3.8	159±1.6	243±2.8	3.58	3.13	2.92	15	4 -15	--
Hexagonia apiaria	138±2.4	174±2.5	250±1.7	5.80	5.74	5.52	10	6 -10	--
Navisporus floccosa	112±2.8	145±2.8	198±2.5	6.25	6.14	5.85	15	6-15	--

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

5.6. Effect of different nitrogen sources

This essential element is used by fungi for functional as well as structural purposes. Chitin, the chief component of cell wall in most of the fungi, is a linear polymer of D-glucoseamine. Similarly proteins, the basis of protoplasm are composed of nitrogenous substance. Purines, pyrimidines, some vitamins and other essential metabolites are also nitrogen containing compounds.

In nature both the organic and inorganic forms of nitrogen are available to fungi but as far as their utilization is concerned they fundamentally differ from each other in their metabolic potentialities. A few utilize atmospheric nitrogen, many utilize nitrate nitrogen and a still grater number utilize ammonium nitrogen. All species are able to utilize some form of organic nitrogen. Owing to the specific response of various fungi towards different nitrogenous substances numerous investigators have classified them into different groups on the basis of their abilities to utilize these sources.

For the few wood rot fungi previously studied both qualitative and quantitative difference have been found in the utilization of known organic and inorganic nitrogen compounds including certain amino acids (Hacskaylo *et al.,* 1954 and Yusef, 1953). Generally ammonium nitrogen is assimilated (Fries, 1950; Lilly and Barnett, 1951; Yusef, 1953). A few wood rot fungi utilize nitrate nitrogen slowly in stationary culture (Lilly and Barnett, 1951; Hacskaylo *et al.,* 1954). Discussing the possible pathway of protein synthesis Lilly and Barnett, (1951) have mentioned, "with the exception of certain amino acids (primary amino acids) and ammonia, most nitrogen sources undergo modifications before entering the synthetic metabolic pathways. Nitrates, nitrites and hydroxylamine are presumably reduced to ammonia before assimilation. Those amino acids (secondary amino acids) which do not enter directly into the metabolic pathways leading to the synthesis of protein are probably deaminated."

In urea there was substantial increase in the average amount of growth from the third to ninth serial subculture, with both the brown rot and the white rots. Presumably this increase reflected some degree of adaptation of certain organisms to the nutrients. *M. americanus* showed a marked increase in growth in casein hydrolysate and in ammonium sulfate and *F. fomentarius* in ammonium carbonate from the third to the ninth serial transfer. The negative data for growth in ammonium chloride, Potassium nitrate and Potassium nitrite were not included, since *T. serialis* was the only culture which could utilize even one of the compounds under the conditions used (Jennison *et al.,* 1955).

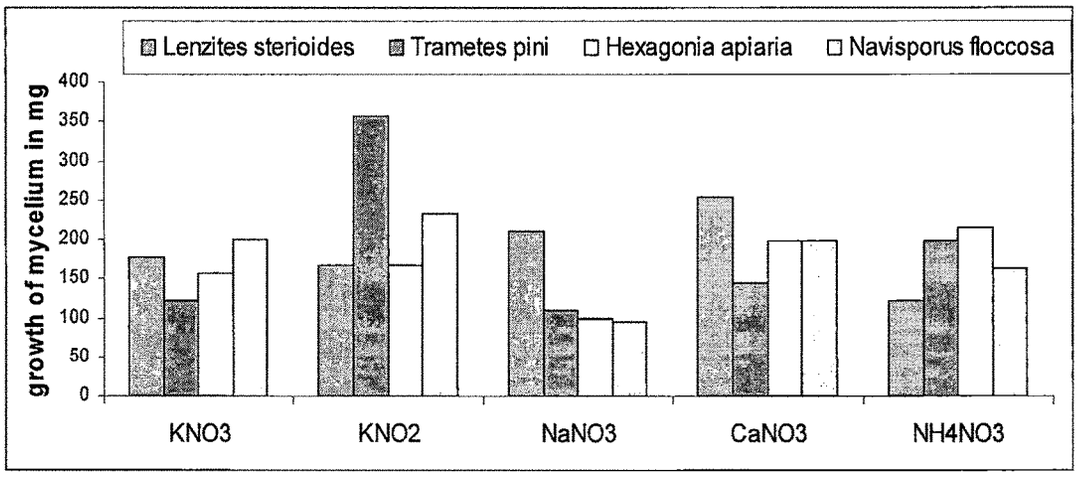
The effect of 5 different nitrogen sources was observed in case of 4 wood rotting fungi, the results are depicted in table 18. The potassium nitrite showed better growth for *T. pini* and *N. floccosa.* The final pH of the medium changed from acidic to slightly neutral nature. The sodium nitrate showed better growth in *L. sterioides* and lowest growth was shown by *N. floccosa.* The calcium nitrate as sole nitrogen source showed better growth in *L. sterioides*, as compared to other fungi. The ammonium nitrate as sole nitrogen source showed better growth in *H. apiaria* and lowest growth in *L. sterioides* respectively (Histogram 8). Based upon growth supporting ability the inorganic nitrogen compounds are grouped as calcium nitrate > Sodium nitrate > Potassium nitrate > Potassium nitrite > ammonium nitrate for *L. sterioides.*

Table 18: Effect of different Nitrogen sources on growth of wood decay fungi

		Lenzites sterioides		*Trametes pini*		*Hexagonia apiaria*		*Navisporus floccosa*	
Treat. No	**Nitrogen source**	**Dry wt***	**Final pH**	**Dry wt***	**Final pH**	**Dry wt***	**Final pH**	**Dry wt***	**Final pH**
1	KNO_3	178±2.8	4.53	122±2.2	4.65	156±1.8	5.70	199±1.5	5.54
2	KNO_2	167±3.4	6.30	357±1.0	4.70	168±1.2	6.76	233±2.5	7.12
3	$NaNO_3$	210±1.5	7.10	110±1.5	6.86	98±1.8	7.26	95±2.8	7.60
4	$Ca(NO_3)_2$	253±2.5	6.20	145±2.5	5.10	198±1.9	5.23	197±3.4	6.18
5	$NH_4(NO_3)_2$	121±2.6	3.58	198±1.7	4.50	215±2.4	6.50	162±3.9	5.18

* indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

Histogram 8: Effect of nitrogen sources on growth of mycelium in different wood rotting fungi



The brown rot fungi as a group and for the white rot species, there was a consistent decrease in average growth-supporting ability for ammonium nitrate (Jennison *et al.*, 1955). In the present study the wood rotting fungi showed the good mycelial growth in

H. apiaria and lowest growth in *L. sterioides* for ammonium nitrate as sole nitrogen sources. The Basidiomyctes with the exception *Polyporus distortus*, grew very slowly on nitrate nitrogen. The species of basidiomycetes tested utilize nitrate nitrogen slowly or not at all, with the time of incubation (Hacskaylo *et al.,* 1954). In the present study the wood rotting fungi showed good growth in nitrate nitrogen. The phenomenon of slow utilization of nitrate nitrogen appears to an exaggerated degree in some of the Basidiomycetes tested (Hacskaylo *et al.,* 1954). In the present study the nitrate nitrogen shown better growth in 15 days of incubation as it take less time for stabilization and the wood rotting fungi i.e., *L. sterioides, T. pini, H. apiaria* and *N. floccosa* showed good growth of mycelial mass in nitrate, nitrite, ammonium nitrogen sources which were incubated for short time.

On the other hand none of these strains could grow on Sodium nitrite. All strains responded moderately to the ammonium compounds studied. However the nitrates were found to support moderate to poor for growth of all strains (Singh and Verma, 1996). In the present study the sodium nitrite showed better growth in *T. pini* and *N. floccosa* and good growth in *L. sterioides* and also in *H. apiaria*.. Similarly sodium nitrite has been shown as a non available nitrogen source to several fungi including *Agaricus bisporus* (Hsu and Hu, 1967) and *Lentinus edodes* (Tokimoto and Kumatsu, 1979). In the present study *L. sterioides, T. pini, H. apiaria* and *N. floccosa* showed better to good growth of mycilial mass when sodium nitrite was used as sole nitrogen source.

In the series of complex and inorganic nitrogen sources, it was observed that inorganic compounds supported moderate biomass production. The best biomass yield was found with ammonium nitrate closely followed by potassium nitrate. This result is contrary to that obtained by (Jonathan and Fasidi, 2001) for *P. atroumbonata* where ammonium nitrate supported insignificant mycelial yield (Gbolagade *et al.,* 2006). In the present study also the ammonium nitrate showed good growth for *H. apiaria* and *T. pini* to moderate growth in *N. floccosa* and *L. sterioides*. So ammonium nitrate is good source of nitrogen. As the incubation period increases the growth mycelium also increased in *Sterium hirsutum* on Ammonium sulphate and Potassium nitrate as sole nitrogen source (Minc, 2005). The potassium nitrate also showed good growth in *N. floccosa* and *L. sterioides* and moderate growth in *T. pini* and *H. apiaria.*

The differences in nitrogen sources requirements may suggest that biomass production in different fungi may be influenced by different nutritional requirements. It was also observed that very low concentration of nitrogen compounds generally supported little

biomass yield while low concentration and above were supportive to high biomass yield (Yajie and Zhong, 2002). In the present study also the wood rotting fungi showed good growth in higher concentration of nitrogen as the free nitrogen was available to the fungi. The mycelial growth of *Cystoderma amianthinum* in Calcium nitrate was 5.6, Potassium nitrate was 16.4, and Sodium nitrate was 30.6 mg (Shim *et al.,* 2005). In the present study the mycelial mass of the *L. sterioides* in calcium nitrate was 25 3mg, potassium nitrate was 178 mg and sodium nitrate was 210mg.

Nitrogen sources $(NH_4)_2HPO_4$, NH_4Cl, NH_4NO_3 $(NH_4)_2S0_4$ were good source of ammonium nitrogen sources whereas the KNO_3 and KNO_2 are poor source of nitrate nitrogen (Niederipruem, *et al.,* 1964). In the present study the ammonium nitrogen sources showed good to moderate growth whereas the inorganic nitrogen sources i.e. KNO_3 and KNO_2 showed better to good growth in all wood rotting fungi under study.

These fungi, *Polyporus adustrzs* and *Liberfella befulincr,* grew better on ammonium nitrate. Utilization of nitrate led to an increase of pH in the medium. The changes in pH of media in which *Polyporrrs adusfus* had grown on ammonium nitrate indicates that the ammonium ion was taken up before the nitrate ion (Henningsson, 1967). In the present study also the pH of the medium is decreasing as incubation period is increasing. This decrease may be due to the release of cations and organic acids into the medium.

6) Biochemical changes of certain Timber Rotting fungi

6.1 Tests for different Enzymes

Among 21 fungi tested (Table 19) lignin degrading fungi were 13 belonging to Basidiomycota and cellulolytic fungi were 8 belonging to Zygomycota, Ascomycota and Mitosporic fungi. It is evident from table 19 that highest ligninolytic activity was shown by *G. lucidum* and lowest by *L. betulina.* Highest laccase activity and peroxidase activity was shown by *G. lucidum* and lowest by *S. commune*. The highest cellulolytic activity was shown by Basidiomycota members. Lowest cellulolytic activity was shown by *L. betulina.* Among the Mitosporic Fungi the highest cellulolytic activity was shown by *T. viride*. Lowest cellulolytic activity was shown by *A. alternate*

Table 19: Enzyme production by different wood inhabiting fungi

Fungal isolate	Lignolytic activity* (Hallo Zone in cm)	Cellulolytic activity* (Hallo Zone in cm)	Laccase activity* (Hallo Zone in cm)	Peroxidase activity* (Hallo Zone in cm)
Zygomycota				
Rhizopus stolonifer	--	9±0.5	--	--
Ascomycota				
Chaetomium globosum	--	4.5±0.36	--	--
Basidiomycota				
Schizophyllum commune	4.8±0.54	9±0.58	1.5±0.2	1.0±0.2
Lenzites sterioides 2	4.6±0.32	9±1.0	2.5±0.25	3±0.34
L. betulina	0.9±0.23	0.5±0.21	--	--
L. exima	7±1.0	9±0.5	2.4±0.26	--
Lenzites sterioides 1	3.8±0.6	9±0.23	--	2.3±0.25
Navisporus floccosa	4.2±0.67	8±0.45	6.5±0.28	1.5±0.75
RS17b	2±0.2	0.9±0.32	5.8±0.14	3.0±0.5
Flavodon flavus	3.4±0.4	9±0.25	3.5±0.45	3.5±0.38
RS17d	6.8±0.24	9±0.45	4.8±0.34	2.5±0.45
Ganoderma lucidum	8.8±0.53	9±0.56	9±0.24	9±0.56
Hexagonia tenuis	6.5±0.65	8.5±0.37	4.3±0.58	4.3±0.68
Tremates pinii	5.8±0.75	9±0.38	2.3±0.87	--
Coriolus versicolor	8.5	9±0.12	5.6±0.68	4.5±0.46
Mitosporic fungi				
Phoma multirostata	--	6.7±0.5	--	--
Thielaviopsis paradoxa	--	7.5±1.2	--	--
Fusarium pallidoroseum	--	3±0.34	--	--
Alternaria alternata	--	2.8±0.48	--	--
Curvularia lunata	--	5.2±0.23	--	--
Trichoderma viride	--	9±1.0	--	--

-- activity not detected

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

Fungi commonly encountered on monocotyledonous substrates were evaluated for their *in vitro* ability to produce enzymes involved in lignocellulose breakdown. Most were capable of utilizing this structural polysaccharide, but few produced enzymes associated with lignin breakdown. None of the monocotyledon- inhabiting fungi produced reactions as strongly as wood decay fungi (Sin *et al.,* 2002). In the present study the fungi inhabiting the timbers of dicotyledonous plants were evaluated for there *in vitro* ability to produce enzymes in wood degradation. All timber degrading fungi were able to produce ligninolytic enzyme. Forty isolates of 18 fungal species isolated from mushroom culture (compost, casing soil, and mushrooms) were tested for their ability to produce hydrolytic enzymes which degrade cellulose, starch and lipids. The pathogenic fungi showed the most variability between isolates of the same species in their ability to produce cellulolytic enzymes. Two-thirds of the species studied exhibited phenolic-oxidase activity (Trigiano and Fergus, 1979). Production of lignin-modifying enzymes for diatrypaceous fungi from Argentina was studied by Pildain *et al.,* (2005). Extracellular production of ligninolytic and cellulolytic enzymes was studied by mycelium growing on solid medium supplemented with different dyes (Malachite green, Azure B, Poly R-478, Congo red, tannic and galic acid, and guayacol). Seven of the eight strains analysed decolourised all the dyes except for the Malachite green. Only one strain of E. scoparia was able to decolourise Malachite green (Pildain *et al.,* 2005).

All *S. commune* and *F. flavus* strains showed positive reactions to tannic acid used in the Bavendamm test. The data from the Bavendamm test provided evidence for the presence of laccase activity in this fungus (Appendix II). In the present study also the *S. commune* and *F. flavus* and other timber degrading fungi showed positive reaction to the tannic acid used in Bavendamm test. Which indicates that all timber degrading fungi were able to produce laccase enzyme. De Vries *et al.,* (1986) studied the production of extra-cellular laccases from *S. commune*. The present study confirms this finding by the aforementioned test. *F. flavus* (strain 312), isolated from decaying sea grass from a coral lagoon off the west coast of India, mineralized nearly 24% of ^{14}C-labeled synthetic lignin to $^{14}CO_2$ in 24 days (Raghukumar *et al.,* 1999). When grown in low-nitrogen medium (2.4 mM N) this fungus produced three major classes of extra cellular Lignin Modifying Enzymes (LMEs): Manganese-dependent Peroxidase (MnP), Lignin Peroxidase (LiP), and Laccase (Raghukumar *et al.,* 1999). The strains of *F. flavus* showed strong positive reactions to tannic acid. So all strains of *F. flavus* produced lignin-modifying enzymes to degrade the lignin in *M. indica* and *S. cumini* woods (Appendix III). In the present study

G. lucidum showed strong positive reaction to tanninc acid. So it has highest capacity to produce the lignin modifying enzymes to degrade the teak wood. The isolates of *F. flavus* possessed high lignin-degrading capacity whereas isolates of *S. commune* possessed low lignin-degrading capacity (Appendix III). In the present study the *S. commune* showed medium range of lignin degrading capacity when compared to the *F. flavus* and other timber degrading fungi and also *S. commune* produced laccase, peroxidase wich indicates that the fungi was mostly ligninolytic.

Bains *et al.,* (2006) studied the evaluation of wood degrading enzymes of some indigenous white rot fungi. All the seven wood rot fungal isolates (*Ganoderma lucidum* I, II, *Pleurotus volvatus*, *Polyporus* sp. I, II, and *Hymenochaete* sp.) possessed ligninolytic enzyme activity and five (*G. lucidum* II, *P. florida*, *P. volvatus*, *Polyporus* sp. I and *Hymenochaete* sp.) were found to possess cellulolytic enzyme activity. In the present study the 21 timber degrading fungi were evaluated for the wood degrading enzymes production. In which 13 timber degrading basidiomycota showed ligninolytic enzyme activity and 21 timber degrading fungi belonging to Zygomycota, Ascomycota, Basidiomycota and Fungi imperfecti showed cellulolytic enzyme activity.In the present study the timber degrading fungi were differentiated into white rot, brown rot, and soft rot based on the reaction with tannic acid. Most of the timber degrading Basidomycota members were usually white rot and very few were brown rot fungi. While the members of Zygomycota, Ascomycota and Fungi imperfecti caused soft rot. All the white rot and few brown rot fungi showed the production of laccase and peroxidase enzymes. *C. versicolor* showed laccase and peroxidase activities were more intense in the peripheral region (White and Boddy, 1992). In the present study also the *C. versicolor* showed strong positive reaction to the laccase and peroxidase activity.

Scientists have found that 89% of marine fungi were cellulolytic (crystalline cellulose utilization). Few isolates of Ascomycetes and their anamorphs were cellulolytic. There were no obvious differences in enzyme production between Ascomycetes or their anamorphs. The ability to produce enzymes involved in lignin degradation *in vitro* varied greatly between taxa used in this study. Of the anamorphs tested by Bucher *et al.* (2004a), 20% decolorized Poly R, whilst none decolorized Azure B and 30% oxidized syringaldazine. Many of the Ascomycetes members decolorized Poly R (77%), with 9% decolorizing Azure B and 17% oxidizing syringaldazine. The reactions of reference white-rot and soft-rot taxa were typical and confirmed the suitability of the assay medium for each test. In the present study the members of

Ascomycota were cellulolytic. In this study the production of ligninolytic enzymes varied in different fungi.

6.2. Enzyme assay

6.2.1. Estimation of ligninolytic enzymes in medium containg different nitrogen sources

The term white rot fungi have been applied to certain ligninolytic basidiomycetes with a relatively high selectivity to degrade lignin in wood. These fungi produced a set of enzymes which are directly involved in lignin decay. Two major families of enzymes are involved in ligninolysis by white rot fungi, those are peroxidases and phenol oxidase termed laccase (Leonowicz *et al.*, 2001). Peroxidases divided into lignin peroxidase (LiP) and Manganese peroxidase (MnP) (Kirk and Farrell, 1987; Farrell *et al.*, 1989; Datta *et al.*, 1991; Reddy, 1993; Reddy and D'Souza, 1994, Cullen, 1997). Each species of white rot fungi secretes a particular assortment of this enzymatic machinery to the medium in which it is growing. Thus some strains produce all of the major families of enzymes, others only two of them or even one (Lobos *et al.*, 2001; Perez *et al.*, 2002). These results suggest that different enzymatic system, formed by enzymes encoded by different genes, are responsible for lignin degradation by white-rot fungi (Varela *et al.*, 2000).

Based on the production of ligninolytic enzymes the wood-rotting fungi can be divided into three groups:

(1) laccase-producing fungi

(2) peroxidase-producing fungi and

(3) those which do not excrete measurable amounts of these oxidative enzymes.

The first group, i.e., laccase-active species, was represented by two white-rot fungi, *C. versicolor* and *P. igniarius*. Their laccases oxidized not only syringaldazine, but also o-dianisidine and vanillylacetone. Vanillylacetone is a model lignin compound, whose oxidation may reflect involvement of laccases in the lignin degradation process. The second group of fungi, consisting of a brown-rot fungus *P. betulinus*, two soft rotters, *C. cellulolyticum* and *C. piluliferum*, and a white rotter, *P. chrysosporium*, expressed only peroxidase activities which may be independent or dependent upon the presence of manganese in the medium. The representative of the last group is *L. trabea*, a brown-rot fungus, which did not produce any extracellular enzymes of the oxidase or peroxidase type (Ander and. Eriksson, 1978). This finding corroborates the results of Harkin and Obst (1973) who found neither peroxidase nor laccase activity in this species.

Some genera of Basidiomycetes, such as *Pleurotus* spp., were found to lack lignin peroxidases (Fukushima and Kirk, 1995), *Lentinus edodes* showed laccase, but not LiP activity (Leatham and Stahmann, 1981).

Basal medium containing KNO_3, $NaNO_3$, $Ca(NO_3)_2$ and $NH_4(NO_3)_2$, was used to test the efficacy for ligninolytic activity, the highest laccase activity was observed in basal medium containing $NH_4(NO_3)_2$, whereas lowest laccase activity was observed in case of $NaNO_3$. The highest aryl alcohol oxidase activity was observed in $NH_4(NO_3)_2$ containing basal medium, where as the lowest aryl alcohol oxidase was observed in case of $NaNO_3$. The highest lignin peroxidase activity was observed in presence of $NH_4(NO_3)_2$, whereas lowest in case of $Ca(NO_3)_2$. The highest peroxidase activity was observed in KNO_3 containing basal medium whereas, lowest in case of $Ca(NO_3)_2$.

It is evident from Tables 20 to 23, that *S. commune* showed highest laccase activity in $NH_4(NO_3)_2$ basal medium than other basal medium and lowest in case of $Ca(NO_3)_2$ basal medium. The highest aryl alcohol oxidase enzyme was observed in $NH_4(NO_3)_2$ medium and lowest in case of $Ca(NO_3)_2$. The highest lignin peroxidase and peroxidase activity was observed in case of $NH_4(NO_3)_2$ and lowest in case of $Ca(NO_3)_2$. *L. sterioides* showed highest laccase activity incase of $NaNO_3$. The highest aryl alcohol oxidase activity was observed in case of basal medium supplemented with $NH_4(NO_3)_2$. *G. lucidum* showd highest laccase activity in case of Ca NO_3 whereas it was lowest in case of Na NO_3. The highest aryl alcohol oxidase activity was observed in case of KNO_3 and lowest in case of $NaNO_3$

N. floccosa showed highest laccase activity in case of $NH_4(NO_3)_2$ and lowest in case of basal medium supplemented with $Ca(NO_3)_2$. The highest aryl alcohol oxidase activity was observed in case of KNO_3 and lowest in case of $Ca(NO_3)_2$ basal medium. *F. flavus* showed highest laccase activity in case of Na NO_3, aryl alcohol oxidase activity in case of KNO_3, lignin peroxidase activity in case of $NH_4(NO_3)_2$ and peroxidase activity in case of KNO_3.

It is evident from Table 20 and histograms 9 to 12, that basal medium having KNO_3 the *S. commune* showed highest laccase activity in 25 days (1.81U/ml), whereas lowest laccase activity was observed in 5 days (0.67 U/ml), the highest aryl alcohol oxidase is observed in 10 days (3.41 U/ml) whereas lowest aryl alcohol oxidase was observed in 5 days (1.27 U/ml), highest lignin peroxidase activity was observed in 10 days (2.62 U/ml) whereas lowest lignin peroxidase activity was observed in 25 days

(1.83 U/ml) and highest peroxidase activity is observed in 5 days (10.52 U/ml), whereas lowest peroxidase activity was observed in 25 days (3.60 U/ml).

The *L. sterioides* shown highest laccase activity in 25 days (1.23 U/ml), while it was 5 days (2.38 U/ml) in case of *G. lucidum*. *G. lucidum* showing the highest aryl alcohol oxidase activity was observed in 10 days (2.76 U/ml) whereas it was lowest in 25 days (0.83 U/ml). The highest lignin peroxidase activity was observed in 10 days (7.08 U/ml), whereas lowest lignin peroxidase activity was observed in 25 days (2.45 U/ml), the highest peroxidase activity is observed in 10 days (17.90 U/ml), whereas it was lowest in 25 days (1.64 U/ml).

The *N. floccosa* showed highest laccase activity in 5 days (1.12 U/ml) wheras it was lowest in 20 days (0.40 U/ml). The *F. flavus* showed highest laccase activity in 20 days (1.63 U/ml), whereas lowest in 25 days (0.70 U/ml). The highest aryl alcohol oxidase activity was observed in 10 days (2.63 U/ml) whereas lowest in 5days (2.23 U/ml). The highest lignin peroxidase activity was observed in 10 days (7.33 U/ml) whereas lowest in 5 days (2.33U/ml). The highest peroxidase activity was observed in 10 days (14.00 U/ml) wheras lowest in 25 days (2.48 U/ml).

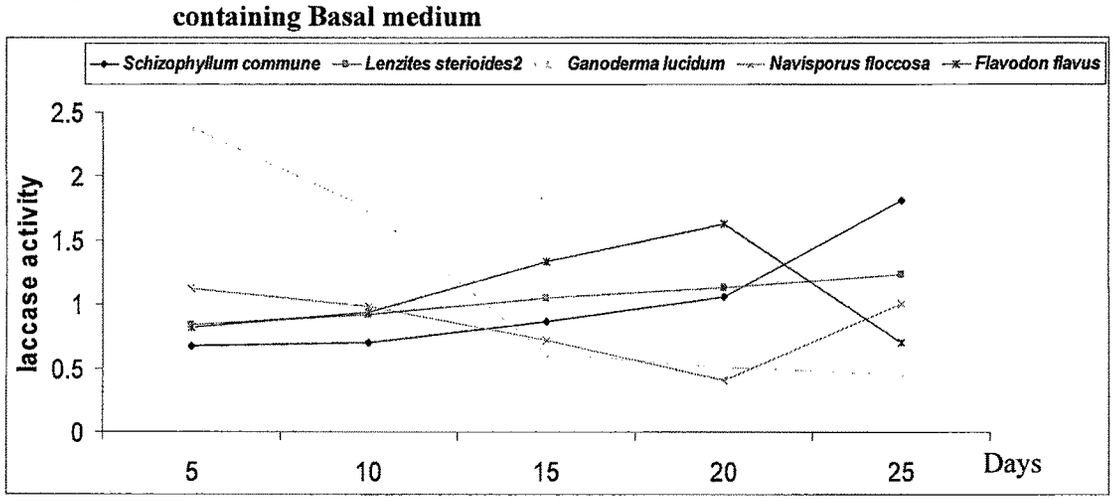
Table 20: Estimation of lignin degrading enzymes of different fungi in basal medium containing KNO_3

Lignin Degrading Enzymes				
Schizophyllum commune				
Days	Laccase* U/ml	Aryl Alcohol oxidase* U/ml	Lignin Peroxidase* U/ml	Peroxidase* U/ml
5	0.67 ±0.2	1.27±0.04	2.12±0.02	10.52±0.04
10	0.70±0.05	3.41±0.06	2.62±0.05	9.56±0.05
15	0.86±0.03	2.63±0.05	2.20±0.04	6.40±0.02
20	1.06±0.04	2.38±0.03	2.00±0.01	5.16±0.06
25	1.81±0.06	2.25±0.01	1.83±0.04	3.60±0.04
Lenzites sterioides2				
5	0.84±0.03	1.32±0.04	1.41±0.07	11.12±0.1
10	0.92±0.02	1.42±0.03	1.83±0.05	11.48±0.2
15	1.05±0.05	1.51±0.02	2.16±0.07	7.16±0.25
20	1.13±0.06	1.59±0.04	3.45±0.03	5.68±0.32
25	1.23±0.06	1.69±0.07	4.20±0.06	3.64±0.40
Ganoderma lucidum				
5	2.38±0.20	1.73±0.08	4.16±0.08	15.48±0.20
10	1.75±0.07	2.76±0.06	7.08±0.01	17.9±0.10
15	0.62±0.10	1.82±0.09	2.95±0.03	12.92±0.23
20	0.51±0.03	1.28±0.05	2.70±0.06	5.56±0.32
25	0.45±0.04	0.83±0.10	2.45±0.08	1.64±0.12

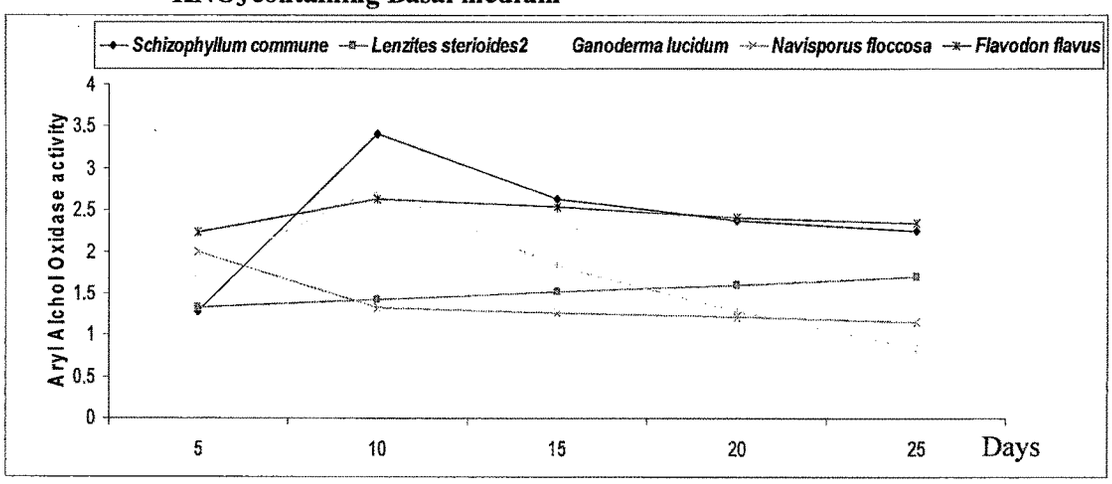
		Navisporus floccosa		
5	1.12±0.05	2.00±0.03	3.70±0.10	12.96±0.15
10	0.98±0.10	1.32±0.05	2.16±0.20	10.16±0.21
15	0.72±0.03	1.26±0.04	2.00±0.24	8.72±0.14
20	0.40±0.04	1.21±0.08	1.41±0.05	2.44±0.05
25	1.00±0.06	1.14±0.07	1.45±0.06	2.16±0.08
		Flavodon flavus		
5	0.82±0.05	2.23±0.06	2.33±0.05	10.83±0.24
10	0.94±0.03	2.63±0.08	7.33±0.02	14.0±0.34
15	1.33±0.10	2.53±0.04	5.70±0.08	10.00±0.24
20	1.63±0.04	2.40±0.03	5.29±0.06	6.16±0.12
25	0.70±0.02	2.34±0.20	4.83±0.07	2.48±0.05

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

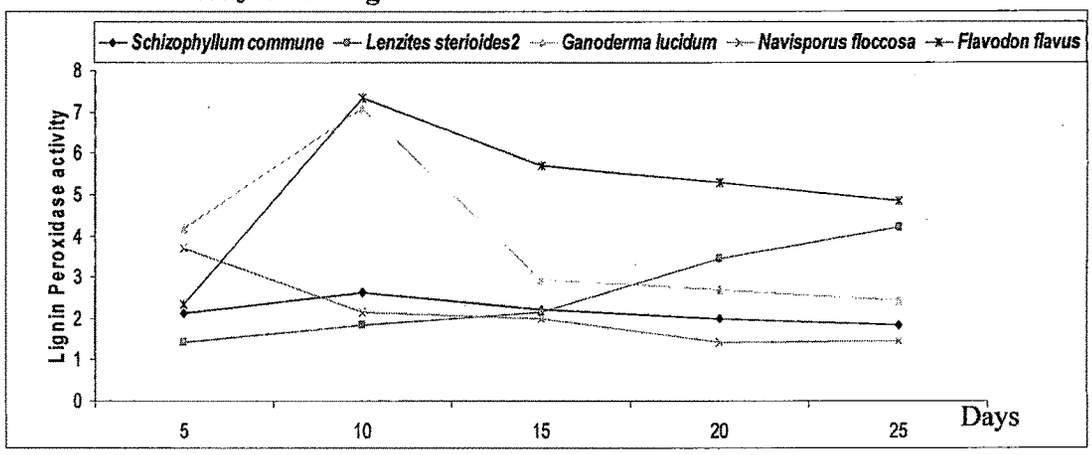
Histogram 9: Laccase activity of different wood rotting fungi in the KNO_3 containing Basal medium



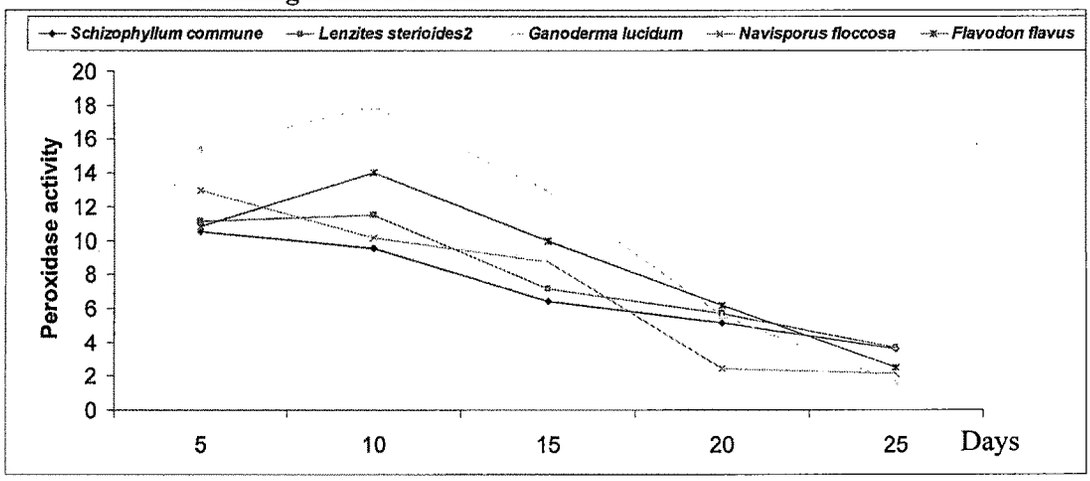
Histogram 10: Aryl Alcohol Oxidase activity of different wood rotting fungi in the KNO_3 containing Basal medium



Histogram 11: Lignin peroxidase activity of different wood rotting fungi in the KNO_3 containing Basal medium



Histogram 12: Peroxidase activity of different wood rotting fungi in the KNO_3 containing Basal medium



It is evident from the Table 21 that in basal medium supplemented with $NH_4(NO_3)_2$ the *S. commune* showesd highest laccase activity in 20 days (12.05 U/ml), whereas lowest in 5 days (0.37 U/ml). The highest aryl alcohol oxidase activity was observed in 20 days (6.85 U/ml) whereas lowest in 5 days (0.17 U/ml). The highest peroxidase activity was observed in 20 days (16.64 U/ml) whereas lowest in 5 days (3.38 U/ml). The highest lignin peroxidase activity was obaerved in 20 days (45.50 U/ml) whereas lowest in 5 days (4.08 U/ml). The *L. sterioides* showed highest laccase activity in 10 days (1.52 U/ml), whereas lowest laccase activity was observed in 25 days (0.13 U/ml). The highest aryl alcohl oxidase activity was observed in 10 days (0.97 U/ml), whereas lowest aryl alcohol oxidase was observed in 5 and 25 days (0.23 U/ml). The highest peroxidase activity was observed in 15 days (8.20 U/ml), whereas lowest peroxidase activity was observed in 25 days (0.63 U/ml). The highest lignin peroxidase activity is observed in 10

days (15.79 U/ml), whereas lowest lignin peroxidase activity was observed in 25 days (2.00 U/ml).

The *G. lucidum* showed highest laccase activity in 5 days (0.95 U/ml) whereas lowest in 25 days (0.20 U/ml). The highest aryl alcohol oxidase activity was observed on 5th day (0.53 U/ml), whereas lowest on 20th day (0.21 U/ml). The *N. floccosa* showed highest laccase activity in 5 days (1.98 U/ml) whereas lowest in 20 days (0.53 U/ml. The *F. flavus* showed highest laccase activity on the 15th day (6.32 U/ml), whereas lowest activity was onserved on 5th day (0.25 U/ml). The highest aryl alcohol oxidase activity was observed on 10th day (1.90 U/ml), whereas lowest aryl alcohol oxidase was observed on 5th day (0.21 U/ml).

Table 21: Estimation of lignin degrading enzymes of different fungi in basal medium containing $NH_4(NO_3)_2$

Schizophyllum commune				
Days	Laccase* U/ml	Aryl Alcohol oxidase* U/ml	Peroxidase* U/ml	Lignin peroxidase* U/ml
5	0.37±0.03	0.17±0.04	3.38±0.08	4.08±0.1
10	6.86±0.04	0.38±0.02	5.32±0.07	13.20±0.2
15	11.15±0.08	1.99±0.08	5.82±0.10	4.08±0.32
20	12.05±0.20	6.85±0.30	16.64±0.25	45.50±1.2
25	5.58±0.15	4.49±0.20	12.74±0.14	26.29±1.5
Lenzites sterioides2				
5	0.28±0.02	0.23±0.04	4.00±0.2	7.75±0.12
10	1.52±0.04	0.97±0.06	6.68±0.26	15.79±0.32
15	0.42±0.05	0.44±0.02	8.20±0.05	2.91±0.21
20	0.33±0.01	0.62±0.07	1.69±0.03	2.25±0.15
25	0.13±0.05	0.23±0.02	0.62±0.04	2.00±0.25
Ganoderma lucidum				
5	0.95±0.04	0.53±0.01	4.28±0.05	9.20±0.15
10	0.45±0.02	0.28±0.04	3.46±0.03	3.54±0.18
15	0.36±0.01	0.25±0.06	2.84±0.07	2.20±0.05
20	0.30±0.08	0.21±0.07	1.74±0.09	1.37±0.08
25	0.20±0.03	0.34±0.02	1.10±0.02	2.54±0.02
Navisporus floccosa				
5	1.98±0.05	0.98±0.03	5.54±0.2	8.00±0.12
10	0.59±0.02	0.28±0.05	4.22±0.1	3.87±0.04
15	0.80±0.06	0.38±0.08	4.82±0.24	4.70±0.20
20	0.53±0.04	0.25±0.06	2.56±0.05	3.75±0.04
25	1.07±0.08	0.73±0.07	4.90±0.21	6.20±0.24
Flavodon flavus				
5	0.25±0.02	0.21±0.02	2.68±0.05	3.25±0.15
10	5.65±0.40	1.90±0.04	7.10±0.07	21.37±0.23
15	6.32±0.50	1.35±0.01	3.84±0.02	13.83±0.35
20	1.46±0.02	0.68±0.08	1.16±0.04	5.54±0.05
25	1.86±0.08	0.96±0.04	0.64±0.01	6.83±0.08

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

It is evident from the table 22 that in Basal medium containing $Ca(NO_3)_2$ the *S. commune* showed highest laccase activity on 10th day (6.71 U/ml), wheras lowest activity was observed on 25th day (0.18 U/ml). The highest peroxidase activity was observed in 20 days (8.26 U/ml), whereas lowest peroxidase activity was observed in 25 days (0.70 U/ml). The highest lignin peroxidase activity was observed in 10 days (2.08 U/ml), whereas lowest activity is observed on 25th day (0.08 U/ml). *L. sterioides* howed the highest laccase activity on 25th days (0.92 U/ml), wheras lowest activity is observed on 5th day (0.37 U/ml). The *G. lucidum* showed highest laccase activity on 10th day (5.04 U/ml), whereas lowest activity was observed on 20th day (0.24 U/ml). The highest aryl alcohol oxidase activity was observed on 10th day (0.57 U/ml), whereas lowest aryl alcohol oxidase activity was observed on 20th day (0.25 U/ml). The highest peroxidase activity was observed on 20th day (7.34 U/ml), whereas lowest peroxidase activity was observed on 25th day (0.78 U/ml). The highest lignin peroxidase activity was observed on 5th day (2.75 U/ml), whereas lowest lignin peroxidase activity was observed on 25th day (0.91 U/ml). The *N. floccosa* showed highest laccase activity on 15th day (0.58 U/ml) whereas lowest on 20th day (0.09 U/ml). It is evident from table 22 that *F. flavus* showed highest laccase activity in 10 days (8.24 U/ml) whereas lowest activity was observed in 25 days (0.14 U/ml).

Table 22: Estimation of lignin degrading enzymes of different fungi in basal medium containing $Ca(NO_3)_2$

Schizophyllum commune				
Days	Laccase* U/ml	Aryl Alcohol oxidase* U/ml	Peroxidase* U/ml	Lignin peroxidase* U/ml
5	0.38±0.02	0.18±0.04	0.82±0.02	1.37±0.03
10	6.71±0.05	1.30±0.02	0.84±0.04	2.08±0.05
15	1.84±0.06	1.43±0.08	2.54±0.08	1.93±0.07
20	0.20±0.08	0.20±0.07	8.26±0.20	1.63±0.08
25	0.18±0.03	1.17±0.05	0.70±0.01	0.08±0.08
Lenzites sterioides2				
	0.37±0.02	0.17±0.04	1.02±0.03	0.79±0.04
10	0.43±0.04	0.30±0.07	1.34±0.04	0.62±0.07
15	0.76±0.07	0.45±0.03	1.24±0.05	1.75±0.08
20	0.83±0.05	0.52±0.01	6.64±0.07	2.33±0.01
25	0.92±0.09	0.41±0.08	1.62±0.08	2.08±0.08
Ganoderma lucidum				
5	0.96±0.03	0.49±0.07	4.00±0.1	2.75±0.08
10	5.04±0.05	0.57±0.04	0.84±0.02	2.56±0.04
15	0.47±0.01	0.36±0.06	1.16±0.05	1.45± 0.06
20	0.24±0.06	0.25±0.04	7.34±0.2	1.00±0.08
25	0.44±0.08	0.28±0.01	0.78±0.02	0.91±0.02

		Navisporus floccosa		
5	0.27±0.08	0.11±0.08	0.24±0.01	0.62±0.04
10	0.30±0.05	0.16±0.06	0.44±0.03	0.54±0.06
15	0.58±0.07	0.21±0.02	0.92±0.05	1.75±0.02
20	0.09±0.02	0.29±0.03	8.74±0.07	1.45±0.04
25	0.46±0.01	0.30±0.05	0.72±0.02	0.08±0.07
		Flavodon flavus		
5	0.36±0.02	0.16±0.03	0.50±0.02	0.91±0.01
10	8.24±0.04	0.86±0.06	1.52±0.04	2.54±0.04
15	0.26±0.05	0.39±0.02	1.12±0.05	2.04±0.06
20	0.24±0.01	0.25±0.04	8.48±0.06	1.75±0.01
25	0.14±0.08	0.24±0.08	0.60±0.01	1.33±0.05

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

It is evident from the table 23 that in basal medium containing Na NO_3 the *S. commune* showed highest laccase activity on 15th day (1.96 U/ml), whereas lowest laccase activity was observed on 5th day (0.37 U/ml). The highest aryl alcohol oxidase activity was observed on 15th day (1.45 U/ml), whereas lowest activity wasobserved on 5th day (0.36 U/ml). The highest lignin peroxidase and peroxidase activity was observed on 10th day (9.12 U/ml) and 20th day (4.62 U/ml) respectively.The *L. sterioides* showed highest laccase activity on 15th day (4.31 U/ml), whereas lowest activity was observed on 5th day (0.24 U/ml). The highest aryl alcohol oxidase activity was observed on 15th day (1.82 U/ml), whereas it was lowest on 5th day (0.15 U/ml).

The *G. lucidum* showed highest laccase activity on 15th day (2.00 U/ml), whereas lowest activity was observed on 25th day (0.16 U/ml). The highest peroxidase activity .was observed on 15th day (5.08 U/ml) and the highest lignin peroxidase activity was observed on 15th day (5.95 U/ml). The *N. floccosa* showed highest laccase activity on 15th day (1.68 U/ml), whereas lowest activity was observed on 20th day (0.12 U/ml). The highest aryl alcohol oxidase activity was observed on 15th day (1.03 U/ml), whereas lowest on 5th day (0.25 U/ml). The *F. flavus* showed highest laccase activity in basal medium containing $NaNO_3$ in 20 days (8.83 U/ml), whereas lowest activity is observed on 25th day (0.81 U/ml).

Table 23: Estimation of lignin degrading enzymes of different fungi in basal medium containing $NaNO_3$

		Schizophyllum commune		
Days	Laccase* U/ml	Aryl Alcohol oxidase* U/ml	Peroxidase* U/ml	Lignin peroxidase* U/ml
5	0.37±0.03	0.36±0.04	1.58±0.1	6.71±0.2
10	1.57±0.05	1.03±0.05	2.44±0.17	9.12±0.3
15	1.96±0.07	1.45±0.07	3.08±0.2	7.10±0.5
20	1.94±0.05	1.04±0.08	4.62±0.3	3.87±0.7
25	0.86±0.06	1.04±0.05	2.76±0.25	3.83±0.3

Lenzites sterioides2				
5	0.24±0.05	0.15±0.02	0.96±0.02	0.95±0.04
10	1.01±0.04	0.90±0.01	2.88±0.2	2.40±0.08
15	4.31±0.07	1.82±0.04	4.68±0.15	8.50±0.12
20	1.85±0.03	0.62±0.08	5.52±0.32	1.12±0.06
25	2.26±0.08	0.62±0.07	5.78±0.45	4.41±0.3
Ganoderma lucidum				
5	0.42±0.07	0.25±0.08	0.74±0.03	1.16 ±0.07
10	0.52±0.08	0.44±0.05	1.66±0.08	2.37±0.15
15	2.00±0.02	0.85±0.02	5.08±0.2	5.95±0.24
20	0.21±0.04	0.20±0.04	4.38±0.1	1.87±0.07
25	0.16±0.05	0.18±0.01	4.20±0.5	1.66±0.08
Navisporus floccosa				
5	0.32±0.04	0.25±0.02	0.80±0.05	1.87±0.08
10	0.80±0.05	0.52±0.04	1.36±0.07	1.83±0.04
15	1.68±0.07	1.03±0.7	6.52±0.08	9.79±0.45
20	0.12±0.04	0.28±0.5	4.36±0.02	2.70±0.1
25	0.73±0.08	0.28±0.3	1.70±0.07	1.00±0.08
Flavodon flavus				
5	1.07±0.07	1.22±0.03	2.08±0.08	10.62±0.48
10	1.44±0.03	1.26±0.01	2.94±0.01	11.54±0.78
15	5.01±0.02	0.70±0.05	2.72±0.04	8.70±0.83
20	8.83±0.04	0.70±0.06	3.06±0.07	3.00±0.2
25	0.81±0.05	0.43±0.07	3.00±0.03	6.50±0.5

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

Secretion of extracellular laccase (polyphenoloxidase, EC 1 .10. 3.2) is a common property amongst basidiomycete fungi. In organisms such as *Sporotrichum pulverulentum* and *Phlebia radiata* a function related to lignin degradation has been proposed (Ander and Eriksson, 1976; Kantelinen *et al.,* 1989). For many fungi such as *Agaricus bisporus, Pleurotus sajor-caju* and *Lentinula edodes*, high laccase activities were observed during the phase of substrate colonization, with a reduction in this activity at the beginning of primordium formation (Bonnen, *et al.,* 1994; Ohga and Royse, 2001; Singh, *et al.,* 2003). In agreement with these studies, it was observed that the growth of *P. ostreatus* on solid substrate influences enzymatic activities and laccase production in *A. bisporus* (Wood, 1985). In contrast, other basiodiomycetes such as *L. edodes* and *S. commune* (Leatham and Stahmann, 1981; De Vries *et al.,* 1986) and *A. mellea* secrete laccase in differentiation of vegetative mycelium and to form rhizomorphs (Worrell *et al.,* 1986). A dikaryon of *S. commune* growing in surface culture at 30°C in the dark produced extracellular laccase (EC 1. 10. 3.2). Little extracellular laccase was formed in the light at 24°C (De Vries *et al.,* 1986). Laccases have implicated in the development of

spore-bearing structures of *S. commune* (Leonard and Phillips, 1973; Phillips and Leonard, 1976). In the present study the five white rot producing fungi belong to the laccase and peroxidase producing group, the laccase is observed during the dikaryotization of *S. commune* mycelium which produce the fruting body. *S. commune* showed highest laccase activity in $NH_4(NO_3)_2$ than other basal medium at 20 days (12.05 U/ml).

Dodson *et al.,* (1987) have isolated a ligninase-like enzyme from *C. versicolor* grown in nitrogen limited medium, able to oxidize veratryl alcohol in the presence of H_2O_2. Under nitrogen-rich medium, however, the enzyme was not detectable in either of the two studied strains of *C. versicolor* (Szklarz *et al.,* 1989). Studies by Garcia *et al.,* (1987) suggest that ligninase is produced by several white-rotters, but is located mainly intracellularly, which might explain our failure to detect this activity in the medium of isolates studied. In the present study, timber degrading fungi were grown in different nitrogens sources like KNO_3, $NaNO_3$, $Ca(NO_3)_2$ and $NH_4(NO_3)_2$ to produced lignase activity. Under high nitrogen source medium like $NH_4(NO_3)_2$ the detectable ligniolytic enzymes like laccase, peroxidase, lignin peroxidase and aryl alcohol oxidase were produced by all white rot fungi studied.

Peroxidase was measured spectroscophically. In an investigation all of the 30 species of wood rotting fungi produced some H_2O_2 on blood agar. No brown rot fungus produced extracellular peroxidase in 3 weeks, but individual isolates of *Lentinus lepideus* and *Coniophora puteana* did so later; 11 of the 23 species of white rot fungi secreted peroxidase. This appears to be the first report of the formation of free extracellular H_2O_2 by fungi. This H_2O_2 may be involved in plant pathogenisis and in degradation of plant constituents by wood destroying fungi. All 12 white rot fungi had laccase activity (Machado *et al.,* 2005). *A. mellea G. lucidum* and *L. tigrinus* did not produced extracellular peroxidase after 3 weeks but did so by the 4^{th} week. Two white rot fungi *P. versicolor* and one isolate *of P. tomentisus* produced peroxidase later. *P. weirii* and *F. robustus* was exceptionally high peroxidase activity (Koenings, 1972).

In the present study with the white rot fungi production of peroxidase was strated on 5^{th} day and reached to maximum on 20^{th} day, after that it declined as the incubation period was increased. The *S. commune* produced maximum peroxidase in 20 days when $NH_4(NO_3)_2$ was added as nitrogen source, *L. strioides* produced maximum peroxidase enzyme on 10^{th} day, *N. floccosa* produced maximum peroxidase enzyme on 5^{th} day, *F.*

flavus and *G. lucidum* showed highest peroxidase production on 10th day when KNO_3 was added as nitrogen source.

Maximal peroxidase and laccase activities were observed at 25 days of growth, followed by a significant reduction in these activities at 28 days. Peroxidases might be inactivated in the presence of H_2O_2, with this inactivation depending on the concentration of H_2O_2 (Aitken and Irvine, 1989; Tonon and Odier, 1988). Under refrigeration, reductions of only 20% peroxidase and laccase activities were observed at 18 days. In contrast, freezing of the *P. ostreatus* extract caused a loss of about 51 and 62% reduction of peroxidase and laccase activities, respectively, Peroxidase activity was only affected after 30 days of refrigeration, with a loss of about 90% of its initial activity at 109 days (Machado and Matheus, 2006). In the present study the timber rot fungi showed decline in the production of peroxidase after reaching the maximum as it grew for long periods because of presence of proteases in growth medium.

Quantitative studies of extracellular phenol oxidase and peroxidase production by eight species of wood-rotting fungi were performed. *C. versicolor*, *Phellinus igniarius* and *Lycoperdon* sp. exhibited high laccase activities. *Chaetomium piluliferum* and *C. cellulolyticum* expressed low peroxidase activities. In *Lenzites trabea* no extracellular oxidase or peroxidase activity was found. For *P. igniarius* and *C. versicolor* (one isolate) maximum specific activity of laccase was observed in trophophase. The marked differences in phenol oxidase activities between two strains of *C. versicolor,* one of them recently isolated and the other from ATCC (Szklarz *et al.,* 1989).

In the present study the five white rot fungi produced the laccase, lignin peroxidase Aryl alcohol oxidase and peroxidase in KNO_3, $NaNO_3$, Ca(NO3)2 and $NH_4(NO_3)_2$ as nitrogen sources. *L. strioides* showed highest laccase and peroxidase activity in $NaNO_3$ and KNO_3 basal medium respectively. In the present study when white rot fungi were cultured for long periods some fungi reached to maximum and lost the ability for enzyme production, few white rot fungi reached to maximum and have less decline perioid. They once again started production of enzymes. Some other white rot fugi showed highest production of enzymes as long as the cultured for long time. Under the experimental conditions the ligninase activity was found in all the white rot fungi studied.

In the present study the *L. sterioides* strated production of LiP in 5 days, reached to maximum on 10th days and decline in production on 25 days of incubation. The maximum enzyme production recorded in *L. strioides* was 15.79 U/ml, 8.50 U/ml, 4.20

U/ml and 2.08 U/ml in $NH_4(NO_3)_2$, $NaNO_3$, KNO_3, and $Ca(NO_3)_2$ respectively. *P. elegans* and *T. versicolor* showed maximum production (900 U/ml) of laccase on 15th day of incubation. *L. betulina* showed laccase production (400 U/ml) only on 15th day and did not show any secretion on 5th day and 10th day of incubation. *M. mucedo* showed (500 U/ml) laccase production on 10th day of incubation (Moturi and Charya, 2009). In the present study the *L. sterioides* showed laccase production in 5th and 10th days of incubation. When compared with the *L. betulina* laccase production on 15th day of incubation the *L. sterioides* produced less laccase (4.31 U/ml) in 15 days of incubation.
LiP attained peaks on day 6. The maximum recorded activity of LiP was 50 nkat liter^{-1} in nitrogen-limited liquid media (Dey *et al.*, 1991). In the present study the LiP attained peaks on 10th day and maximum recorded activity was 15.79 U/ml in nitrogen rich liquid media i.e. $NH_4(NO_3)_2$ as nitrogen source. *P. ostreiformis* produced the LIP enzyme involved in ligninolysis of lignocellulose substrates. Two other brown rot fungi, namely *Poria monticola* NCIM 1090 and *Lenzites trabea* NTCC 1314, failed to produce this enzyme (Dey *et al.*, 1991). First reported lignin peroxidase (LIP) production by *Polyporus ostreiformis* (Dey *et al.*, 1991). In the present study the *L. sterioides* able to prodce all ligninolytic enzymes in different nitrogen sources. The presence of laccase, peroxidase, lignin peroxidase and aryl alcohol oxidase were reported for the first time from *L. sterioides.*

The involvement of extracellular oxidases in biotransformation of low-rank coal was assessed by correlating the ability of nine white-rot and brown-rot fungi to alter macromolecular material in alkali-solubilised brown coal with the spectrum of oxidases they produce when grown on low-nitrogen medium (Ralpha *et al.*, 1996). In the present study the KNO_3, $NaNO_3$, $Ca(NO_3)_2$ and $NH_4(NO_3)_2$ was used as nitrogen sources for production of ligninolytic enzymes from *G. lucidum.* In 15-ml cultures, *Gloeophyllum trabeum, Lentinus lepideus* and *Trametes versicolor* produced little or no lignin peroxidase, laccase activity and caused no change to SWC6 coal. *G. applanatum* and *Pycnoporus cinnabarinus* also produced no detectable lignin or laccase yet increased the absorbance at 400 nm of SWC6 coal (Ralpha *et al.*, 1996). In the present study the *G. lucidum* produced the ligninolytic enzymes like laccase, peroxidase Lignin peroxidase and aryl alcohol oxidase in all nitrogen sources. *G. applanatum,* which produced veratryl alcohol oxidase, also increased the modal apparent molecular mass (Ralpha *et al.*, 1996). *Ganoderma* sp. strains have been reported to produce ligninolytic enzymes by using of several agricultural wastes and food industry wastes in submerged fermentation.

In the present study the *G. lucidum* highest laccase activity (5.04 U/ml) was on 10th day of incubation with $Ca(NO_3)_2$ as nitrogen source. The highest aryl alcohol oxidase (2.76 U/ml) was observed on 10th day of incubation with KNO_3 as nitrogen source, the highest lignin peroxidase activity (9.20 U/ml) was observed on 5th day of incubation with $NH_4(NO_3)_2$ as nitrogen source and the highest peroxidase activity (17.90 U/ml) was observed on 10th day of incubation with KNO_3 as nitrogen source.

White rot fungi *G. lucidum* was able to prodce all ligninolytic enzymes when compared to *G. applanatum*. So it may be used in transformation of low rank coal. The quantifcation of enzymes produced in parallel assays during the same incubation period showed that *G. australe* produced only laccase while *C. subvermispora* strains produced both laccase and peroxidase (Mendonça *et al.*, 2008). In the present study the *G. lucidum* able to produce laccase, peroxidase, lignin peroxidase and aryl alcohol oxidase. In *C. subvermispora*, laccase only detected when the fungus was grown in presence of easily assimilable carbon and nitrogen sources (Ferraz *et al.*, 2003; Vicuña *et al.*, 1996). In the present study also when different nitrogen sources were used for the production of ligninolytic enzymes so the *G. lucidum* was able to produce all ligninolytic enzymes.

Marine basidiomycetes fungus *F. flavus* was isolated from sea grass at Mjimwema in the Western Indian Ocean off the Coast of Dar es Salaam, Tanzania. Protein content and lignocellulosic enzyme activities were measured by photometric methods. The fungal filtrate had maximum lignin peroxidase (LiP), and Laccase (Lac) activities of 42, and 15 U/mL, respectively (Mtui and Nakamura 2008). In the present study the *F. flavus* showed highest lignin peroxidase activity in $NH_4(NO_3)_2$ as nitrogen source and highest laccase activity was observed in $NaNO_3$ as nitrogen source.

Raghukumar *et al.*, (1999) and Kondo *et al.*, (2004) demonstrated that the ability of some coastal marine fungi to produce major lignocellulolytic enzymes. In the present study the ligninolytic enzymes were produced after 5th day of incubation. The LiP increase sharply (3.25 U/ml) on 5th day, reached maximum (21.37 U/ml) on 10th day and declined as cultutre was grown for long period. The Lac production strated on 5th day, medium on 15th day and reached to maximum (8.83 U/ml) on 20th day and decline further. The amounts of LiP and Lac are comparable to the amounts produced by the Tanzania's terrestrial mushrooms (Mtui *et al.*, 2003). The decrease in enzyme activities after their peaking was attributable to the production of protease in the medium (Nakamura *et al.*, 1999; Mtui and Nakamura, 2002). In the present study also the decrease in activity after reaching maximum was may be due to the production of proteases in the medium.

6.2.3. Estimation of ligninolytic enzymes in medium containing carbon sources

The lignocellulases in broth cultures of the Basidiomycete *Panus tigrinus* indicates that laccase enzymes were produced. In stationary culture at 28°C, the greatest laccase activity was observed after growth for approximately 9 days. Laccase production was dependent on the presence, and the particular brand, of malt extract in the growth medium. Preliminary studies of static cultures at 28°C in MMG containing CRL malt extract showed a steady increase in laccase from day 5 to day 10, after which activity declined. The substitution of this malt extract by that of HIMedia, | max at 265 nm in the MMG supported growth but not production of laccase. Although not shown, in the absence of malt extract, growth was poor and no laccase activity was observed. Being produced in the absence of their substrates, both enzymes appeared to be constitutive in nature in this *P. tigrinus* strain. From the contrast between the good growth of the fungus and the high laccase activity observed with the malt extract of CRL, | max at 280 nm, and the HIMedia malt extract it appeared that the constituents of the malt extract were crucial for enzyme production. A | max at 280 nm for CRL malt extract could be indicative of the presence of amino acids which were lacking in the other malt extract and that these amino acids may have promoted synthesis of laccase (Nazareth, and Sampy 2003). Laccase activity accumulated during rhizomorph development in malt extract cultures of *A. mellea* (Rehman and Thurston 1992). *T. trogii* laccase isoenzymes were similar in media with different C/N ratio and Cu-concentrations. Likewise, identical laccase isoforms were consistently seen in cultures of *G. lucidum* grown in low N synthetic medium, malt extract or wood (De'Souza *et al.*, 1999).

In Malt extract medium the malt was used as carbon source and the production of ligninolytic enzymes by timber rotting fungi was observed in table 24. *S. commune* had highest capacity to degrade the lignin than *L. sterioides, G. lucidum, N. floccosa* and *F. flavus*. In all the fungi tested lignin peroxidase activity was more and least activity was shown by aryl alcohol oxidase enzyme. It was evident from the table 24 that the *S. commune* showed highest laccase activity in 5 days (6.22 U/ml), whereas lowest laccase activity was observed in 25 days (1.90 U/ml). The highest aryl alcohol oxidase activity was observed on 5th day (2.59 U/ml), whereas lowest aryl alcohol oxidase was observed in 25 days (1.28 U/ml). The highest peroxidase activity was observed on 5th day (7.54 U/ml), whereas lowest peroxidase activity was observed on 20th day (1.98 U/ml). The highest lignin peroxidase activity was observed in 15 days (18.08 U/ml), whereas lowest lignin peroxidase activity was observed in 5 days (13.41U/ml).

L. sterioides showed highest laccase activity on 25th day (5.28 U/ml), whereas lowest laccase activity was observed on 5th day (1.52 U/ml). The highest aryl alcohol oxidase activity was observed on 10th day (1.99 U/ml). Whereas lowest aryl alcohol

oxidase was observed on 5th day (0.72 U/ml). The highest peroxidase activity was observed on 10th day (6.72 U/ml), whereas lowest peroxidase activity was observed on 25th day (2.94 U/ml). The highest lignin peroxidase activity was observed on 20th day (15.87 U/ml), whereas lowest lignin peroxidase activity was observed on 5th day (9.20 U/ml).

G. lucidum showed highest laccase activity on 5th day (2.57 U/ml), whereas lowest laccase activity was observed on 25th day (0.52 U/ml). The highest aryl alcohol oxidase activity was observed on 5th day (1.38 U/ml), whereas lowest aryl alcohol oxidase activity was observed on 25th day (0.35 U/ml). The highest peroxidase activity was observed on 15th day (5.66 U/ml), whereas lowest peroxidase activity was observed on 25th day (1.00 U/ml). The highest lignin peroxidase activity was observed on 5th day (11.04U/ml), whereas lowest lignin peroxidase activity was observed on 25th day (5.70 U/ml).

N. floccosa showed highest laccase activity on 5th day (2.01 U/ml), whereas lowest activity was observed on 25th day (0.54 U/ml). The highest peroxidase activity was observed on 15th day (5.24 U/ml), whereas lowest peroxidase activity was observed on 25th day (0.82 U/ml). The highest lignin peroxidase activity was observed on 10th day (11.62), whereas lowest lignin peroxidase activity was observed on 25th day (7.00 U/ml). *F. flavus* showed highest laccase activity on 5th day (2.27 U/ml), whereas lowest laccase activity was observed on 25th day (0.80 U/ml).

Table 24: Estimation of lignin degrading enzymes of different fungi in Malt extract medium

Schizophyllum commune				
Days	Laccase* U/ml	Aryl Alcohol oxidase* U/ml	Peroxidase* U/ml	Lignin peroxidase* U/ml
5	6.22±0.1	2.59±0.04	7.54±0.08	13.41± 0.3
10	3.42±0.3	1.61±0.02	7.50±0.06	14.58±1.2
15	3.27±0.5	1.63±0.06	7.08±0.05	18.08±1.5
20	3.10±0.25	1.57±0.08	1.98±0.07	16.00±1.8
25	1.90±0.08	1.28±0.01	3.84±0.02	14.04±2.5
Lenzites sterioides2				
5	1.52±0.05	0.72±0.04	4.18±0.01	9.20±1.5
10	1.83 ±0.06	1.99±0.06	6.74±0.04	11.00±2.8
15	2.56±0.02	1.03±0.08	5.72± 0.8	13.50±2.5
20	2.91±0.07	0.90±0.02	5.04±0.3	15.87±2.9
25	5.28±0.03	0.75±0.04	2.94±0.1	14.41±3.5
Ganoderma lucidum				
5	2.57±0.04	1.38±0.03	3.16±0.4	11.04±1.6
10	1.31± 0.06	0.63±0.02	3.44±0.5	8.33±1.8
15	1.11±0.07	0.73±0.05	5.66±0.2	9.41±2.6
20	1.00±0.02	0.40±0.06	1.52±0.08	5.87±2.4
25	0.52±0.05	0.35±0.07	1.00±0.06	5.70±2.3

		Navisporus floccosa		
5	2.01±0.08	0.95±0.07	2.78±0.04	8.70±2.5
10	1.78±0.07	1.01±0.03	4.38±0.2	11.62±2.3
15	1.75±0.03	0.94±0.05	5.24±0.5	10.45±3.2
20	1.65±0.05	0.78±0.03	2.08±0.7	10.29±3.5
25	0.54±0.02	0.36±0.08	0.82±0.02	7.00±2.2
		Flavodon flavus		
5	2.27±0.07	1.61±0.08	2.82±	10.91± 1.5
10	1.31±0.05	1.98±0.07	6.20±	13.87±2.5
15	1.27±0.02	0.99±0.02	3.24±	13.91±3.2
20	0.88±0.06	0.56±0.04	1.74±	17.66±3.5
25	0.80±0.08	0.53±0.07	0.80±	9.50±2.8

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

In Basal medium containing teak wood sawdust, all the fungi were able to produce more lignin peroxidase responsible for the degradation of lignin. Highest laccase, aryl alcohol oxidase, peroxidase, lignin peroxidase activity was shown by *S. commune* than the other fungi. Lowest ligninolytic enzymes activity was showed by *F. flavus*. The *S. commune* showed highest laccase activity on 2nd day (0.85 U/ml), whereas lowest activity was observed on 10th day (0.70 U/ml). The highest aryl alcohol oxidase activity was observed on 10th day (0.68 U/ml), whereas lowest aryl alcohol oxidase activity was observed on 2nd day (0.45 U/ml).

L. sterioides showed highest laccase activity on 10th day (0.64 U/ml), whereas lowest laccase activity was observed on 2nd days (0.45 U/ml). *G. lucidum* showed highest laccase activity on 8th day (0.81 U/ml), whereas lowest laccase activity was observed on 2nd day (0.64 U/ml). The highest aryl alcohol oxidase activity was observed on 10th day (0.50 U/ml), whereas lowest aryl alcohol oxidase activity was observed in 2nd day (0.35 U/ml). The highest peroxidase activity was observed on 6th day (0.82 U/ml), whereas lowest peroxidase activity was observed on 10th day (0.40 U/ml). The highest lignin peroxidase activity was observed on 8th day (6.00 U/ml), whereas lowest lignin peroxidase activity was observed on 2nd day (5.58 U/ml).

N. floccosa showed highest laccase activity on 6th day (0.85 U/ml), whereas lowest laccase activity was observed on 2nd day (0.61 U/ml). *F. flavus* showed highest laccase activity on 8th day (0.61 U/ml), whereas lowest laccase activity was observed on 2nd days (0.43 U/ml). The highest aryl alcohol oxidase was observed on 10th day (0.38 U/ml), whereas lowest aryl alcohol oxidase activity was observed on 2nd day (0.27 U/ml). The highest peroxidase activity was observed on 4th day (0.78 U/ml), wheras lowest

peroxidase activity was observed on 10th day (0.60 U/ml). The highest lignin peroxidase activity was observed on 8th day (5.58 U/ml), whereas lowest lignin peroxidase activity was observed on 10th day (4.29 U/ml).

Table 25: Estimation of lignin degrading enzymes of white rot fungi in Basal medium containing Teak wood sawdust

Schizophyllum commune				
Day	Laccase* U/ml	Aryl Alcohol oxidase* U/ml	Peroxidase* U/ml	Lignin peroxidase* U/ml
2	0.85±0.02	0.45±0.01	0.90±0.04	6.33±1.2
4	0.81±0.04	0.47±0.04	1.00±0.07	7.00±0.9
6	0.80±0.06	0.48±0.06	1.16±0.05	9.20±1.5
8	0.78±0.01	0.54±0.07	1.10±0.04	7.54±2.3
10	0.70±0.07	0.68±0.02	1.00±0.07	6.91±1.8
Lenzites sterioides2				
2	0.45±0.06	0.24±0.02	0.64±0.05	4.16±2.6
4	0.52±0.02	0.28±0.03	0.56±0.03	4.79±1.5
6	0.56±0.03	0.31±0.04	0.48±0.02	4.83±1.8
8	0.62±0.07	0.33±0.06	0.44±0.05	4.45±2.6
10	0.64±0.08	0.34±0.07	0.40±0.07	4.29±2.8
Ganoderma lucidum				
2	0.64±0.08	0.35±0.04	0.64±0.02	5.50±2.9
4	0.67±0.02	0.36±0.05	0.70±0.04	5.58±1.5
6	0.71±0.05	0.43±0.08	0.82±0.07	5.83±1.3
8	0.81±0.03	0.44±0.02	0.78±0.02	6.00±2.8
10	0.75±0.06	0.50±0.07	0.72±0.07	5.87±1.6
Navisporus floccosa				
2	0.61±0.02	0.36±0.07	0.48±0.07	5.04±1.8
4	0.66±0.06	0.37± 0.04	0.52±0.08	5.25±2.7
6	0.85±0.08	0.38±0.02	0.68±0.02	7.58±2.3
8	0.76±0.02	0.40±0.05	0.78±0.05	5.33±3.2
10	0.68±0.03	0.49±0.08	0.84±0.08	5.45±2.3
Flavodon flavus				
2	0.43±0.02	0.27±0.02	0.62±0.02	4.45±1.3
4	0.45±0.04	0.28±0.05	0.78±0.05	4.62±1.4
6	0.52±0.06	0.31±0.06	0.58±0.06	4.75±1.8
8	0.61±0.08	0.36±0.08	0.58±0.03	5.58±2.8
10	0.44±0.01	0.38±0.01	0.60±0.08	4.29±1.8

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

Stajic *et al.*, (2006) detected lowest Lac activity in medium containing 10 g/l glucose at the level of 24 ± 13 U/l for P. eryngii and 45.4 ± 5.8 U/l for *P. ostreatus*. In the present study the *G. lucidum* was grown on malt extract medium for the production of ligninolytic enzymes i.e. laccase, lignin peroxidase, ary alcohol oxidase and

peroxidase. The *G. lucidum* was producing lacase activity in the 1% malt extract is 2.57U/ml for 5 days and then decreasing later.

C. versicolor strain RC3 produced LiP and laccase 0.17 and 0.04U/g substrate, respectively. However, laccase was produced at 0.12U/g substrate, while LiP decreased to 0.09U/g substrate when 4% (w/v) glucose solution were added (Khanongnuch *et al.*, 2004). In the present study the *L. sterioides* produced LiP and laccase activity 1.99 and 5.28 U/ml respectively and all the wood rotting fungi produced ligninolytic enzymes in 1% malt extract medium. These results indicate the difference in physiological responses to nutrient parameters in *C. versicolor* strain RC3 and *P. chrysosporium* ATCC 34541 (Khanongnuch *et al.*, 2004). In the present study also the physiological conditions influenced the production of ligninolytic enzymes in wood rotting fungi under study.

Laccase is only the ligninolytic enzyme produced by *Ganoderma* sp. KU-Alk4 in Kirk medium. Laccase production started on day 3 with the addition of 0.85 mM veratryl alcohol as inducer (Teerapatsakul *et al.*, 2007). In the present study the laccase production started on 5th day for all the wood roting fungi under study with the addition of malt as inducer. Higher activity of laccase was detected in G1% than in G4% culture. The fungus produced overall laccase activity (U/mL) about two fold higher in G1% medium than in G4%. However, the mycelial dry weight of fungus in G1% medium was 1.6-fold lower than that in G4% at day 9, the time at which the maximal laccase activities were observed. The specific activities of laccase at day 9 in G1 and G4% media were 126.8 U/mg cell dry weight and 33.3 U/ mg cell dry weight, respectively (Teerapatsakul *et al.*, 2007). In the present study as the incubation perioid is increasing the laccase activity is increasing in case of *L. sterioides*2 whereas decrease in activity was observed in case of *S. commune, G. lucidum, N. floccosa* and *F.flavus.* Of the five carbon sources tested, glucose supported the highest fungual mycelial biomass yield of (88.32±1.50mg/cm3), while mannose supported the highest laccase enzyme production with 47.5 ±1.85 (U/min) of laccase ($p \leq 0.05$) (Adejoye and Fasidi, 2009). In the present study the maltose induced good growth of mycelial mass as well as the highest laccase activity.

To reveal the most favorable cultivation conditions for laccase production for a number of selected strains this study has been initiated. 29 strains of 25 Basidiomycetes species were studied in stationary and submerged cultures using several liquid nutritional media. A number of the selected strains included species well-known as laccase producers belonging to the genera *Lentinus, Hypholoma*, and *Trametes*. On the contrary,

several of the fungal isolates belonged to genera such as *Lentinellus, Lenzites, Oudemansiella, Polyporus, Steccherinum*, and *Tubaria* that have not been studied yet for laccase production. Liquid alewort, malt extract and two glucose-peptone media with different mineral components were used for stationary and submerged cultivations. Each isolate had individual priorities for nutritional media and cultivation method regarding its laccase production. Growth on malt extract showed high laccase activity in *Lenzites betulina, Oudemansiella mucida, Tubaria* sp., and *Polyporus squamosus*. *Lenzites betulina* revealed high laccase production under both stationary and submerged cultivation on liquid malt extract, but not on glucose-peptone LN-AS medium (Belova *et al.*, 2006). In the present study the *L. sterioides* showed highest laccase activity after 25 days of incubation when malt extract is used as carbon source. Whereas all other wood rotting fungi showed highest laccase activity on 5^{th} day and decreased their activity as incubation perioid increase. In the present study the lacase activity in *L. sterioides* was 5.28U/ml at 25days, in *S. commune* was 6.22 U/ml in 5 days, in *G. lucidum* was 2.57 U/ml at 5 days, in *N. floccosa* was 2.01U/ml and in *F. flavus* was 2.27 U/ml at 5 days respectively.

The extracellular production of lignin peroxidase was observed in the liquid culture growth media amended with lignin containing natural substrates, like corncob, coirdust, sawdust, wheat straw, and bagasse inoculated with mycelia of *P. sajor caju* MTCC–141.The control experiment was run in parallel, which has similar medium composition except the natural lignolytic substrate. The results indicate that the presence of lignin containing natural substrates in liquid media enhanced the extracellular secretion of lignin peroxidase. The order of induction of lignin peroxidase production in the liquid culture medium by *P. sajor caju* was bagasse > sawdust > coirdust > wheat straw > corncob > control (Yadav *et al.*, 2009). In the present study the malt and teak saw dust was used as carbon source for induction of lignin peroxidase. The LiP activity was more in malt than in sawdust for all the wood rotting fungi under the study.

Lentinula edodes, Volvariella volvacea and *Pleurotus sajor caju* are three important commertially cultivated mushrooms which exhibit varying abilities to utilize different lignocellulosic as growth substrates. *L. edodes* was cultivated on highly lignified substrates such as wood or sawdust produce two extracellular enzymes like laccase (Buswell *et al.*, 1996). *V. volvacea* which prefer high cellulose and low lignin containing substrates produce more cellulaes and no lignin degrading enzymes (Buswell *et al.*, 1996). In the present study all the wood rotting fungi able to utilize different

lignocellulose sbstrated for the production of extracellular laccase, lignin perozidase, ary alcohol oxidase and peroxidase and wood rotting fungi under study prefer high lignin containing subastrate and low cellulose containing substrate for production of lignin degrading enzymes.

LME production was also seen in media prepared with artificial seawater. This was the first report on the production of all three major classes of LMEs by *F. flavus* and points to the bioremediation potential of this organism in terrestrial as well as marine environments (Raghukumar *et al.,* 1999). In the present study when *F. flavus* was grown in teak sawdust medium it produced good levels of laccase and LiP. *G. carnosum* was analysed for its ability to produce laccase (Lac) in wheat straw, corn stem, oak and grapevine sawdust as plant raw materials. The obtained Lac activity was very low in the medium with wheat straw (1.80 U/l), while it was not detected in the presence of other three analyzed plant raw materials (Simoniã *et al.,* 2009). In the present study the *G. lucidum* produced good laccase activity in malt and moderated levels in teak sawdust.

G. lucidum was studied for the production of laccase in malt extract, or wood-grown cultures. Laccase production was readily seen in cultures grown with pine or poplar (100-mesh-size ground wood) as the sole carbon and energy source. Cultures containing both pine and poplar showed 5- to 10-fold-higher levels of laccase than cultures containing pine or poplar alone. Since syringyl units are structural components important in poplar lignin and other hardwoods but much less so in pine lignin and other softwoods, pine cultures were supplemented with syringic acid, and this resulted increased laccase levels comparable to those seen in pine-plus-poplar cultures. No LiP production was observed in cultures of *G. lucidum* grown with pine, poplar, or pine plus poplar (De'souza *et al.,* 1999). In the present study the moderate levels of laccase enzyme and good levels of LiP enzyme was produced in teak sawdust medium as carbon source.

Wood is the natural substrate for *G. lucidum,* which was known to cause extensive delignification of various species of hardwoods worldwide (Adaskaveg *et al.,* 1990). Yet a large majority of the previous studies on the production of LMEs have been carried out with defined media (Boominathan and. Reddy. 1992, Kirk and Farrell 1987), and none has been reported for *G. lucidum*. Recent results show that LME production when white rot fungi are grown in wood-containing media (Schlosser *et al.,* 1997). In the present study all the wood rotting fungi under study showed moderate to good levels of laccase enzyme in the presence of teak sawdust and malt as carbon source.

Researchers have shown that low-molecularweight aromatic acids, such as syringic acid, that are structurally related to individual phenolic moieties in lignin serve as good inducers of LMEs (Eggert, *et al.,* 1996a; Yaver *et al.,* 1996). They used commercially available syringic acid as a substitute for syringyl moieties of lignin (in the same way as ferulic acid is often used as a substitute for coniferyl alcohol). Addition of syringic acid to pine cultures of *G. lucidum* resulted in laccase activity (14.7 mkat/liter) comparable to that seen in pine-plus-poplar cultures (14.8 mkat/liter) but was much higher than that produced in the medium with pine alone or poplar alone. These data suggest that the stimulation of laccase production in pine-pluspoplar cultures of *G. lucidum* is probably due to the syringyl units contributed by poplar lignin (De'souza *et al.,* 1999). In the present study the laccase production is moderate because of low syringyl moieties in teak sawdust.

In the present study the *S. commune* produced good levels of laccase and peroxidase enzymes in both malt and teak sawdust as sole carbon source. In the present study as the growth period increase the laccase production increase upto 8 days and later on decreasing. In the present study the wood rotting fungi showed moderate to good production of laccase when teak sawdust is used as carbon source. In the present study the G. lucidum isolate showed production of ligninolytic enzymes in 5 days and least activity is noted in 25 days. The maximum LiP activity was notice in 5days 11.04U/ml and for laccase 2.57 U/ml for malt as sole carbon source.

A total of 45 isolates of white rot fungus *S. commune* taken from different wood species and various environment were investigates as to exsistence and activity of some wood degrading enzymes. Most of the isolates produced laccase and peroxidase (Schmidt and Liese, 1980). *G. australe* strains produced only laccase during biodegradation of *E. globulus* with values reaching 60 IU kg¡1 of wood within 15 days of biodegradation. With the increase of the biodegradation period, the amount of laccase produced by *G. australe* decreased to 20–30 IU kg¡1 wood after 45–60 days of incubation (Mendonça *et al.,* 2008). C. subvermispora did not show laccase activity during wood biodegradation of hardwood or softwood when there was no addition of carbon to the culture medium (Souza-Cruz *et al.,* 2004; Vicentim and Ferraz, 2007).

An indigenous novel strain of *G. lucidum* IBL-06 was investigated for the production of ligninolytic enzymes including lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase using different lignocellulosic substrates in still culture solid-state fermentation (SSF) (Asgher *et al.,* 2010). Samples were harvested after every

48 h to study the profile of ligninolytic enzymes produced by the fungus on different substrates. Maximum enzyme activities were noted on 10th day of incubation on rice straw. *Ganoderma lucidum* IBL-06 produced highest activities of lignin peroxidase (LiP) among the lignolytic enzymes. By optimizing the SSF process, maximum activities of LiP (2185 IU/ml) and laccase (338 IU/ml) were achieved after three days incubation of rice straw at using fructose as carbon source (Asgher *et al.,* 2010). Lignin peroxidase showed highest activity on 10^{th} day of fermentation. The level of enzyme activity was compared with submerged fermentation for *P. ostreatus* with wheat straw as the substrate (Gupte *et al.,* 2007). In the present study the different wood rotting fungi showed highest activity of laccase and lignin peroxidase in 25 days.

In the present study the *F. flavus* showed good amount of laccase activity in 5 – 6 days when malt and teaksawdust used as carbon source. *F. flavus* (NIOCC strain 312) isolated from decomposing leaves of a sea grass, decolorized pigments in molasses spent wash (MSW) by 80% after 8 days of incubation, when used at concentrations of 10% and 50% (Raghukumar and Rivonkar, 2001). The addition of MSW decreased the production of laccase by *F. flavus*. However, the addition of Kraft paper mill bleach effluent increased the production of laccase in this culture (Raghukumar, 2000).

6.2.4. Estimation of ligninolytic enzymes in artificially inoculated woods with wood rotting fungi

It is evident from Table 26 and histogram 13 to 16, that 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 in case of teak infected with *T. pini*. The highest lignin peroxidase activity was observed in case of *Adina* infected with *C. versicolor,* whereas lowest in case of the *T. crenulata* infected with *L. sterioides*.

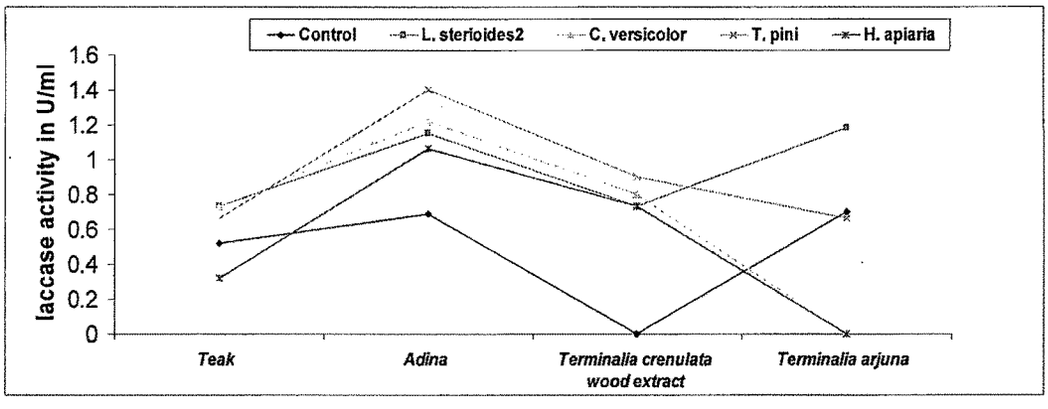
Table 26: Estimation of lignin degrading enzymes of white rot fungi in infected woods

	Teak wood extract			
Fungi	Laccase* U/ml	Aryl Alcohol oxidase* U/ml	Peroxidase* U/ml	Lignin peroxidase* U/ml
Control	0.52±0.02	0.54±0.03	0.66±0.05	6.79±1.2
L. sterioides2	0.73±0.04	0.65±0.05	0.78±0.02	9.04±2.4
C. vesicolor	0.73±0.05	0.55±0.07	0.98±0.07	12.83±2.5
T. pini	0.66±0.02	0.50±0.08	0.90±0.06	8.95±2.7
H. apiaria	0.32±0.08	0.27±0.04	0.74±0.01	8.75±1.6

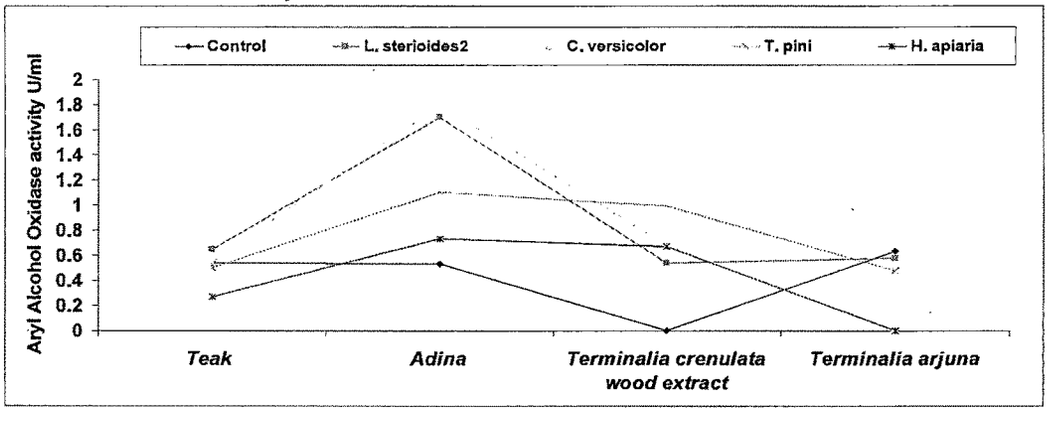
Adina wood extract				
Control	0.69±0.04	0.53±0.02	1.58±0.06	8.08±2.5
L. sterioides2	1.15±0.05	1.70±0.04	1.80±0.02	13.29±1.5
C. vesicolor	1.22±0.06	1.80±0.06	2.96±0.07	16.66±2.6
T. pini	1.40±0.08	1.10±0.02	2.90±0.05	14.79±2.8
H. apiaria	1.06±0.03	0.73±0.01	1.30±0.03	10.95±1.9
Terminalia crenulata wood extract				
Control	--	--	--	--
L. sterioides2	0.73±0.04	0.54±0.04	0.88±0.04	6.41±2.8
C. versicolor	0.80±0.06	0.68±0.02	1.42±0.05	9.66±1.7
T. pini	0.90±0.07	0.99±0.06	2.58±0.06	11.33±2.5
H. apiaria	0.73±0.08	0.67±0.07	1.34±0.07	9.91±2.7
Terminalia arjuna wood exteact				
Control	0.70±0.02	0.63±0.06	1.14±0.03	5.75±2.7
L. sterioides2	1.18±0.06	0.58±0.03	1.48±0.07	9.66±2.5
C. vesicolor	--	--	--	--
T. pini	0.66±0.06	0.48±0.05	1.94±0.05	6.45±1.6
H. apiaria	--	--	--	--

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

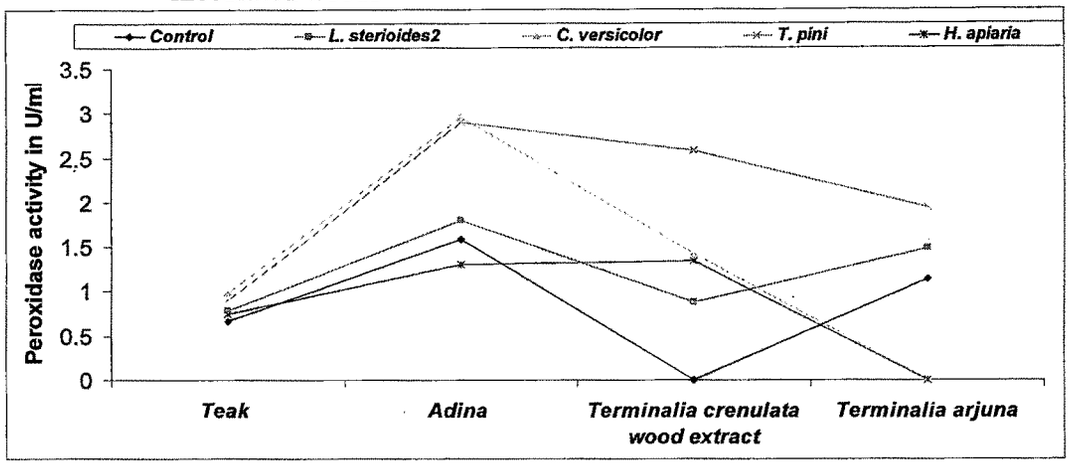
Histogram 13: Laccase activity of different wood rotting fungi in the artificially inoculated woods



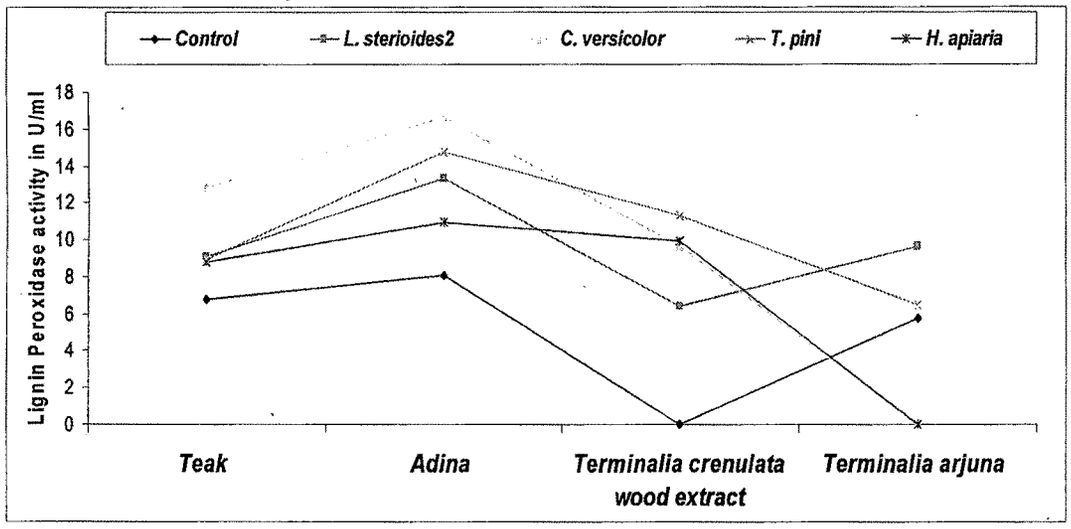
Histogram 14: Aryl alcohol oxidase activity of different wood rotting fungi in the artificially inoculated woods



Histogram 15: Peroxidase activity of different wood rotting fungi in the artificially inoculated woods



Histogram 16: Lignin peroxidase activity of different wood rotting fungi in the artificially inoculated woods



The production of lignin degrading enzymes (laccase and lignin peroxidase (LiP)) of two Basidiomycetous fungi, the white-rot producing *Phlebia radiata* and the litter-degrading *Agaricus bisporus* were examined on lignocellulose containing (pulp mill wastewater, straw, bran, compost leachate) media. In pulp bleaching wastewater and pulp mill wastewater-containing media *P. radiat* secreted LiP and laccase. Laccase was the main ligninolytic enzymes secreted by *A. bisporus* in compost. The laccase from compost was a blue laccase (Lankinen, 2004). In the present study the different wood rotting fungi produced ligninolytic enzymes i.e., laccase and lignin peroxidase, aryl alcohol oxidase and peroxidase. The role of laccases recently has been reevaluated because of 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 enzymes secreted by the *Pleurotus ostreatus* have shown that the concerted action of laccase and aryl-alcohol oxidase, produces significant reduction in the molecular mass of soluble lignosulphonates (Marzullo *et al.*, 1995). The preferential degradation of lignin by *P. ostreatus* strain V-184 has been demonstrated (Delgado *et al.*, 1992, Ginterova´ *et al.*, 1992, Klibansky *et al.*, 1993). In the present study the different wood rotting fungi were artificially inoculated in teak, *Adina Terminalia* woods which showed degradation of wood because of secretion of different ligninolytic enzymes.

The lignin degrading enzymes produced by the fungal strains and removal of colour indicates that LiP from *P. chrysosporium* ATCC 34541 may play an important role for bleaching activity as it is only one kind of lignin-degrading enzyme. In case of *C. versicolor* strain RC3, LiP was produced in higher levels in the absence of nutrients and this resulted in higher bleaching activity of rubber wood chips (Khanongnuch *et al.*, 2004). However, LiP was reported to be an unimportant enzyme in the biological bleaching and delignification of unbleached kraft pulp by *Trametes versicolor* (Archibald, 1992).

For initial screening, ligninolytic enzyme activity was checked in culture filtrates. Both *P. pinophilum* TERI DB1 and *A. gaisen* TERI DB6 were positive for all the three enzymes tested viz., laccase and LiP, while only laccase activity was detected in *P. florida* EM 1303. In SSF mode, enzyme production by fungi again varied according to the substrate used. Both water and a buffer were used to check if there is a difference in the yield of the enzyme extracted. Ingeneral, extraction with water led to better recovery of enzymes. For laccase, maximum activity for *P. pinophilum* TERI DB1 was detected when grown on corncob with molasses as moistening agent and extracted with water. Similar was the case with *A. gaisen* TERI DB6, when maximum activity was detected in same substrate. However, for *P. florida* EM 1303, maximum laccase activity was observed when extracted with buffer. In case of LiP, significantly high activity was found when *P. pinophilum* TERI DB1 was grown on wheat straw with effluent and extracted with water. Due to extraordinarily high activity detected in the above treatment, there was no significant difference in rest of the treatments. Similar was the case with *A. gaisen* TERI DB6 where again highest activity was observed when grown on wheat straw with effluent followed by water extraction (Pant and Adholeya, 2007). In the present study highest laccase, aryl alcohol oxidase, lignin peroxidase and peroxidase activity was observed in *Adina* wood.

6.2.5. Estimation of protein content in medium containing Teak sawdust

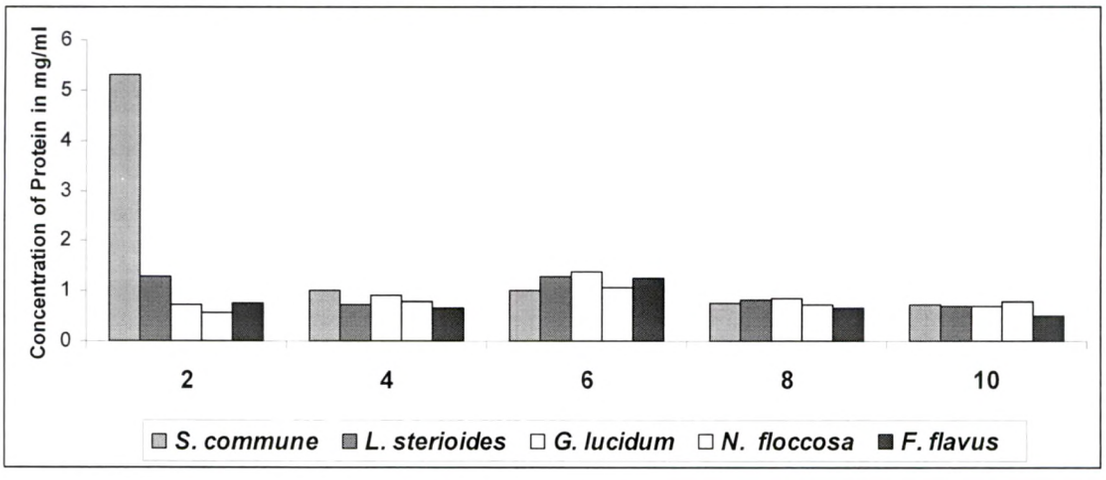
The concentration of protein was highest in case of *S. commune* inoculated flasks where as lowest in case of *F. flavus* inoculated flasks. For the production of highest concentration of protein *S. commune* took only 2 days, whereas all remaing fungi i.e. *L. sterioides G. lucidum, N. floccosa* and *F. flavus* took 6 days (Table 27 and Histogram 17).

Table 27: Estimation of protein content of different white rot fungi in teak wood sawdust medium

Days/ fungi	*S. commune** mg/ml	*L. sterioides* *mg/ml	*G. lucidum** mg/ml	*N. floccosa** mg/ml	*F. flavus** mg/ml
2	5.30±1.5	1.29±0.04	0.71±0.03	0.57±0.07	0.75±0.05
4	1.02±0.05	0.71±0.06	0.91±0.05	0.80±0.03	0.65±0.07
6	0.99±0.07	1.30±0.08	1.38±0.03	1.06±0.06	1.25±0.02
8	0.74±0.08	0.83±0.02	0.86±0.07	0.71±0.04	0.66±0.04
10	0.72±0.02	0.69±0.05	0.68±0.06	0.80±0.07	0.49±0.07

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

Histogram 17: The concentration of protein produced by different white rot fungi in basal medium containing teak sawdust



The progressive production of protein was observed by *Lentinus squarrosulus,* which of great economic value using untreated wood sawdust. With increase in hydraulic retention time, production of protein increased. The use of acid pretreated lignocelluloseic material enhances the production of protein from a maximum of 0.55 to 0.94 mg/ml solution mixture representing a lift of 71.0% (Shide *et al.,* 2004). In the present study the teak sawdust was used for the production of protein content by different wood rotting fungi i.e., *S. commune, L. sterioides, G. lucidum, N. floccosa* and *F. flavus.*

The *S. commne* showed maximum protein content in 2 days that is 5.30 mg/ml, while other three fungi maximum protein content on 6th day.

6.2.6. Estimation of protein content in artificially inoculated woods

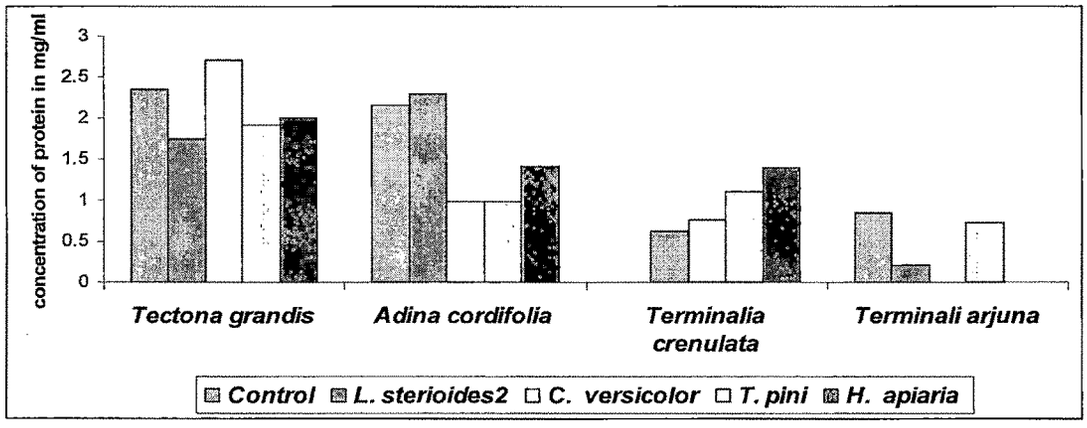
Table 28 showed that the concentration of protein was highest incase of teak wood infected with *C.versicolor* where as lowest incase of *T. arjuna* wood infected with *L. sterioides. L. sterioides* showed highest concentration of protein in infected *Adina* wood, whereas lowest incase of *T. arjuna* wood. The C. *versicolor* showed highest concentration of protein in infected teak wood, whereas the lowest incase of *T. crenulata* and no production of protein in *T. arjuna.* The *T. pini* showed hight concentration of protein in infected teak wood, whereas lowest in case of *T. arjuna* wood. The *H. apiaria* showed highest concentration of protein in infected teak wood whereas lowest in case of *T. crenulata* and no protein content in *T. arjuna* wood (Histogram 18).

Table 28: Estimation of protein content of different white rot fungi infected woods

Fungi/wood	*Tectona grandis**	*Adina cordifolia**	*Terminalia crenulata**	*Terminalia arjuna**
Control	2.34±0.05	2.15±0.08	--	0.85±0.08
L. sterioides2	1.75±0.08	2.29±0.09	0.62±0.07	0.21±0.04
C. versicolor	2.70±0.04	0.99±0.03	0.76±0.05	--
T. pini	1.91±0.07	0.98±0.04	1.10±0.02	0.73±0.03
H. apiaria	2.00±0.02	1.42±0.06	1.39±0.05	--

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

Histogram 18: The concentration of protein in different woods blocks infected with white rot fungi



In the present study the different kinds of woods were artificially inoculated with wood rotting fungi. Study showed good amount of protein production in teak wood, whereas poor amount of protein was observed in *T. arjuna* wood. The *L. sterioides*

produced maximum amount of protein conten in *Adina* wood, and the *C. versicolor* in Teak wood. The *T. pini and H apiaria* produced maximum amount of protein in teak wood. Simoniã *et al.* (2009) found that maximum protein content (0.06 mg/ml) was noted in the medium where oak sawdust was used as carbon source and nitrogen concentration was 20 mM. The total protein conten (15.mg/l) was produced by *C. versicolor* – CLU in 13 days. The protein content of *C. versicolor* – ATCC was 17.2 mg/l in 19 days. The protein content of *P. igniarius* –CLU was 2.8 mg/l in 9 days. The protein content produced by *Lycoperdon sp.*- CLU was 28 mg/l in 16 days. The protein content produced by *P. chrysosporium* was 37.3 mg/l in 17 days. The protein content produced by *P. betulinus* was 4.6 in 13 days. The protein content produced by *C. cellulolyticum* was 31.5 mg/l in 8 days. The protein content produced by *C. piluliferum* is 18.1 mg/l in 10 days (Szklarz *et al.*, 1989).

7) Studies on Decay of Valuable Timbers

7.1. Wood chips method

Percentage decay of seven different woods caused by *S. commune, L. sterioides. H. apiaria* (White rot fungi) and *T. viride* (soft rot fungi) was observed. As compared to the other white rot and soft rot fungi, teak and sissoo wood was efficiently degraded by *L. sterioides*, where the percentage weight loss was 34.6 and 44.6 % after 40days. Whereas in case of *T. arjuna, T. bellerica, A. cordifolia, A. arabica,* and *P. longifolia* the woods were efficiently degraded by *H. apiaria,* where the percentage weight loss was 62.0, 66.4, 60.4, 52.5 and 65.4% respectively in 40 days. When compared to the all woods decays after 40 days of incubation, *S. commune* showed 29.1% decay in *A. arabica,* whereas the *L. sterioides* showed 54.3 % decay in *T. arjuna.* The fungus *H. apiaria* showed 66.4 % decay in *T. bellerica* while *T. viride* showed 22.3 % decay incase of *A. arabica.* The minimum amount of decay was observed in teak wood *i.e* 3.9% in 20 days by *S. commune* (Table 29 and histogram 19).

According to ASTM method of classification of decay resistant classes the teak wood was very resistant in case of *T. viride,* and *Lenzites* sp. upto 3 weeks of incubation. Moderate resistance was observed in case of *H. apiaria and L. sterioides.* Bakshi *et al.* (1967) conducted wood decay test with *Polyporus hirsutus* Fr., *P. sanguineus* L. ex Fr., *P. versicolor* L. ex Fr., *P. palustris* B. & C. and *Irpex flavus* Klotzsch. The results showed that in case of teak wood outer heart wood varies in decay resistance from very resistance to moderate resistance (weight loss 1.98-25.63%) (Bakshi *et al.* 1967). In the

present study the teak wood was moderate resistant to not resistant when different wood rotting fungi were tested. After 5 months, the Basidiomycetes plus composite inocula caused significantly more weight loss of inoculated wood than members of Basidiomycetes alone. Marked stimulatory effects on mycelial growth were evident in treatments combining bacteria and yeasts with Basidiomycetous fungi. The amount of glucosamine, which reflects the amount of chitin present, was higher in treatment with combining composite inocula and members of Basidiomycetes than in those with Basidiomycetes alone. Wood chips lees than 1 year old had the most striking difference, with over 200% increase in glucosamine observed for *C. versicolor*. Although the weight loss for the composite inocula was only 1-4% greater than basidiomycetes alone, the large increase in mycelia represents additional decomposition of substrate. Controls showed significant weight loss and low level of glucosamine. Treatments with bacteria and yeasts alone did not result in significant weight loss or detectable glucosamine (Blanchette and Shaw, 1978). But in the present study the all white rot fungi showed significant weight loss in different samples.

After 5 months wood chips from slash less than 1 and 2 year old were substantially decayed (approx. 40%) by the brown rot fungus *P. placenta*. The decay caused by the white rot fungus, *C. versicolor* was approximately half than the caused by *P. placenta*. *Hirschioporus abientinus* caused approximately 20% weight loss in wood chips in less than 1 year old but 10% in 1 and 2 year old chips. In wood chips over 25 years old an even more striking difference was observed between brown and white rot fungi; *P. placenta* caused a 10 fold increase in decay compared to that caused by treatments with white rot fungus. In treatments combining *P. placenta* or *H. abientinus* with composite inocula. The total weight loss in chips from slash over 25 year was less for all treatments than for comparable treatments with less than 1 or 2 years old (Blanchette and Shaw, 1978). In the present study the increase in weight loss *was* caused by *H. apiaria* in only 40 days.

Fomes fomentarius caused significantly greater pellet weight reduction than those caused by the other fungi. Analysis according to Duncan's multiple range test showed degrees of association between the weight losses induced by the other fungi in this test. All test fungi, except *L. lepideus* produced weight losses which were significantly different from the control (Martin and Dale, 1980). In the present study teak and sissoo woods were efficiently degraded by *L. sterioides*, whereas, the percentage weight loss was 34.6 and 44.6 in 40 days., *T. arjuna, T. bellerica, A. cordifolia, A. arabica,* and *P.*

longifolia these woods were efficiently degraded by *H. apiaria* where the percentage weight loss was 62.0, 66.4, 60.4, 52.5 and 65.4% respectively in 40 days only.

Histogram 19: Showing the percentage weight loss in different woods shavings infected with four wood rotting fungi

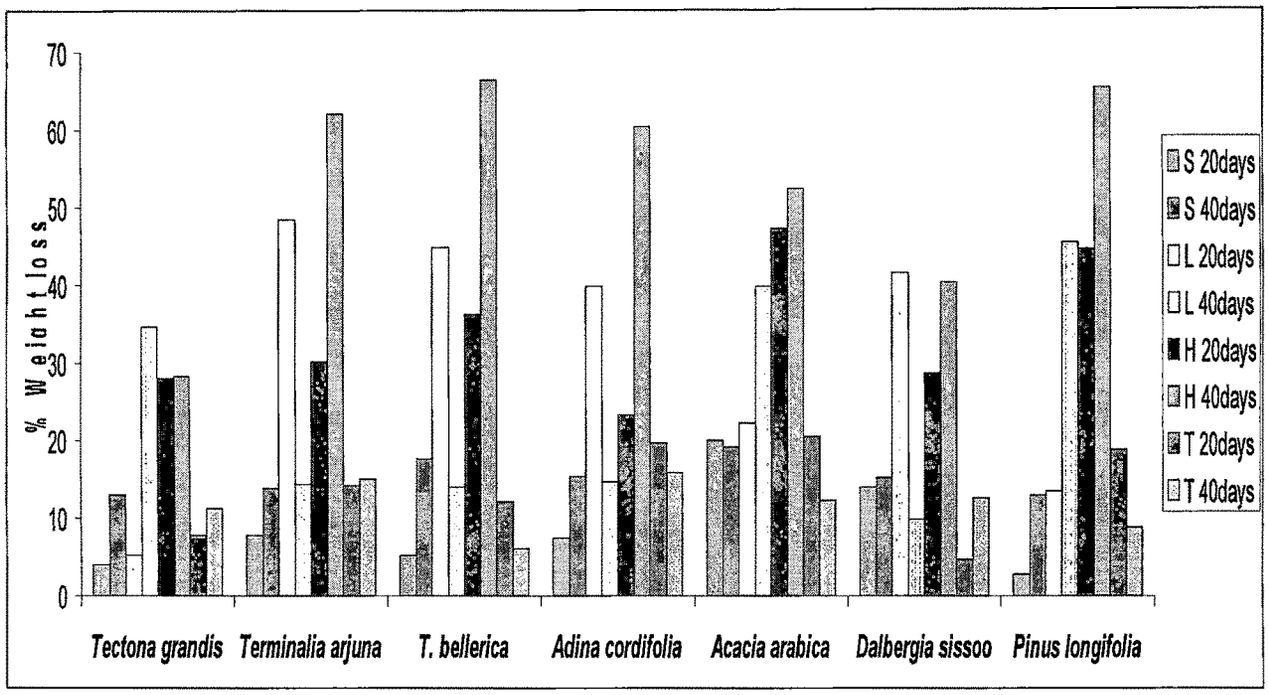


Table 29: Percentage weight loss of wood shavings of seven different trees by four different fungi

Percentage weight loss																
	Schizophyllum commune				*Lenzites sterioides*				*Hexagonia apiaria*				*Trichoderma viride*			
	*20 days	pH	*40 days	pH	*20 days	pH	*40 days	pH	*20 days	pH	*40 days	pH	*20 days	pH	*40 days	pH
Acacia arabica	20±1.0	5.22	29.1±1.5	4.9	22.2±1.2	3.5	39.8±5.6	4.0	47.3±2.4	4.09	52.5±1.3	4.39	20.6±2.5	8.33	22.3±2.5	9.0
Adina cordifolia	7.3±1.5	5.69	15.4±2.7	6.1	39.8±1.8	4.3	44.6±3.5	4.5	23.3±1.8	4.15	60.4±2.7	4.5	19.6±2.6	5.91	15.8±1.9	5.87
Dalbergia sissoo	14.0±2.5	5.93	15.2±2.6	6.0	39.9 ±2.5	4.9	44.6±4.8	5.3	28.6±2.6	4.32	40.3±2.6	4.4	4.7±3.8	7.62	12.6±1.4	8.08
Pinus longifolia	2.8±1.0	5.46	13.0±2.3	5.4	13.4±2.4	4.2	45.5±4.5	3.9	44.7±4.8	4.62	65.4±5.3	5.26	18.8±2.4	9.04	18.8±2.6	10.0
Tectona grandis	3.9±0.9	5.36	12.9±2.5	5.2	5.1±2.5	4.9	34.6±2.5	4.0	27.9±2.4	4.25	28.3±2.3	5.20	7.7±1.8	3.34	11.2±1.8	9.00
Terminalia arjuna	7.8±2.5	6.39	13.8±1.8	6.6	48.4±3.2	5.0	54.3±3.2	5.1	30.2±4.9	4.72	62.0±5.1	5.50	14.2±1.7	8.86	15.0±3.4	8.97
T. bellerica	5.2±1.6	5.97	17.6±1.5	5.8	44.9±3.8	4.4	54.0±4.3	4.3	36.2±1.8	4.35	66.4±5.6	4.26	12.1±2.1	7.04	16.0±2.5	8.24

∗indicates each component values are based on the three replicates. Uninoculated wooden blocks were incubated for 20 and 40 days to act as a control.
± Results were significant at $P < 0.05$ level by one way ANOVA

7.2. Wooden Block Method

Wood decay caused by four test organisms was observed in wood blocks of *T. grandis* and *T. arjuna* wood blocks after every 20, 40 and 60 days. The maximum decay was shown by *L. sterioides* in case of *T. arjuna* after 60 days. The minimum decay was observed in case of *T. arjuna* due to *S. commune* after 20 days. In initial stages of decay the percentage of moisture was more whereas in advanced stages of decay the % moisture reduced. As the percentage moisture was less the percentage weight loss was also less, this indicates that the decay capacity of the fungi depends on the % moisture content in wood (Table 30 and Histogram 20).

An experiment was conducted to study the percentage weight loss by wood block (1x1x1cm) method. In this method to keep the moisture for long time a double layer of filter paper was placed. The filter paper was moistened with sterile-distilled water at regular intervals during incubation period. Percentage wood decay of three different woods was observed by three white rot fungi *i.e. L. sterioides, C. versicolor* and *H. apiaria.* After 12 months 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* after 3 months of inoculation (Table 31 and Histogram 21).

Biological agar block method allowed wood samples to be evaluated and monitored in terms of colonization and development of the decay by Basidiomycetes fungi (*C. versicolor*) and classified based on mean mass loss. In this research, the *in vitro* decay of five commercial woods by *C. versicolor* was studied by the agar block method. The selected wood samples were *Abies alba. Populus alba, Fagus orientalis, Platanus orientalis* and *Ulmus glabra.* The results demonstrated the strong resistance of *U. glabra* and lowest resistance in *F. orientalis.* There were also a high correlation between the mass loss and apparent damage. Therefore biological evaluation of wood regarding biodegradation and the selection of wood types for various applications will be of high priority (Olfat *et al.,* 2007). In the present study the maximum decay was showed by *L. sterioides* in *T. arjuna* wood.

The Basidiomycetes, *Poria carbonica* and *C. versicolor,* caused substantial wood weight loss over the test period. These results are usually obtained by soil or vermiculite burial methods (Morrell and Zabel 1985). Weight losses were greater in *Ponderosa* pine

sapwood, a more decay-susceptible material than douglas fir heart wood (Lin *et al.* 1989). The decreasing weight in studied samples showed that *C. versicolor* can grow quickly and may rapidly affect the appearance and degraded the wood. The lowest weight loss decreasing was observed in *U. glabra* and highest value in *F. orientalis*. This was true for the study of crude oil and beech wood caused by *C. versicolor* (Olfat and Karimi 2005) and the study of wood decay which has been colonized by *C. versicolor* (Heilmann and Boddy 2005). In the present study also the growth of the *C. versicolor* was quick and degrade the wood very fatly when compared other wood rotting fungi tested.

Wood samples were collected from a ten-year old plantation of *Pinus caribaea* (morelet) in Ijaiye Forest Reserve, 38 km northwest of Ibadan, Nigeria. The wood samples were inoculated separately with two species of white-rot fungi; *Corioliopsis polyzona* and *Pleurotus squarrosulus*, and two species of brownrot fungi; *Lentinus lepideus* and *Gleophyllum, striatum*. Wood weight loss due to biodegradation varied from 1.5 – 48.1% for *Corioliopsis polyzona*, 9.6 – 58.0% for *Pleurotus squarrosulus*, 40.4 – 78.1% for *Lentinus lepideus* and 6.8 – 49.2% for *Gleophyllum striatum* degrading activities (Emerhi *et al.*, 2008). In the present study the weight loss due to degradation varied from one to 7% for *S. commuine*, 2 to 12% for *L. sterioides*, 3-9% for *H. apiaria* and 1-6.5% for *T. viride*

The percentage weight loss in inner heart wood and outer heart woods of New guinea teak was 44 , 54 and 12 , 21 for *Coniophora olivacea* and *C. versicolor* respectively. The percentage weight loss in inner heart wood and outer heart wood of Indonesian teak was 54, 55 and 22,21 for *C. olivacea* and *C. versicolor*. The percentage weight loss of inner heart wood and outer heart wood of Burma teak was 4, 8 and 4, 3 for *C. olivacea* and *C. versicolor* respectively. The percentage weight loss in inner heartwood and outer heart wood of *Pinus radiata* was not recorded incase of *C. olivacea* and *C. versicolor* (Guilley *et al.*, 2004).

Based on percentage weight loss, the American Society for Testing Materials (ASTM) classified the resistance of wood. Highly resistant wood showed weight loss of zero to 10%, resistant wood shows weight loss of 11 to 24%, moderately resistant wood showed 25 to 44% weight loss, and nonresistant wood showed 45% or greater weight loss. In the present study the teak wood is moderately resistant to non resistant when

infected with different wood rotting fungi. Twelve hundred samples from 31 trees were exposed to four fungi: *Pycnoporus sanguineus, Antrodia* sp., *Gloephylum trabeum,* and *Coriolus versicolor.* Tests showed that *Antrodia* sp. and *C. versicolor* resulted in <20% mass loss, whereas all samples were rated as durable or highly durable with regard to *P. sanguineus* and *G. trabeum.* Inner heartwood was found to be the most resistant to pathogen attack and outer heartwood the least (Kokutse *et al.,* 2006). In the present study the different woods infected with different wood rotting fungi showed variable degrees of resistant. So, the *in vitro* wood decay test cannot be taken as absolute evidence for the behavior of lignin-degrading fungi, they are useful to determine their wood-destroying properties.

Weight loss of yellow-poplar samples incubated with *T. versicolor* in a soil-block test was significantly higher than that in an agar-block test. Therefore, the soil-block test was more sensitive to detect fungal decay in yellow-poplar under the conditions of the experiments (Schirp and Wolcott 2005). In the present study also the most effective method is agar block method

Table 30: Percentage moisture loss and weight loss of two different wood blocks caused by three fungi

	Schizophyllum commune					
	20 days		**40 days**		**60 days**	
Wood	% Moisture Loss*	% wt. loss*	% moisture loss*	% wt. loss*	% moisture loss*	% wt. loss*
Tectona grandis	28.34±0.7	4.1±0.8	4.1±0.2	4.7±0.7	3.33±0.5	6.96±0.8
Terminalia arjuna	21.25±0.2	1.0±0.5	3.3±0.5	2.0±0.8	5.36±0.8	3.86±0.9
	Lenzites sterioides					
T. grandis	3.76±0.6	2.3±0.1	3.71±0.7	3.8±0.8	5.48±0.8	6.57±0.3
T. arjuna	5.84±0.8	7.2±0.4	3.16±0.2	11.4±0.5	6.53±0.5	9.25±0.5
	Hexagonia apiaria					
T. grandis	10.05±0.3	5.85±0.3	7.85±0.7	8.25±0.4	4.89±0.3	9.12±0.6
T. arjuna	16.08±0.8	3.5±0.7	10.24±0.5	4.5±0.2	8.98±0.2	8.05±0.3
	Trichoderma viride					
T. grandis	25.17±0.5	4.1±0.2	15.87±0.8	4.4±0.5	3.77±0.6	6.41±0.7
T. arjuna	47.08±0.8	1.3±0.3	3.18±0.2	2.2±0.3	2.81±0.7	2.5±0.5

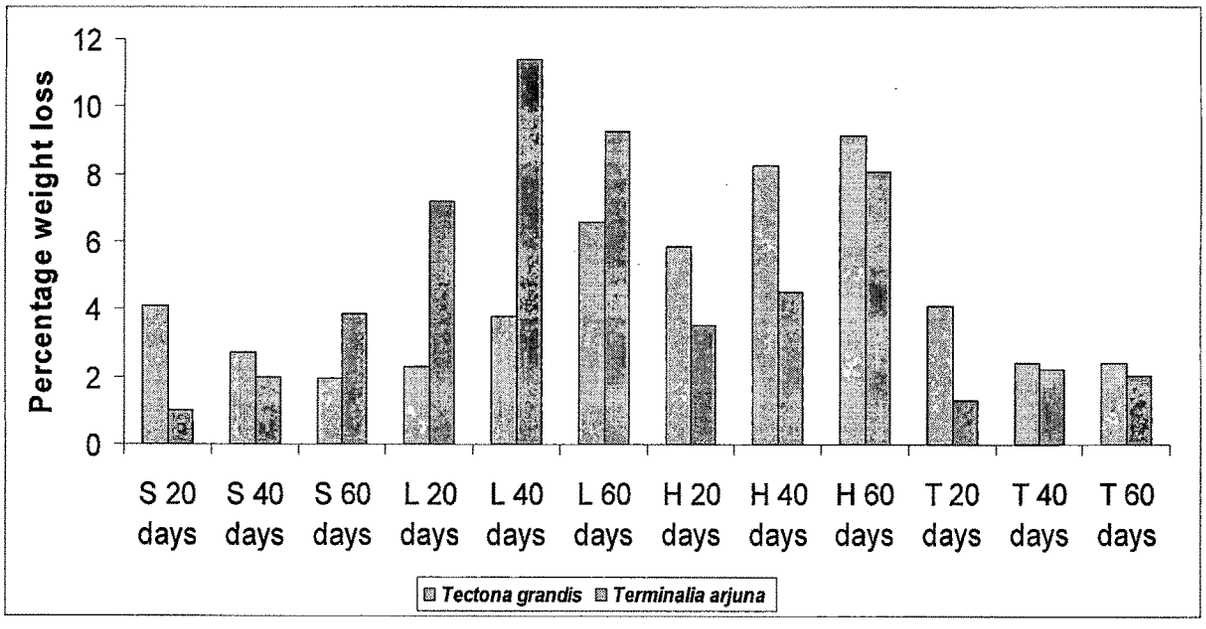
*indicates each component values are based on the three replicates. Uninoculated wooden blocks were incubated for 20 and 40 days to act as a control.
± Results were significant at $P < 0.05$ level by one way ANOVA

Table 31: Percentage weight loss of three different wood block caused by Three different fungi

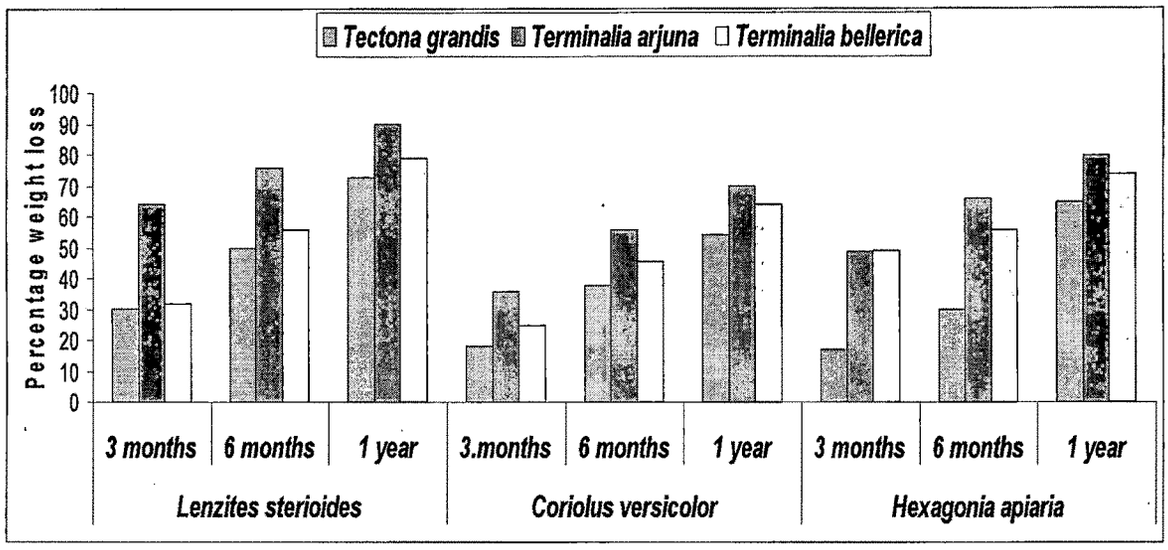
	% Weight loss								
Wood	*Lenzites sterioides*			*Coriolus versicolor*			*Hexagonia apiaria*		
	*3 months	*6 months	*1 year	*3 months	*6 months	*1 year	*3 months	*6 months	*1 year
T. grandis	30.07±1.0	50.00±2.8	72.68±2.8	18.23±2.5	37.84±1.5	54.15±1.4	16.82±1.5	29.80±2.6	64.88±2.8
T. arjuna	64.04±1.5	75.70±2.4	90.00±2.5	35.56±3.5	55.74±1.6	70.00±1.8	48.59±1.3	65.87±2.4	79.80±1.8
T. bellerica	31.64±2.5	55.57±4.3	78.71±3.5	24.53±2.4	45.67±1.4	63.78±1.5	48.83±2.8	55.82±2.6	73.82±2.4

*indicates each component values are based on the three replicates. Uninoculated wooden blocks were incubated for 20 and 40 days to act as a control.
± Results were significant at $P < 0.05$ level by one way ANOVA

Histogram 20: The percentage weight loss in different wood blocks infected with wood decay fungi



Histogram 21: The percentage weight loss in different wood blocks infected with white rot fungi



8) Biochemical analysis of certain artificially inoculated woods

8.1. Analysis of Infected wood blocks

The physicochemical analysis of teak, and pinus woods infected with wood decay fungi was done. The details are recorded in Table 32 and 33, highest percentage of moisture was showen by teak wood infected with *H. apiaria* whereas it was lowest in case of Pine wood infected with *S. commune*. Highly acidic nature was showen by teak wood infected with *H. apiaria* in 20 days. Almost neutral nature was shown by teak wood infected with *T. viride*. The percentage loss of ethanol - benzene soluble substarates was more in case of teak wood decayd by *H. apiaria* for 40 days, whereas, the lowest was observed in Pine wood decayed by *L. sterioides*1 and *T. viride*. The percentage loss of acid insoluble lignin was observed in case of teak wood decayed by *T. viride,* whereas, lowest in case of teak wood inoculated with *H. apiaria* for 40 days. The percentage loss of holocellulose was more in case of teak wood infected with *L. sterioides*2, whereas, lowest in case of pine wood decayed by *S. commune*.

Table 32: Physico- chemical analysis of sound wood of *Tectona grandis*, *Adina cordifolia* and *Terminalia bellerica*

				% of dry weight				
Plants	*Moisture %	pH	*Swelling capacity%	*Acid insoluble lignin	*Holocellulose	*Ethanol -benzene soluble substrate	*Hot water soluble substrate	*Ash (g)
Tectona grandis 1	6.1±0.26	5.8	45.1±0.7	29.3±0.9	143.2±1.8	26.7±1.8	7.8±1.0	0.06±0.01
T. grandis 2	6.5±0.25	4.4	40.0±2	42.0±0.24	112.5±2.5	37.4±1.6	9.5±1.4	0.10±0.03
T.grandis 3	5.1±0.24	5.2	--	43.2±1.5	--	17.2±2.7	5.6±2.5	--
Adina cordifolia	5.7±0.23	5.3	35.5±1.4	35.0±2.4	141.5±2.3	32.1±2.4	19.4±0.5	0.21±0.04
Terminalia bellerica	7.8±0.51	5.2	31.5±1.8	20.3±1.2	153.0±2.8	28.2±1.8	20.7±0.35	0.06±0.01
Pinus longifolia	5.8±0.4	5.1	--	42.4±1.8	--	11.0±2.5	4.4±0.2	--

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

Table 33: Chemical analysis of decayed woods of *Tectona grandis* and *Pinus longifolia* by different fungi.

Plant	fungi	days	* % Moisture	pH	*Hot water soluble substrate	*Ethanol-benzene soluble substrate	*Acid insoluble lignin	*Holo - cellulose
Pine	*Control		5.8±0.8	5.10	4.4±0.2	11.0±1.4	42.4±1.5	4.5±0.8
	Lenzites sterioides 2	20	5.9±0.4	5.57	4.3±0.4	8.2±1.8	11.6±1.2	7.5±1.5
	L. sterioides 1	20	5.7±0.2	5.16	3.8±0.8	7.1±1.6	25.6±1.8	10.0±1.3
	Schizophyllum commune	20	4.5±0.6	5.30	4.7±0.5	7.3±2.4	19.6±1.6	5.0±1.8
	Trichoderma viride	20	6.2±1.2	6.05	3.5±0.4	7.1±2.6	26.0±2.3	11.5±1.4
	H. apiaria	20	5.5±2.5	6.5	4.7±0.6	8.5±1.5	30±2.6	15±1.0
Teak	Control		5.1±0.2	5.26	5.6±0.9	17.2±2.2	43.2±2.8	12.5±1.6
	L. sterioides 2	20	5.9±0.7	6.01	4.9±0.3	13.8±1.8	13.0±1.5	19.5±1.4
	L. sterioides 1	20	5.5±0.6	5.05	4.5±1.5	12.2±1.4	26.8±1.4	10.5±1.8
	S. commune	20	5.9±0.1	5.70	5.9±1.8	17.4±2.3	19.2±1.8	19.0±2.0
	T. viride	20	6.2±1.5	6.90	4.6±0.4	14.0±2.5	31.0±1.6	14.0±2.5
	H.apiaria	20	5.3±1.8	4.89	5.5±0.7	15.6±1.8	18.8±1.9	7.0±1.0
	H. apiaria	40	9.6±2.5	5.27	16.0±2.3	18.4±1.5	8.4±2.5	15.5±2.3

∗indicates each component values are based on the three replicates. Uninoculated wooden blocks were incubated for 20 and 40 days to act as a control.
± Results were significant at $P < 0.05$ level by one way ANOVA.

Three white rot fungi *Daedalea elegans*, *Polyporus glaganetus*, and *Lenzites betulina* were screened for their lignin degrading abilities on rice straw, maiz cob, sawdust of *Terminalia superba* and sugarcane bagasse at different time intervals (30, 60 and 90 days). All the fungi demonstrated varying levels of ligninolytic capability with different degrees of lignin degradation in all the fermented substrates. The highest lignin reduction of 92.9% was recorded in maize cob fermented with *Daedalea elegans* after 90 days (Adejoye and Fasidi, 2009). But in the present study only 26% of reduction in lignin was observed in pinus and teak wood blocks

When a brown-rot-causing fungi *Polyporus palustris* was infected to *Mangifera indica* wood shavings for considerable periods, approximately 40 to 50% lignin loss was observed in two years (Ananthanarayanan *et al.*, 1970). The *Mangifera* wood blocks were infected with white-rot-causing fungi for 90 days, the utilization of lignin was 26% by *F. flavus* and 20% by *S. commune* (Appendices II). But in the present study the maximum lignin loss was recorded in teak than in pine by *L. sterioides*.

8.2. Artificially inoculated wooden blocks

The artificially inoculated wood log of *Tectona*, *Adina*, *Terminalia crenulata* and *T. arujna* were chemically analyzed. It is evindent from Table 34 that 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 acidic nature was shown by *T. crenulata* wood decayed by *C. versicolor*, whereas, basic nature was shown by teak wood infected with *L. sterioides*. 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* in 5months. The percentage loss of acid insoluble lignin was more in case of *T. crenulata* wood decayed by *L. sterioides* for 5 months. The percentage loss in holocellulose was more in case of *Adina* wood decayed by *C. versicolo* (Table 34 and Histogram 22).

Table 34: Chemical analysis of different decayed woods by white rot fungi for 5 months.

Plant	Fungi	Months	*% Moisture		Percentage loss			
				pH	*Hot water soluble substrate	*Ethanol - benzene soluble substrate	*Acid insoluble lignin	*Holo - cellulose
Tectona grandis	*Control	3 and 5	5.1±0.8	5.26	5.6±0.6	17.2±1.5	43.2±1.3	12.5±0.8
	L. sterioides	3	4.0±0.4	9.08	10.5±0.3	17.7±1.3	20.8±1.8	19.5±1.5
	C. versicolor	3	3.5±0.6	8.30	10.3±0.7	27.9±1.8	30.4±2.5	14.0±1.4
	T. pini	5	4.2±0.2	8.10	5.8±0.8	27.9±1.6	36.4±2.4	18.5±1.7
	H. apiaria	5	3.8±0.5	6.87	2.5±0.4	21.7±2.5	27.2±3.0	22.5±3.2
Adina cordifolia	*Control	3 and 5	5.7±0.1	5.30	19.4±0.8	32.1±2.3	35.0±2.5	41.5±3.6
	L. sterioides	3	3.6±0.3	5.98	8.6±0.2	19.9±2.8	42.4±2.4	24.0±2.5
	C. versicolor	5	5.0±0.4	4.60	15.9±0.5	38.1±2.5	33.6±1.6	48.5±2.7
	T. pini	5	4.7±0.6	4.67	18.4±0.6	25.7±1.8	33.8±1.4	25.5±3.4
	H. apiaria	3	4.9±0.7	5.60	9.3±0.2	20.7±1.6	29.2±1.8	26.5±1.8
Terminalia crenulata	*Control	5	6.5±0.7	5.20	25.7±0.4	30.0±2.5	40.5±2.5	35.0±1.6
	L. sterioides	5	5.5±0.2	5.86	22.2±0.7	23.7±1.9	74.0±2.8	20.0±2.5
	C. versicolor	5	4.3±0.4	4.50	9.2±0.3	24.4±2.5	55.0±2.9	10.5±2.5
	T. pini	5	4.8±0.8	4.56	16.5±0.8	20.4±2.6	24.8±2.5	28.5±1.8

*indicates each component values are based on the three replicates. Uninoculated wooden blocks were incubated for 20 and 40 days to act as a control.
± Results were significant at $P < 0.05$ level by one way ANOVA.

When *S. commune* was grown on liquid media containing 14C-lignin-labeled wood, the degradation of lignin was low and variable (Boyle *et al.*, 1992). *S. commune* has the ability to produce lignindegrading enzymes for degradation of lignocellulosics materials (Appendices II). In the present study as the incubation period is increase the loss of lignin also increase. After five months, the highest lignin lost was observed in *T. crenulata* infected with *L. sterioides*.

The chemical analysis of artificially inoculated wood blocks for 1 year was studied (Table 35). 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 (Histogram 23).

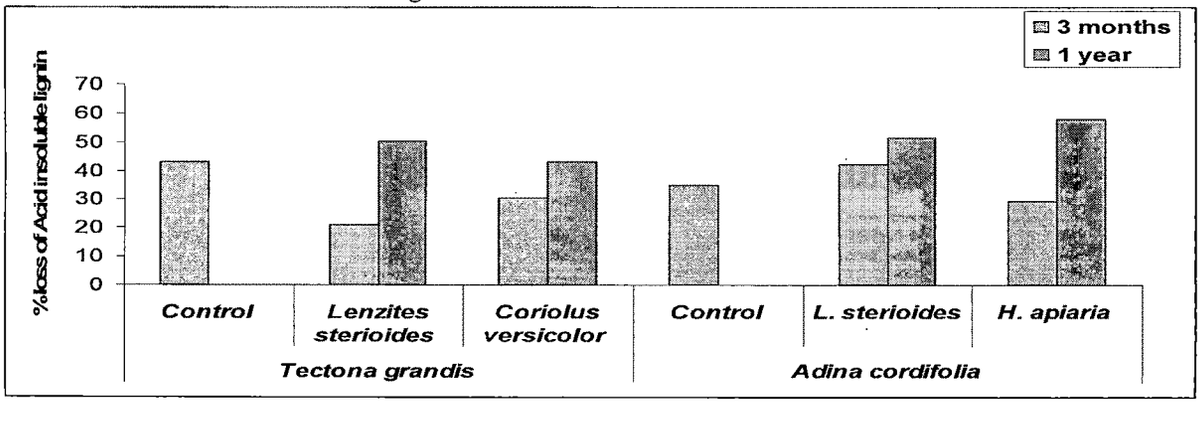
Table 35: Chemical analysis of different decayed woods by white rot fungi for one year.

Plant	fungi	*% Moisture	pH	Percentage loss			
				*Hot water soluble substrate	*Ethanol-benzene soluble substrate	*Acid insoluble lignin	*Holo - cellulose
Tectona grandis	Control	5.1±0.8	5.26	5.6±1.2	17.2±1.2	43.2±2.5	12.5±1.2
	L. sterioides	5.7±0.2	5.66	10±1.6	17.86±1.5	50.4±2.8	8.0±1.4
	C. versicolor	4.80.4	5.88	6.6±1.2	17.33±1.9	43.0±2.6	12.5±1.6
	T. pini	4.8±0.5	5.54	6.0±0.8	18.66±1.3	50.4±2.8	16.5±1.8
	H. apiaria	4.5±0.6	5.36	7.4±0.3	15.20±1.7	50.8±2.5	9.0±1.5
Adina cordifolia	Control	5.7±-0.2	5.30	19.4±1.8	32.1±1.4	35.0±2.1	41.5±1.8
	L. sterioides	6.1±0.1	4.93	12.8±1.4	18.53±1.6	51.4±2.6	15.5±1.6
	C. versicolor	35.0±1.5	4.89	39.6±2.4	28.53±1.8	31.2±2.7	46.2±2.5
	T. pini	14.8±1.4	4.99	16.4±1.6	13.80±1.4	56.6±2.4	15.0±1.4
	H. apiaria	19.5±1.8	4.95	23.0±1.5	7.20±1.6	57.8±2.8	14.0±1.9
Terminalia crenulata	Control	6.5±0.6	5.20	25.7±1.3	30.0±1.3	40.5±2.6	35.0±2.8
	L. sterioides	7.1±1.2	5.42	13.4±1.7	18.60±1.6	41.0±2.7	23.0±2.4
	C. versicolor	8.9±1.4	4.91	16.6±1.8	19.20±1.4	47.0±2.4	28.5±2.7
	T. pini	14.7±2.5	4.92	19.8±2.6	16.20±1.7	35.8±2.6	6.0±0.8
	H. apiaria	10.2±1.5	5.10	13.8±2.8	11.80±1.5	48.2±2.8	7.0±0.6
T. arjuna	Control	2.2±0.4	5.50	9.3±2.5	22.5±1.0	50.0±2.5	22.0±2.4
	L. sterioides	6.7±1.2	5.50	13.8±2.8	10.60±1.4	41.2±2.2	16.5±2.8
	T. pini	7.7±1.8	5.21	13.4±2.4	28.73±1.2	49.0±2.7	14.0±2.7

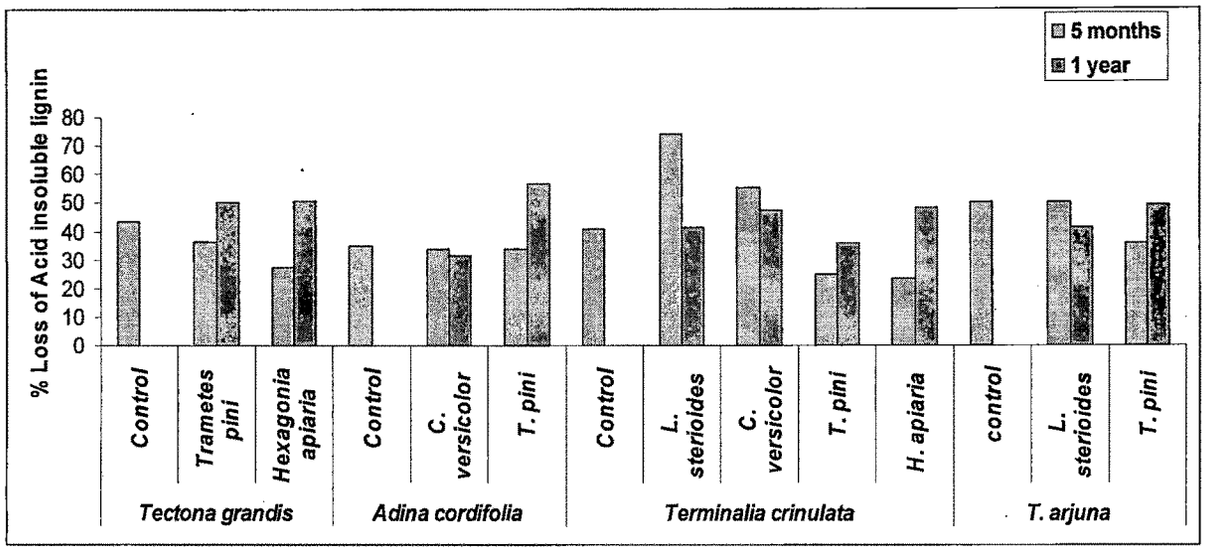
*indicates each component values are based on the three replicates. Uninoculated wooden blocks were incubated for 20 and 40 days to act as a control.

± Results were significant at $P < 0.05$ level by one way ANOVA.

Histogram 22: The percentage loss of Acid insoluble lignin of 2 woods infected with white rot fungi



Histogram 23: The percentage loss of Acid insoluble lignin in different wood blocks infected with different white rot fungi



White rot fungi *F. flavus* and *S. commune* selectively degraded the lignin of *Syzygium cumini* rather than the holocellulose component, whereas simultaneous degradation of lignin occurred in the case of *M. indica* (Apendices II). In the present study the *L. sterioides, C. versicolor,* and *H. apiaria* showed the selective degradation of the lignin in all the artificially inoculated woods, whereas, the *T. pini* showed simultaneous degradation of lignin in all the woods tested. After 90 days of pretreatment with *F. flavus*, loss in lignin content was 25.7% in *M. indica* wood. However, 8% loss of holocellulose was caused by *S. commune* in *S. cumini* wood. (Apendices II). In the present study after 1 year of pretreatment with *H. apiaria*, loss in lignin content was 58% in *Adina* wood. However 6% loss of holocellulose was caused by *T. pini* in *T. crenulata* wood.

Adaskaveg *et al.* (1990) observed selective delignification and simultaneous decay in oak wood infected with *Ganoderma* isolates. In decay of oak wood, for simultaneous decay, the ratio of Klason lignin (%KL) to Chlorite Holocellulose (%CHC) obtained was 1:1 by *G. meredithiae*; for moderate amount of delignification the ratio was 1.5:1 by *G. zonatum*; and for high amount of delignification 2.5 to 5:1 by *G. colossum* and *G. oregonense*. After 90 days of incubation, both the white-rot fungi degraded a

moderate amount of lignin in *M. indica* wooden blocks, while in *S. cumini* a moderate amount of delignification was shown by *F. flavus* and *S. commune* (Appendice II). In the present study the white rot fungi degraded highest amount of lignin in teak, *Adina,* and *Terminalia* woods.

9) Histological Studies

Teak wood has been classified as Class 1 according to EN 350-1, and is believed to have a service life up to 40 years. The anti-decay compound was tectoquinone (Haupt *et al.* 2003; Thulasidas and Bhat 2007). White rot fungi are capable of removing all of the cell wall components of wood, i.e., lignin, cellulose, and hemicellulose (Cowling, 1961). They have developed two distinct ways to attack wood: (i) the simultaneous degradation of lignin, hemicelluloses, and cellulose and (ii) the selective degradation of lignin and hemicelluloses (Blanchette *et al.,* 1985, Kirk and Moore, 1972). Extensive investigations by Blanchette, (1984b) and Otjen and Blanchette, (1982) clearly demonstrated that both types of decay can occur side by side in the same substrate. Variations in the physical, chemical, mechanical and anatomical properties of teak caused by the white rot fungus *Rigidoporus* sp. in Costa Rica has been studied by Elia and Julieta, (2004). Bhat *et al.*, (2005) studied wood durability of home-grown teak against brown-rot and white-rot fungi caused by *Polyporus palustris* and *Gloeophyllum trabeum.* Bakshi *et al.,* (1967) studied natural decay resistance of Indian timbers *Shorea robusta* Gaertn and *Tectona grandis* L. by Soil – block method to evaluate the natural decay resistance of timber under laboratory conditions.

The effect of timber 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 and is whitened in contrast to normal wood. In addition, it becomes soft and spongy in texture and retains in fibrous structure into advanced stages and will absorb and retain a considerable amount of water. This type of decay is called white rot. All the species of Hymenochaetaceae and most of the species of Polyporaceae members are associated with white rots (Sharma, 1995). In the present study also the teak wood infected by *L. sterioides C. versicolor, H. apiaria* and *T. pini* showed changes in colour and texture i.e. the infected wood becomes white than the normal wood, softened and spongy. A wood from which the cellulose components have

been removed, is darker than the normal wood, will be dry, brittle and of charcoal consistency. This type of decay is called brown rot (Sharma, 1995). Formation of boreholes by specialized cell wall degrading hyphae has been described in the literature (Liese 1970; Schwarze 2004). These were initiated by fine penetration hyphae, less than 0.5 mm diameter, which penetrated the cell wall by means of lignolytic enzymes (Schwarze 2004).). The young hyphae lysis the cell walls and produces furrows in it. The degradation products of various cell wall layers are completely absorbed by the hyphae. White rot fungi successively depolymerise cell wall substances only to the extent that the products can be utilized consecutively for metabolism (Cowling, 1961).

9.1. Anatomy of Healthy Teak wood

Light Microscopy of sound/normal wood specimens revealed that growth ring boundaries are distinct with simple perforation plates. Vessels are generally distributed evenly across the growth ring, often aggregative of two to several and with distinct bordered pits (Plate VII Fig A). The vessels were mostly 350 - 800 µm wide and there walls are 300 µm thick and consisting of three layers S1 S2 and S3. Tyloses were common. S1 and S3 layers were thin and S2 layers are thick. Vessels were surrounded by thick walled fiber tracheids where it shared a common middle lamella. Fiber cells consist of simple to minutely bordered pits and were thin- to thick-walled. Axial parenchyma was vasicentric and four (3- 4) cells per parenchyma strand. Fiber cells were 900-1600 µm wide. Multiseriate ray cells were attached by rigid walls with intercellular spaces and simple bordered pits.

9.1.1. Decay caused by *Lenzites sterioides*

The teak wood infected by fungi like *L. sterioides, C. versicolor, H. apiaria* and *T. pini* contains a relatively high remainder of cellulose and the infected wood is whitened in contrast to normal wood. In addition, it becomes soft and spongy in texture and retains in fibrous structure into advanced stages of decay. It also absorb and retain a considerable amount of water.

L. sterioides showed selective delignification processes in infected teak wood blocks. In transverse sections, delignification was easily observed in vessels and fiber cells. Many of them showed a concentric delignification starting from the lumen surface in vessels and fiber cells. Due to the advanced delignification complete degradation of

fiber cells, wall of the vessel and associated cells occurred (Plate VII Fig. B). The larger delignified secondary walls become increasingly soft, lose their typical form and became kidney shaped (Plate VII Fig. C). The fungal hyphae penetrate through bore holes and spread in the S2 layer of vessel in circular manner producing chlamydospore (Plate VII Fig. E). In fiber cells centrifugal delignification starts towards the lumen surface. Instead of middle lamellae, the secondary walls stained blue with fast green and cell corners stained pink with safranin (Plate VII Fig. D). The circular bore holes in the lignified cell wall of the vessel were distinctly observed. The cell wall gets cracked due to degradation of the lignified wall layers (Plate VII Fig. F). Wood decay is characterized by formation of bore holes. Individual hyphae advanced through bore holes from one cell to the neighbouring one and produces chlamydospores in chains. The thick walled chlamydospores remain in the lumen. The lignified fiber walls get cracked (Plate VII Fig. G). The xylem vessel element wall shows bore holes and canals through which hyphae penetrate forming conical cavities. Cell wall degradation along the surface of the hyphae leads to narrow conically pointed cavities in the wall. The wall of vessel element becomes thin walled and the lumen is field with hyphae growing in a circular manner. (Plate VII Fig. H). With an advance in delignification only outline of the multiseriate ray cells remained intact, remaining cells were disfigured and consequently the entire cells got degraded (Plate VII Fig I). Conical shaped holes in the ray cell walls were observed due to the decay (Plate VII Fig. J). The cell wall showed discolouration. The hyphae of *L. sterioides* was observed in vessel elements, fiber cells, ray cells, and axial parenchyma cells.

9.1.2. Decay caused by *Coriolus versicolor*

The decay caused by *C. verscicolor* mainly occurs in the multiseriate rays, vessel elements, fiber cells and medullary rays. The hyphae enter through pit apertures and they become circular to degrade secondary layers of the vessel element. Numerous round to oval bores holes occur in the fiber cells close to vessel elements. The fungi bore in through these holes and penetrate the lignified xylem vessel walls. The delignification of the fiber cells in concentric manner was observed (Plate VIII Fig. A). A complete delignification of lignified layer in the vessel element indicates that the *C. versicolor* is selective degrader of lignin (Plate VIII Fig.D). The hyphae enter into the intracellular

spaces of ray cells to degrade cell wall layers by secreting enzymes (Plate VIII Fig. B). In some portion of the sample, separation among cells started due to the complete degradation of the middle lamella (Plate VIII Fig. C). The round bore holes created by the fungus were observed in the axial parenchyma cells. The fungi degrade entire fiber cell with secondary walls stained green with safranin fast green whereas the middle lamellae with remnants of lignin stained red (Plate VIII Fig. E). The hyphae penetrate through bore holes in the fiber cell to initiate degradation process so that cell wall layers selectively degrade. As a result of this cells separate leaving only some portion of the cell wall layers. In advanced stages of degradation the cells loosen and lost their rigidity and strength (Plate VIII Fig. F).

9.1.3. Decay caused by *Hexagonia apiaria*

H. apiaria was able to degrade vessels, fiber cells, xylem parenchyma cells and ray cells completely in teak wood. The degradation of the lignin occurred in both directions *i.e.* centripetal and centrifugal in vessels (Plate VIII Fig. G-I). In vessels selective delignification occurs in centripetal direction due to the release of the ligninolytic enzymes. The hyphae enters into the lumen of vessel elements through pits, accumulates into entire lumen and start degrading lignin. The nearby fiber cells were completely degraded. In fiber cells, first middle lamella degraded, followed by degradation of lignin in centrifugal direction leading to the complete degradation of cells and leaving only outline of cell wall. Some of these are under degradation process (PlateVIII Fig. H). In some vessel elements middle lamella and cellulose fibrils were degraded leaving lignin layers (S3 – S4). The accumulated hyphae creates bore holes in cell wall of vessel element in which delignification and complete degradation of the vessel wall was observed (Plate VIII Fig. J). In xylem parenchyma, bore holes were created by tips of hyphae which are active centre for growth and degradation of cell walls nearby releasing ligninocellulolytic enzymes. In fiber cells, hyphae penetrate through pits, accumulate in lumps to form clamydospores and degrade cell wall layers partially (Plate VIII Fig. I). In advanced stages of decay bore holes merged, all cell wall layers degraded leaving skeleton of fiber cells (Plate VIII Fig. K). In another section of fiber cells they were partially degraded (Plate VIII Fig. L).

9.1.4. Decay caused by *Trametes pini*

T. pini was able to degrade vessels, fiber cells, and ray cells completely and parenchyma cells partially. Selective delignification occurred in vessels and fiber cells. Simultaneous decay occured in ray cells and some fibers cells (Plate IX Fig. A-F). In vessels, lignin was degraded first in centripetal direction and then cell wall layers were completely degraded. Adjacent fiber and parenchyma cells were also affected. They undergo degradation process, and the infected cells got separated first due to decay of the middle lamella and other layers (Plate IX Fig. A,B). The linear longitudinal cavities were also created by the hypahe in fiber cells. Fungal hyphae was observed penetrating from one cell to the other through the pits and traveling parallel to the wall. The circular boreholes were created by the active centers of hyphal tips in parenchyma (Plate IX Fig. D). In advancement stage of infection fiber cells were completely degraded creating the diamond shaped cavity. The partially degraded fiber cells were stained with fast green and appear green in colour. The hyphae were traveling intercellularly in fiber cells (Plate IX Fig. E). Axial parenchyma cells were also partially degraded in advancement of decay and they were completely degraded. Ray cells at some other sites were degraded towards the lumen of cells, leading to the separation of S2 layer after degradation of middle lamella. In advanced stage all the cell wall layer were degraded leaving only outline of cells (Plate IX Fig. F).

White rot fungi degrade cellulose and hemicellulose at approximately the same rates relative to the original amounts present (Kirk and Highley, 1973) where as the lignin is decomposed at a similar rate or usually somewhat at faster rat on a relative basis (Blanchette, 1980; Setiff and Eudy, 1980). In wood products and slash, there is a strong tendency for soft woods to be degraded primarily by brown rot and hardwoods by white rot fungi (Scheffer, 1964). This is probably related to the fact that the lignin in hardwoods is easier to biodegrade than that in coniferous wood (Yang *et al.*, 1979) and not due to the difference in the hemicellulose components (Highly, 1976). In the present study lignin in infected teak woods is easily degraded by white rot fungi. In the present study the hyphae of the timber degrading fungi were mostly seen in vessels and ray cells and the hyphae invade other cells from ray cell and vessels via pits. Wilcox, (1970) and Liese, (1970) also found that the 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 cells walls.

In the present study four timber decaying fungi were found to produce cellulases and ligninase systems, which were secreted at hyphal tips. The ligninase depolymerize the lignin layer and forms bore holes in it. The bore holes were observed in cell walls of xylem vessels and fiber cells which are degraded by *L. sterioides*, in xylem vessel and axial parenchyma cell of teak wood degraded by *C. versicolor*, in xylem vessels, xylem parenchyma and fiber cells of teak wood degraded by *H. apiaria* and in xylem parenchyma cells of teak wood degraded by *T. pini*. Similar results were obtained by Schwarze, (2004). The action of the enzyme system of white rot fungi is restricted to the cell wall layers in the immediate vicinity of the hyphae in contrast to the brown rot fungal enzymes which apparently have a deep diffusion into the inner layers of cell walls (Sharma, 1995). In the present study also the cell wall layers in teak wood are infected by four timber decay fungi and degraded them severely.

In selective delignification, lignin is degraded earlier in the decay process than cellulose or hemicellulose. The hyphae grow in the cell lumen in some cases, so that the lignin is dissolved out of the adjacent cell wall. In other cases, hyphae penetrate the cell walls and initially delignify the middle lamella so that the cells tend to separate. Cellulose was left relatively unaltered during selective delignification, at least in the early stages of decay (Schwarze, 1995; Schwarze, 2004). The different patterns of cell wall degradation during selective delignification can be observed under the light microscope. Firstly a degradation of the middle lamella occurs in conjugation with extensive lignin degradation in the secondary wall. At the later stage, individual cells become separated from their matrix (Blanchette, 1984a). According to Hartig, (1878) and Peek *et al.*, (1972), lamellar collapse of the secondary wall (S2) into submicroscopic layers is also possible. Moreover, extensive delignification may occur in the S2 layer leading to the accentuation of radial structures (Schwarze and Engels, 1998).

In simultaneous white rot the hyphae grow in the lumen on the S3 layer and the cell wall is broken down in the immediate vicinity of the hyphae, which leads to the formation of erosion furrows. The hyphae sink into the cell wall like a river in its bed. Simultaneous removal of all cell wall components at about equal proportions (Highly and

Murmanis, 1987; Messner and Stachelberger, 1984). This decay is characterized by the fact that the enzymes released by the fungal hyphae degrade all the main constituents of the lignified cell wall (Liese 1970; Rayner and Boddy 1988; Eriksoon *et al.,* 1990). This localized cell wall degradation is caused by a slime coating around the hyphae through which the enzyme gain closer contact with the wall substance. As a consequence of many erosion furrows merging together, the thickness of the lignified cell walls gradually decrease from the inside (lumen) to the outwards (Middle lamella; Schwarze, 1995, Otjen and Blanchette, 1986b). Fungi such as *C. versicolor* typically produce simultaneous delignification in Brich wood (Blanchette, 1987). In present study teak wood showed selective removal of lignin in the vessels and fibers.

A simultaneous rot was the predominant type of decay present in the wood, highlighting the fact that not only a selective delignification but also the simultaneous rot may be caused by a single fungus, but that the two processes often occur side by side (Blanchette, 1980). Samples inoculated with *P. sanguineus* showed a selective delignification, whereas those inoculated with *G. lucidum* exhibited a combination of simultaneous decay and selective delignification (Luna *et al.,* 2004). In the present study the selective delignification was observed in case of *Lenzites* and *Coriolus* whereas, combination of selective and simultaneous delignification was observed in case of *Hexagonia* and *Trametes* infected teak woods.

Natural decay resistance of home grown teak depends on the planting site, fungal species, radial position from the centre to the periphery of the heartwood and their interaction (Bhat *et al.,* 2005). In Gujarat teak plantation in dry deciduous forest was affected by different white rot causing fungi. In present studies *L. sterioides* was found associated with teak wood. It degraded all cell wall components and causes white rot.
Natural decay resistance of juvenile teak wood grown in high input plantations were studied by Bhat and Florence, (2003). The weight loss of wood specimens due to attack of both the *Trametes hirsuta* and *T. versicolor* (*C. versicolor*) fungi was around 21 percent at the age of 5 years. But in the present study, teak wood inoculated with *C. versicolor* had shown delignification and degradation of ray cells, vessel elements and fiber cells. The advanced degradation was observed in case of fiber cells and ray cells. The fungal hyphae were seen in all cells particularly in ray cells and in lumen of vessel in

circular manner. For the determining the resistance of wood to decay anatomical studies will have to be conducted.

9.2. Anatomy of Healthy *Adina* wood

Light microscopic study of sound wood of *Adina* revealed that the growth ring boundaries were distinct. Wood is diffuse-porous with vessel outline angular. The vessels contain simple perforation plates. Within the vessels, inter-vessel pits were alternate, polygonal, 4 - 7 µm thick. Vessels were 40 - 100 per sq mm, 350 - 800 µm in size and 800 µm in thickness (Plate IX Fig. G). In both radial and tangential walls, pits were common and with distinctly bordered pits, Fiber cells are non-septate, thin- to thick-walled, 900-1600 µm in size, 1600 µm in thickness. Axial parenchyma diffuse-in-aggregates, Eight (5-8) cells per parenchyma strand. Multiseriate rays are 1 to 3 cells width and cells were procumbent with over 4 rows of upright and / or square marginal cells. Ray parenchyma cell wall is disjunctive.

9.2.1. Decay of *Adina* wood caused by *L. sterioides*

The *L. sterioides* decay is characterized by loosening of the ray cells and fiber cells from the neighbouring cells due to preferential degradation of middle lamella. Subsequently lignin degradation from fiber cell walls take place. Cell walls of the fibers have become noticeably thin (Plate IX Fig. J, K). Depletion of lignin occurs from outer layers towards lumen (i.e. S1 layer). This is very clearly depicted by the dark stained middle lamella which indicates its intactness. Delignification of secondary wall gradually brings about complete discoloration of vessel walls (Plate IX Fig. H). The hyphae after penetration produce numerous chlamydospores within the ray parenchyma cells (Plate IX Fig. L). Lignin degradation in the vessel element leads to the cracking of the lignified wall partially and remains hanging into the lumen of vessel element (Plate IX Fig. I).

9.2.2. Decay *Adina* wood caused by *C. versicolor*

C. versicolor is able to degrade all cells of *Adina* wood. Mostly vessels, ray cells and fiber cells were degraded up to some extent and the xylem parenchyma cells also damaged (Plate X Fig. A). Selective delignification of xylem vessels and fiber cells occurred, leaving behind the cellulose fibrils in S2 layer. The middle lamella was degraded first and after that lignin in the cell wall was degraded in centripetal manner. In advanced stage of decay all the components of the cell wall were degraded in fiber cells

leaving only the outline of the cell wall (Plate X Fig. B). In advanced stages of decay the fiber cells were severely degraded only outer cellulose layer was remaining. The ray cells also degraded with some bore holes in the cell wall (Plate X Fig. C). The hyphae entered into fiber cells and ray cells through intercellular spaces. All the ray cells were completely degraded leaving only outline of the cells (Plate X Fig. D).

9.2.3. Decay caused by *H. apiaria*

Decay caused by another fungus *H. apiaria* is characterized by presence of dark phenolic deposits especially in the ray cells and ray parenchyma cells. In these parenchyma cells the hyphae cause brown to dark brown discolouration. The middle lamella has been degraded which is very evidently noticed in the ray cells bringing about loosening and separation of cells. The hyphae are difficult to be recognized because of the dark polyphenolic deposits in the cells (Plate X Fig. E, F). Vessel elements become disfigured due to the completely degradation of fiber cells associated with it. The vessel element appears buckled (Plate X Fig. G). The hyphae branch and penetrate through bore holes and enter into the neighbouring fibers. The middle lamella between the fiber cells were degraded so they show separation from each other which is a clear indication of selective delignification (Plate X Fig. H). Cluster of chlamydospores were formed within the lumen of xylem vessel (Plate X Fig. I).

9.2.4. Decay of *Adina* wood caused by *T. pini*

T. pini was able to degrade the vessels, fiber cells and ray cells in *Adina* wood. Vessels were severely degraded and fiber cells and ray cells and xylem parenchyma cells were degraded moderately. In cell wall layers the middle lamella was completely degraded so that cells separated easily (Plate XI Fig. A-C). In advanced stage of decay the lignin layers i.e. S2 to S4 were degraded in vessels. The hyphae entered through pits into lumen of vessels, where selective delignification process occurred due to release of ligninolytic enzymes. Middle lamella also degraded by the release of hemicellulolytic enzymes, thus sepearting fiber cells. In advanced stages cell wall components of fiber cells were degraded completely and some fiber cells were seen under the processes of degradation (Plate XI Fig. B, C). In the processes of decay, hyphae entered through pits where it released the lignocellulolytic enzymes to degrade fiber cell wall components, and increased there size, and produced more number of hyphae to enhance the process of

decay (Plate XI Fig. D). Fiber cells and xylem parenchyma walls were degraded. The bore holes were seen present in fiber cell walls. Chlamydospores were also produced by hyphae in fiber and ray cells (Plate XI Fig. E). Ray cells were also degraded, hyphae entered into fiber cell and parenchyma cell through pits from vessel lumen (Plate XI Fig. F). The simultaneous degradation occurred in fiber cells, ray cells and xylem parenchyma cells.

Wilcox, (1964a,b) used vital stains and light microscopy to study the progressive stages of colonization by white and brown rot fungi. These studies led him to predict that light microscopy could be used to detect decay at weight losses 5%. Wood which has experienced little or no loss in mass may have lost as much as 60% of its bending strength (Wilcox, 1978). Degradation of lignin does not occur in the absence of wood carbohydrates, and lignin is probably of limited importance as a carbon source (Kirk and Fenn, 1982).

In the present study the *Adina* wood contains more amount of starch, which is used by the timber degrading fungi in colonization. Successful colonization of wood depends largely on the ability of fungi to spread rapidly by using nonstructural carbohydrates; the hyphae of both white- and brown-rot fungi was widespread in wood before any significant weight losses were detectable (Hulme and Shields, 1970). In the present study the timber degradation of *Adina* by *L. sterioides, C. versicolor, H. apiaria* and *T. pini* showed presence of hyphae in vessels, ray cells, fiber cells, axial and ray parenchyma cells. Hyphae penetrate from cell to cell through natural openings in the wood (pits) or by boring holes in the cell wall (Wilcox, 1970). In the present study the hyphae of the *H. apiaria* penetrated into neighboring fiber cells through bore holes. Early decay by brown- and white-rot fungi was characterized by the presence of at least one hyphal strand in each cell, suggesting that extracellular enzymes may not be able to diffuse outside the wood cells in which they were secreted (Wilcox, 1970). In the present study the early decay of *Adina* was characterized by the presence of hyphal strand in vessels, fiber cells and ray cells for timber decay fungi.

The wood decayed by *C. versicolor* has been studied extensively to its biochemistry of degradation and microscopy at the light microscopic level (Cowling, 1961; Greaves and Levy, 1965; Wilcox, 1968,1970). Light microscopic studies shown that it degrades wood

in a manner typical of white rotters, starting the attack from the S3 layer and progressing through the middle lamella. Similar type of decay was noticed in case of *Adina* wood decayed by *L. sterioides, C. versicolor, H. apiaria* and *T. pini* in almost of all the cells. Wood decay fungi such as *C. versicolor* typically produce simultaneous type of white rot. The decay is usually localized near hyphae or around the circumference of the cell lumen. All cell wall layers are progressively degraded from the lumen towards the middle lamella (Cowling, 1961; Liese, 1970; Otjen and Blanchette, 1986b). In the present study the *C. versicolor* infected *Adina* wood showed selective delignification in xylem vessels and fiber cells, leaving behind the cellulose fibrils in S2 layer. The middle lamella was degraded first and then the lignin of the cell wall was degraded in centripetal manner. Selective delignification in ray and fiber cells was observed in case of *L. sterioides* and *H. apiaria* infected wood. Selective decay of lignin in cell wall layers of vessels was observed in case of *T. pini* infected woods. The simultaneous decay was also observed in case of *T. pini* infected woods where the ray, fiber and axial parenchyma cells were degraded.

It has been found that Formation of chlamydospores is crucial for survival of the fungus under dry or moist conditions for a range of wood decay fungi. Both *Oligoporus placenta* (*Poria placenta*) and *Antrodia carbonica* produce chlamydospores, which facilitate their survival during prolonged exposure to elevated temperatures and other adverse conditions (Powell, 2002). The brown rot fungus *Lactiporus sulphureus*, formed chlamydospores in heartwood of robinia (*Robinia pseudoacacia*). During early stages of colonization, most hyphae grew within the lumen of the libriform wood fibres. Individual hyphae grew transversely via minute bore holes to adjacent cells and subsequently produced single chlamydospores in the cell lumen (Schwarze, 2004). These thick-walled resting spores allow survival of the fungus in time rather than space. In the present study also the chlamydospores were formed because of high moisture in the *Adina* wood. The hyphae after penetration produce numerous chlamydospores within the ray parenchyma cells was observed in case of *Lenzites* infected wood. Cluster of chlamydospores were observed in the lumen of xylem vessels infected with *Hexagonia.* The formation of chlamydospores was observed in fiber and ray cells infected with *Tramates* but it is absent in caseof *Coriolus* infection.

9.3. Anatomy of Normal *Terminalia crenulata* wood

Growth ring boundaries are distinct in *Terminalia*. Wood is porous with simple perforation plates. Five vessels per sq mm with 350µm width were present. Tyloses were common in vessels (Plate XII Fig. A). Fibers with simple to minutely bordered pits were present. Fiber pits were common in both radial and tangential walls. Fibers may be thin- to thick-walled and septate. Axial parenchyma diffused with 4 cells per strand. Ray cells were uniseriate with one row of procumbent cells. Prismatic crystals were present in procumbent ray cells.

9.3.1. Decay caused by *L. sterioides*

L. sterioides has the ability to degrade vessels, fiber cells, xylem parenchyma and ray cells completely and axial parenchyma cells partially. The hyphae penetrate through both intercellular spaces and pits into the cells. It accumulates in the lumen and coils like bundles or lumps (Plate XI Fig. G-L). Selective delignification was mostly observed in vessels and partially in fiber cells. Simultaneous decay was observed in fibers and ray cells. During delignification processes at hyphal tips and around the hyphal cell lignolytic enzymes were secreted. They act on lignin and degrades them into smaller, low molecular weight compounds. Cell wall of vessel element and xylem parenchyma cells undergo decay, due to which they lose their strength and finally break. Mostly hyphae spread through xylem parenchyma cells (Plate XI Fig. G, H). In fiber cells hyphae spreads intracellularly where it colonizes heavily there by degrading all cell wall layers as a result cells lose mechanical strength and break (Plate XI Fig. I). In cell wall of vessels, conical shaped bore holes were created as a result of enzymes produced by hyphal tips. All bore holes merge together to create a big cavity and thus whole cell wall was degraded (Plate XI Fig. J). In advanced stages of infection, fiber cells were degraded completely creating big cavities. In ray cells, bundles of hyphae degrade all cell wall layers (Plate IX Fig. K). In ray cells and axial parenchyma cells, simultaneous decay occurred in which lignocellulolytic enzymes were mostly active so all the cell wall components were degraded leaving only outline of the cells. Lumps of hyphal remnants were observed in the cells (Plate XI Fig. L).

9.3.2. Decay of *T. crenulata* wood caused by *C. versicolor*

C. versicolor degrades vessels, fiber cells, xylem parenchyma and ray cells completely (Plate XII Fig. B-F). Selective delignification processes was observed only in

vessels, whereas in fiber cells and ray cells simultaneous decay was observed. In this processes removal of lignin takes place in centripetal direction and in advancement of decay cell wall layers get completely degraded and lose their strength and break. Infected xylem parenchyma cells also undergo simultaneous decay in all cells and were completely degraded (Plate XII Fig. B). In fiber cells hyphae enters through pits where it degrades middle lamella, cellulose fibril layer (S2) and finally lignin (S3 –S4), only leaving out line of the cells (Plate XII Fig. C). Complete degradation of fiber cells occurred to create large gaps between the cells, while some cells were intact together (Plate XII Fig. D). In longitudinal section of the fiber cells they were under the processes of degradation, where cells were degraded partially to create longitudinal cavities (Plate XII Fig. E). In ray cells hyphae entered through intercellular spaces and pits where it degraded wall layers partially to enlarge the pit hole as round shape (Plate XII Fig. F).

9.3.3. Decay of *T. crenulata* wood caused by *H. apiaria*

H. apiaria degrades vessels, fiber cells, ray cells and axial parenchyma cells completely in *T. crenulata* wood. Delignification was observed in vessels where at an advanced stage infected part gets completely degraded and spreads infection to surrounding cells. Xylem parenchyma and fiber cells simultaneously degrade in which all cell wall components degrade equally and completely (Plate XII Fig. G, H). In parenchyma cells the circular bore holes were created by hyphae. In advanced stages all the bore holes were merged and created conical shaped cavity. Lignin was degrades towards the lumen side (Plate XII Fig. I). In fiber cells, hyphae entered in both ways, where it degrades lignin selectively (Plate XII Fig. J). Degradation appeared to occur in a concentric manner creating space around the cells. In ray cells hyphae enters through pits and degrade all walls by secreting lignocellulolytic enzyme at their wall surface. Fiber cells also undergo degradation, where all cell wall layers degraded completely (PlateXII Fig. K, L).

9.3.4. Decay of *T. crenulata* wood caused by *T. pini*

T. pini completely degraded vessels, fiber cells and rays cells completely. The hyphae enters in both ways to accumulate in lumen and cause degradation of lignin towards the cell wall. In advanced stages all cell wall layers were degraded completely to create a gap. The fiber cells also got infected and degraded completely after accumulation

of hyphae (Plate XIII Fig. A, B). In fiber cells hyphae entered through pits and accumulated to degrade the middle lamella and lignin towards the lumen (Plate XIII Fig. C). In ray cells the wall layers were infected and degraded badly to lose their function. In advance stages all the ray cells were completely lost. In fiber cells hyphae spreads intracellularly, where it degrade lignin and cellulose leaving behind middle lamella. At advanced stages all wall components were degraded completely (Plate XIII Fig. D, E,). In advanced stages all cell wall layers degraded completely, creating severe gaps (Plate XIII Fig. E, F).

The occurrence of *Ganoderma* on different perennial crops as host was recorded by Sankaran, (2005). *Terminalia alata* Roth is native tree to India. *G. lucidum* was found on timber, *T. bellerica* (Gaertner) Roxb. was native tree to India having damage due to *G. applanatum*, *T. crenulata* Roth. was native tree to India. *G. lucidum* was found on timber. To explore the range of variation that each of these encompass, as shown partly by our own use of improved techniques for the preparation and examination of decayed wood by light microscopy (Schwarze, 2004). In the present study the light microscopy was used for decay identification. The application of these techniques has provided a detailed picture of the decay patterns that occur amongst a wide range of host/fungus combinations (Schwarze, 2007).

A sample of *Terminalia* wood recovered from an ancient Polynesian canoe thought to be approximately 1000 years old, was examined by light microscopy to determine the extent and pattern of degradation. Bacteria were the main cause of decay (Donaldson and Singh, 1990). In the present study the timber degrading fungi was main cause of *Terminalia* wood decay. The secondary walls of fibers, vessels and parenchyma cells were extensively degraded but the compound middle lamella remained relatively intact (Donaldson and Singh, 1990). In the present study *L. sterioides* degraded vessels, fiber cells, xylem parenchyma and ray cells completely and axial parenchyma cells partially in *T. crenulata*, *C. versicolor* severely infected the vessels, fiber cells, xylem parenchyma and ray cells and degraded them completely, *H. apiaria* also degraded vessels, fiber cells, ray cells and axial parenchyma cells completely in *T. crenulata* and *T. pini* mostly degraded vessels, fiber cells and rays cells. Vestures in intervascular pits were preserved, presumably by vinue of their high lignin concentration. Plasmodesmata were

also preserved by infiltration with extractives thought to be tannins (Donaldson and Singh, 1990). The ability to resist bacterial degradation of a similar layer in *Terminalia* suggested presence of high lignin (Singh *et al.,* 2002) and tannin content (Donaldson & Singh, 1990). In the present study the wood of *T. crenulata* infected with all timber degrading fungi showed changes in colour and texture i.e. it became white, soft and spongy. The ability of white rot fungi infected was seen in *Terminalia.* Lignin present in the wood was degraded which was not degraded even by bacteria.

In the present study the selective delignification was mostly observed in vessels and partially in fiber cells and simultaneous decay was observed in fibers and ray cells of *Lenzites* infected *T. crenulata* wood, Selective delignification processes was observed only in vessels whereas in fiber cells and ray cells simultaneous decay was observed in *Coriolus* infected wood, In *Hexagonia* infected wood, the xylem parenchyma and fiber cells simultaneously degrade in which all cell wall components degrade equally and completely and In *Trametes* infected wood the hyphae entered through pits in fiber cells, it accumulates their to degrade the middle lamella and lignin towards the lumen so all neighbouring fiber cell get separated. In the present study hyphae enters through pits in fiber cells and ray cells where it get degraded, enlarged and created rounded pit hole.
According to the Anagnost, (1998) the selective delignification has only single character i.e. Cell separation is common; erosion channels are sometimes present and simultaneous white rot shows erosion channels on the lumen surface. Erosion channels rounded with U-notches; hardwoods or softwoods, Erosion appears as elongate channels beneath lumen hyphae; bore hole and pit erosion are common; cell separation may be evident; cavities in the S2 are rare; if present, cavities not in chains, penetrating bore hole is wider than hyphae. In slide cultures of birch attacked by *Trametes versicolor* showed the rounded pit erosion persisted from early to advanced decay stages (Anagnost, 1998).

9.4. Anatomy of Normal *T. arjuna* Wood

Growth ring boundaries were distinct in arjuna tree. Wood was diffuse to porous with simple perforation plates. Intervessel pits were alternate and vessel-ray pits have distinct borders; similar to intervessel pits in size and shape throughout the ray cell. Vessels were 100 - 200 µm in width, 5 - 20 vessels, per sq mm. Tyloses wascommon in vessels (Plate XIII Fig. G). Fiber cells contained simple to minutely bordered pits

which were common in both radial and tangential walls. They were septate and very thick-walled. Axial parenchyma is aliform with seemingly marginal bands. Two cells were present per parenchyma strand. Uniseriate ray with width of 1 to 3 cells were present. All ray cells were procumbent. Body ray cells were procumbent with one row of upright cells having prismatic crystals.

9.4.1. Decay caused by *L. sterioides*

L. sterioides degraded vessels, fiber cells and xylem parenchyma completely, whereas ray and axial parenchyma cells were degraded partially. Selective delignification was observed in fiber cells and vessels (Plate XIII Fig. H,). In advanced stages of decay all fiber cells and wall layers of vessel were degraded completely. Removal of lignin takes place in centripetal manner and cell wall breaking was observed in vessel element. With advancement in decay, fiber cells completely got degraded creating big cavities. The xylem parenchyma cells were also infected with accumulating hyphae in masses. In severe infections only some cells were completely degraded leaving S2 layer (Plate XIII Fig. I). Ray cells also contain massese of hyphae which degraded lignin in centrifugal manner. In fiber cells complete degradation was observed as a result of which longitudinal cavities were formed with in fibers of cellulose layer (Plate XIII Fig. J, K). In ray cells, conical cavities were created due to the activity of hyphal tips which were not seen in advanced stages (Plate XIII Fig. L).

9.4.2. Decay caused by *T. pini*

T. pini mostly affects vessels, fiber cells and rays cells and degrades them completely. Mostly hyphae spreaded intracellularly in parenchyma cells (Plate XIV Fig. A-D). Hyphae entered through pits in vessels, where it accumulated to degrade, lignin selectively leaving the cellulose layer intact. The vessel wall breaks due to loss of S2- S4 layers. Xylem parenchyma cells were filled up with masses of hyphae to secrete lignocellulolytic enzymes for complete degradation of wall layers (Plate XIV Fig. A, B). In fiber cells selective delignification was observed where middle lamella was degraded and all the cells got separated. The degradation processes take places in centripetal manner *i.e.* towards lumen of fiber cells. In advancement of decay all the wall layers were degraded completely to create cavities at these sites (Plate XIV Fig. C). In parenchyma cells selective delignification occurred, where hemicellulose layers did not degrade while in some cells delignification was seen towards lumen (Plate XIV Fig. D). In uniseriate

ray cells hypha enters through pits and accumulate to degrade lignin in centrifugal direction. Fiber cells also degraded completely in advanced stage of infection (Plate XIV Fig. E). Axial parenchyma cells also degraded in same manner as xylem parenchyma and in advancement of decay all wall layers were degraded completely forming cavities (Plate XIV Fig. F).

Wood of *Terminalia arjuna* (Arjun) is durable and field test of decay resistant showed 3-4 class of decay (Tewari, 1978). In the present study the *Arjuna* wood was infected by two timber degrading fungi like *L. sterioides* and *T. pini.* The arjuna wood was severely degraded in which *L. sterioides* degrades vessels, fiber cells and xylem parenchyma completely, whereas ray and axial parenchyma cells were degraded partially. Selective delignification was observed in fiber cells and vessels of *Lenzites* infected *Arjuna* wood, whereas in *Trametes* infected *Arjuna* wood the selective delignification was observed in veseels, fibers and xylem parenchyma cells. Accumulation of starch was a common feature in all the species studied (Rajput and Rao, 1999). In the present study also the starch granules were observed in normal wood, whereas in infected woods of *Arjuna* the starch granules were not observed they might have been utilized by timber degrading fungi.

9.5. Histo-Chemical Studies using Light Microscopy

9.5.1. Changes in lignin content of teak wood by *L. sterioides*

When the vessels and fiber cells were stained with Phloroglucinol-HCl it gave intense red to pink colour, whereas, the *L. sterioides* infected wood showed least colouration in the degraded areas of fiber and vessels. In ray cells also the delignified portions were not stained with Phloroglucinol –HCl. The xylem vessels when stained gave intense pink colour in the lignified layers of S1-S3, whereas the infected cells which were delignified did not stained pink. The xylem parenchyma cells also stained dark pink The cell wall layers remained unstained, which indicated that *L. sterioides* selectively degraded the lignin in severely infected tissues. The fiber cells also showed light coloured areas, where the delignification processes was at the initial stages.

9.5.2. Change in lignin content of teak wood by *C. versicolor*

Teak wood sections were stained with Phloroglucinol – HCl which gave intense dark pink colour. Only small amount of lignin was intact to the cell walls of fiber cells

which were stained light pink. In xylem parenchyma cells the selected regions were light in colour but most of the wall layers were pink in colour. In the delignification processes xylem parenchyma was in the direction of the lumen side. Some of the fiber cells which were severely degraded and look like kidney shaped, these were stained light pink. The ray cells gave intense pink colouration with some regions of light pink which indicates that the delignification process is in the initial stage. The bore holes in xylem parenchyma cells were visible. The cell walls of the vessels when stained with Phloroglucinol gave black colour which indicated that the lignin is completely degraded by *C. versicolor* and the vessel wall layers were severely infected.

Histologically, the Wiesner reagent (Phloroglucinol-HCl) is used as a general to detect p-hydroxycinnamyl aldehyde end groups in macromolecular lignin of plant tissues. Although frequently cited as specific for coniferyl aldehyde (e.g., Sarkanen and Ludwig, 1971), the reagent reacts with all three p-hydroxycinnamyl aldehydes (Pomar *et al.,* 2002; Jourdes *et al.,* 2007). Some of the very early investigations for evidence of lignin degradation in wood (Zeller *et al.,* 1919) employed the qualitative test described by Czapzik, (1899) i.e. the extraction of a substance from rotted wood designated "hadromal" which gave an intense red color with Phloroglucinol -HCl.

A colorimetric detection of aldehydes, based on phloroglucinol-HCI staining, showed a stronger coloration in stem sections of down-regulated CAD plants, suggesting increased aldehyde content in the lignin. This positive result of phloroglucinol test shows the coupling reaction of some aldehyde group of the sediment with phloroglucinol (Trojanowski and Leonowicz, 1962).The Wiesner test (Phloroglucinol-HCI staining), which is generally considered to be indicative of aldehyde end groups (not only the C6-C3 cinnamyl aldehydes but also the C6-C1 benzaldehydes; (Garcia and Latge, 1987 and Monties, 1989), revealed that the typical pink color of the cell wall of the control. In the present study also the teak wood degraded by *Lenzites* and *Coriolus* showed positive reaction. Different cells in teakwood are heavily infected by white rot fungi and degradation products were giveing positive pink colouration which indicates the presence of aldehyde groups in the lignin.

Selective delignification was characterized by the unstained or "bleached" (terminology of Ross and Corden, 1973) appearance of cell walls when treated with the

Phloroglucinol reagent. At first, selectively delignified walls displayed no observable structural degradation but were later thinned centrifugally (nonsclerenchymatous elements) or by cavity enlargement (sclerenchymatous elements). The middle lamellae, however, were often degraded, resulting in separation of fibers (Gao and Chamuris, 1993). In the present study also the selectively delignification is observed in vessels ray cells and fiber cells of *Lenzites* infected teak wood whereas The infected fiber cells only small amount of lignin was intact to the cell walls of fiber cells which were stained light pink. The cell walls of the vessels when stained with Phloroglucinol gave black colour which indicated that the lignin is completely degraded by *C. versicolor*. Nonselective wall degradation was characterized by gradual wall erosion in the nonsclerenchymatous phloem elements and cavity enlargement in sclerenchymatous elements; in both cases walls retained their reactivity in phloroglucinol. In the present study also initiation of selective delignification was observed in xylem parenchyma and fiber cells of *Lenzites* infected wood whereas in *Coriolus* infected wood the ray cells and xylem parenchyma is partially delignified. Both *Dendrothele acerina* and *Mycena meliigena* displayed two patterns of delignification. This dual strategy for delignification has been reported for a number of wood-inhabiting basidiomycetes (Blanchette, 1984, Gao and Chamuris, 1993). In the present study also timber degrading fungi showed dual strategy for delignification i.e. both selective and simultaneous delignification in infected teak wood.

The observation that selective delignification was more pronounced in blocks in contact with agar, and therefore subject to diffusion of solutes from the agar, may be related to repression of the cellulase system by carbohydrates in the malt extract (Ander and Eriksson, 1977). Once delignified, the cellulose present in the cell walls became detectable, agreeing with similar results reported by Peek *et al.,* (1972). Since lignin appears to be masking cellulose, only selectively delignified areas tested positive for cellulose. In areas undergoing nonselective wall degradation, cell walls were progressively thinned and retained their affinity for phloroglucinol (Gao and Chamuris, 1993). In the present study the selectively delignified cells shown positive reaction to the cellulose test in advanced condition the remaining celluloses was degraded. The areas undergoing initiation of selective delignification and the cell walls retained affinity for Phloroglucinol giving light pink colour.

Wood decayed by *Ganoderma tsugae* typically showed a mottled rot consisting of two distinct types of decay large uniformly white areas of delignified wood containing

black spindle shaped zones and yellow or tan areas that were white rotted with numerous small holes filled with fungal mycelium. These two types of decay were easily differentiated when phloroglucinol-HCl was applied to the surface of the wood block. A bright carmine red coloration indicated the presence of lignin. Black zones within white areas lost color intensity and gradually disappeared. Sections through several annual rings of decayed wood demonstrated that early wood tracheids were preferentially delignified. Positive staining with phloroglucinol – HCl suggested substantial amounts of lignin remained in latewood cells; however, the adjacent early wood tracheids were free of lignin (Blanchette, 1984a).

In the present study the late wood was degraded by timber degrading fungi like *L. sterioides* and *C. versicolor* when stained with Phloroglucinol –HCl, some cells gave positive reaction and some cells gave negative reaction which indicated that some cells were in initial stage were delignified and some were not in selectively delignified.

Sections cut from nondelignified areas and examined by scanning electron microscopy showed tracheid cell walls with intact middle lamellae and exposed lumens. In contrast, tracheids from delignified wood readily separated and could not be cut to exposed the cell lumen. Ray parenchyma cells were completely destroyed. Late wood that stained a deep carmine color with phloroglucinol had a decay that resembled a typical white rot; lignin and carbohydrates were removed simultaneously from isolated areas. Holes were present in the tracheids cell walls, and large voids resulted from the gradual coalition of degraded areas; these were filled with fungal mycelium. Ray parenchyma cells also contained hole similar to the white rot degradation of the tracheids (Blanchette 1984a). In the present study also the degradation processes was typical of white rot in which both selective and simultaneous decay was observed in same wood so selectively delignified areas showed negative reaction and simultaneous degraded areas showed positive reaction to the Phloroglucinol test for in both the timber degrading fungi associated with teak wood.

9.6. Localization of cellulose in Teak wood

When the normal wood was stained with Potassium-iodide-iodine, sulphuric acid method it gave intense yellow colour, whereas the infected wood also gave a little bit of light yellow colouration which indicated that degradation of cellulose was minimum. As both the white rot fungi tested preferentially degraded the lignin. Only little bit of change were observed in case of cellulose degradation.

10) Ultra-structural studies of Teak wood samples deteriorated with wood rotting fungi

To better understand how the wood components are degraded by fungi on the molecular level it is important to investigate the morphology of fungal attack on wood. Such studies have been undertaken both on the micromorphological level and on the ultrastructural level using scanning electron microscopy (SEM) (Bravery, 1971). The effect of microorganisms on wood and the patterns of wood cell wall lysis have been observed (Findlay and Levy, 1969; Jutte and Sachs, 1976; Jutte and Zabel, 1974) with wood blocks inoculated with one organism and consequently did not demonstrate the association that exist between microorganism during the wood decay process. Little information is available at the ultra structure level (Cowling 1961, Wilcox, 1970). Degradation by *C. versicolor* differed in sweetgum and hemlock. Sweetgum was attacked by hyphae from both the lumen and cell corners. Hemlock was attacked only from the lumen (Highley and Murmanis, 1987). In the present study the teak was attacked by hyphae from the lumen. The middle lamella of hemlock was not attacked until removal of the secondary wall completely and the cell corners were particularly resistant to degradation. In sweetgum the middle lamella and cell corners were severely degraded without appreciable degradation of adjacent cell wall material (Highley and Murmanis, 1987). In the present study the middle lamella was first degraded and after that all the wall layer were degraded in vessels and fiber cells of teak wood.

SEM studies indicated that ***L. sterioides*** has both selective delignification and simultaneous decay with in the same section. Selective delignification in vessel walls with decay of the middle lamella was distinctly observed. In advanced stages the whole lignin layer was degraded and remaining cellulose fibrils here separated from cell wall. S1 and S2 layers of the vessel elements degraded partially. Hyphae spreaded intracellularly in the lumen of vessels and blocked the conductivity of the vessels (Plate XV Fig. A). Simultaneous decay of fiber cells was observed. In axial parenchyma fungal hyphae could be distinctly observed running parallel to the longitudinal axis of parenchyma cells. It was seen emerging out from the pit and becoming branched and in advanced stages of infection, complete degradation of all wall layers was observed (Plate XV Fig. B, C). In fiber cells also middle lamella was degraded and cells were separating, in advancement of decay, complete degradation of infected cells was observed. Some

partially degraded cells form thin fibrils in between due to selective delignification. At advanced stages of decay remnants of cells were observed.

Coriolus versicolor inoculated wood logs showed simultaneous degradation of ray cells, along with selective delignification of fiber cells with in the same section. In vessels hyphae enter through bordered pits and occupy the complete lumen. It decayed lignin in centrifugal direction leaving S2 layer intact. In advanced stages, all wall layers were degraded (Plate XVI Fig. A). Decay of middle lamella was also observed in vessels. In initial stages the middle lamella was degraded first, followed by lignin. Stiffness of the wall reduced and the walls were found to be loosely hanged into the lumen. The ray parenchyma cells were also simultaneously degraded (Plate XVI Fig. B). In tangential section, fiber cells were separated due to the decay of middle lamella, whereas ray cells also degraded simultaneously and with the advancement of decay whole ray cell were degraded to create a gap. These regions were identified only by spindle shaped spaces (Plate XVI Fig. C, D). In fiber cells along with selective delignification the circular bore holes were created by active centers of hyphal tips (Plate XVI Fig. E). Magnified portion of vessels showed blackening of vessels with degradation of the wall layers completely. The lumens were filled with remnants of degraded wall particles. Regions with advanced decay showed complete absence of any cells and the identity of the cells had been lost completely (Plate XVI Fig. F).

Trametes pini was able to degrade vessels and fibers selectively and ray cells simultaneously. Transverse section of degraded teak wood shows the entry of hyphae through pits where it forms the tyloses to block conduction. Selective delignification occurs in centrifugal manner where the middle lamella gets degraded and cellulose fibril separated. The surrounding cells of vessels also degraded simultaneously (Plate XV Fig. D). In initial stage of infection within fiber cells a gummy substance was secreted whereas in advanced stage of infection middle lamella degraded and cells got separated. The ray parenchyma cells also degrade selectively in which middle lamella degrades. In the advancement of decay all wall layers (S2-S4) were degraded and increased the cavities (Plate XV Fig. E). Tangential section of decayed teak wood shows vessels filled with mycelium, which blocks them to nearly 100-µm length. Selective and simultaneous decay of cells were observed with in the same section in different cells. In fiber cells the

middle lamella was degraded as a result, cells got separated and lignin was removed selectively. In advanced stage of decay whole fiber cell were degraded. In ray cells hyphae enters through pits where it releases lignocellulolytic enzymes to degrade all the cell wall layers simultaneously as a result cavities are formed (Plate XV Fig. F).

Ganoderma sp was the main white rot fungus associated with the decay of *Eucryphia cordifolia* and *Nothofagus dombeyi*. Ultrastructural studies showed that the delignification process was diffuse throughout the cell wall. Lignin was first removed from the secondary wall nearest the lumen and then throughout the secondary wall toward the middle lamella. The middle lamella and cell corners were the last areas to be degraded (Agosin *et al.*, 1990). In the present study the same results were observed in teak wood affected with *L. sterioides*.

In general, white rot basidiomyctes degrade lignin either selectively or in parallel with cellulose and other polysaccharides (Krik and Farrell, 1987). As to *Lentinus edodes*, Vane *et al.*, (2003) studied decay of oak wood by Micrmorphologically and found that the mode of wood cell wall degradation by *L. edodes* differs with tree species and was strongly influenced by the differences in portion, morphology and growth pattern of the hyphae as well as in the physicochemical properties of the wood cell wall (Tsuneda *et al.*, 1987, 1989, 1991). In the present study also based on the type of cells the degradation is also different. No preferential removal of matrix substances (lignin and hemicelluloses) was evident (Vane *et al.*, 2003). In the present study the formation of bore holes and cell wall thinning from lumen towards middle lamella occurs incase of vessels infected with *L. sterioides*. The wood rotting fungi showed both type of degradations i.e., selective and simultaneous. In rays, the middle lamellae were first removed and then bore holes developed in the seconday wall (Vane *et al.*, 2003). In the present study the rays cells infected with *T. pini* showed simultaneously decay and with *C. versicolor* showed simultaneous decay as well as selective decay. Unlike in vessels and rays, matrix material of secondary wall fibers appeared to be preferentially removed and the walls became porous and swollen before heavily degraded by hyphae (Vane *et al.*, 2003). In the study, the removal of secondary wall fibers removed preferentially, without the swelling of cell walls by *L. sterioides* and *C. versicolor*.

Many white-rot fungi that selectively remove lignin from wood do not uniformly degrade the substrate. This type of decay is commonly referred to as a white-mottled rot (Otjen and Blanchette 1986b). In the present study the teak wood infected with *L. sterioides* showed preferential lignin degradation in some areas. All white-rot fungi that cause preferential lignin degradation probably can also produce a simultaneous rot. *P. tremellosus* may also become less specific for lignin removal and cause a degradation of all cell wall components under certain growth conditions. Some cellulase activity was evident in advanced stages of decay, resulting in the depletion of the secondary wall (Otjen and Blanchette 1986b). In the present study the *T. pini* showed less specific for lignin removal in vessels. In advanced conditions all wall layers of the vessels were degraded.

White-rot fungi have the capacity to produce two distinct types of degradation (Otjen and Blanchette 1986b), decay by these fungi in vitro may have to be evaluated by using different substrates and environmental conditions to obtain a more complete understanding of the degradative process. In the present study also both type of decays were observed for wood rotting fungi i.e., *L. sterioides, C. versicolor* and *T. pini.*

The ultrastructural features of the hyphal sheath and associated extracellular hyphal structures of the brown-rot fungus *P. placenta*, and the white-rot fungi *T. versicolor* and *P. chrysosporium* were studied by SEM. In general, the hyphal sheath of both brown-and white-rot fungi appeared extensive and similar (Green *et al.,* 1990). In the present study the wood rotting fungi were not capable to produce hyphal sheaths. Immune-scanning electron microscopic localization of extracellular wood-degrading enzymes within the fibrillar sheath of the brown-rot fungus *Postia placenta* (Green III *et al.,* 1992). In the present study also different wood degrading enzymes were produced by different wood rotting fungi under the study. In the present study the fungal hyphae colonized in the cell lumina, the degraded cell have inter cellular spaces. These wood rotting fungi have the capacity to enter through pits. *P. chlamydospora, P. aleophilum* penetrated the fungal hyphae in cell walls of host xylem cells degrading the hemicellulose, cellulose and lignin components (Valtaud *et al.,* 2009). In the present study also degradation of hemicellulose, cellulose and lignin compound were observed with different wood rotting fungi under study.

11) Bio-control of certain Timber Degrading fungi

Among the most valuble of all our natural resources are our forests not only because of their monetary value due to the lumber business but because of the many thousands of useful products obtained from trees or made from the wood. Different group of fungi cause diseases of living trees as well as deterioration of timber and timber products. Fungi may get entry through roots or stem/bark. Fungi may cause stains or damge to the cells or tissues making them soft or unfit for further use. To control such damages scientists have suggested use of chemicals, fumigants, use of γ irradiations and application of UV rays and heat treatments etc. Gray (1959) reported use of creosote to prevent rail, roads and telephone poles and sodium fluoride and zinc chloride (Wolman salts) to treat the wood used in mines. Terpentine, 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.

Considering the harmful effects of chemicals it was thought desirable to use following eco-friendly alternatives

i) Leaf extract

ii) Oils *(Cymbopogon*, Cahewnuts, Cotton seed. Linseed.)

iii) Gel (*Aloe vera)*

11.1. Use of Leaf Extracts

Spencer *et al.* (1957) made pioneering efforts to suggest use of harmless non phytotoxic and biodegradable plant extracts to be used as antifungal agents. Neem leaves possess bioactive compounds like Meliantriol (Lavie *et al.,* 1967) and Azadirachtin (Butterworth and Morgan, 1968), which have antifungal and feeding deterent property for insects. A large number of plants like *Azadirachta indica* A. Juss., *Melia tosendens* L., *Melia azadirach* L., *Swetenia mahogani, Annona squamosa L., Tagetes erecta* L., *T. tatula* L. and *Tripterygium wilfordii* Hook are reported by numerous investigators as botanical pesticides. All plants contain medicinal compounds, which are toxins and antitoxins, oxidants and antioxidants, nutrients and antinutrients depending on their action or reaction on the pest. The effect of such compounds may change the membrane permeability of a microbe thus affecting its metabolism, or there may be inhibition in metabolism due to one or other reasons. The plants have active principles like alkaloid,

glucoside, saponin, toxic proteins or other metabolites in aromatic oils present in flowers. The degree of their biocide character decides, whether it is a potential phytobiocide or not. These are less pollutive as they are broken down in nature after some time. The cells exposed to these substances may create ultrastructural changes like retraction of plasmalemma, breakdown of organelles, disintegration of cytoplasm, loss of turgor, weakening and ultimately death of host cells (Arya and Arya, 2009). Lal and Srivastva (2002) found 51 plants as effective biopesticides against microbes and insect pests.

Table 36 includes names of plants and their active ingradients which were tried against five fungal pathogens

Table 36: Plants used as botanical pesticides and their activie ingradients

S.No	Plant	Family	Active ingradient
1	*Thevetia peruviana* (Pers.) Schum. (Pili kaner)	Apocynaceae	Peruvoside cannogenin
2	*Tagetes erecta* L. (Merigold)	Asteraceae	L- thevetoside Trimer of thiophene
3	*Eucalyptus globulus* Labill.	Myrtaceae	Cineole , α – inene limonene
4	*Azadirachta indica* A. Juss	Meliaceae	Azadirachtin
5	*Prosopis juliflora* (Sw.) DC.	Mimosaceae	
6	*Saraca indica* L. (Sita Ashoka)	Caesalpiniaceae	
7	*Lantana camara* L.	Verbenaceae	Because of it antifungal property Ocean Agro Ltd has produced a biofungicide Ovis.
8	*Biota sinensis* L. (Thuja)	Cupressaceae	B licins a group of tropolon monoterpenoids
9	*Cymbopogon citrates* (Nees) Stapf. (Citronella)	Poaceae	Citronellal Geraniol, Eugenol Borneol
10	*Callistemon linearis* DC.	Myrtaceae	
11	*Datura metel* L.	Solanaceae	
12	*Parthenium hysterophorus* L.	Asteraceae	Sesquiterpene parthenin

Table 37: Effect of methanolic and aquous plant extracts on 5 different wood decay fungi by Poisoned food technique.

***Thevetia peruviana* (Pers.) Schum.**						
	Methanolic			**Aqueous**		
Fungi	**5%**	**10%**	**25%**	**5%**	**10%**	**25%**
Lenzites sterioides	35.13	81.56	93.04	34.37	65.0	78.75
Trametes pini	66.76	90.76	96.92	31.42	57.14	71.42
Schizophyllum commune	41.52	69.55	93.35	29.52	48.57	61.90
Ganoderma lucidum	36.09	54.88	94.58	19.11	36.76	48.52
Sterium hirsutum	62.77	79.44	100	0	0	0
***Tagetes erecta* L.**						
L. sterioides	25.42	68.92	100	35.83	50.0	68.33
T. pini	36.92	70.76	100	49.03	60.57	66.34
S. commune	14.06	64.84	100	50.0	58.33	66.66
G. lucidum	40.0	81.37	100	49.09	63.63	74.54
S. hirsutum	83.88	90.55	100	27.77	63.88	88.88

***Eucalyptus globulus* Labill.**						
L. sterioides	61.66	75.0	100	11.58	28.39	40.24
T. pini	62.37	79.20	100	40.14	47.88	57.74
S. commune	35.83	56.66	100	18.11	29.13	37.0
G. lucidum	51.81	77.27	100	32.0	42.4	53.6
S. hirsutum	27.77	100	100	11.11	16.66	27.77
***Azadirachta indica* A. Juss.**						
L. sterioides	61.98	94.15	100	69.90	100	100
T. pini	41.42	87.14	100	85.91	90.14	94.36
S. commune	21.42	60.0	100	35.77	48.62	63.30
G. lucidum	45.45	77.27	100	67.27	81.81	90.90
S. hirsutum	27.77	100	100	11.11	55.55	100
***Prosopis juliflora* (Sw.) DC.**						
L. sterioides	100	100	100	47.05	52.94	69.93
T. pini	46.51	100	100	63.41	74.79	82.92
S. commune	78.33	100	100	57.98	68.42	72.92
G. lucidum	73.77	100	100	85.87	93.22	97.74
S. hirsutum	100	100	100	81.11	94.44	97.77
***Saraca indica* L.**						
L. sterioides	53.29	85.02	100	5.55	46.66	76.66
T. pini	67.21	96.72	100	15.58	59.74	77.92
S. commune	13.63	81.81	90.9	21.17	58.82	85.88
G. lucidum	47.91	86.11	100	8.88	32.22	55.55
S. hirsutum	3.33	16.66	70.0	11.11	33.33	75.55
***Lantana camara* L.**						
L. sterioides	13.25	26.34	30.68	18.89	29.56	60.00
T. pini	40.24	48.45	62.46	20.25	30.65	48.28
S. commune	50.00	82.34	100.00	23.25	50.26	55.68
G. lucidum	44.35	68.24	100.00	24.86	42.46	63.34
S. hirsutum	41.24	53.25	85.68	15.80	28.05	58.25
***Biota sinensis* L.**						
L. sterioides	50.00	55.34	65.24	10.00	15.00	50.00
T. pini	18.50	25.56	54.89	48.89	60.25	65.34
S. commune	10.00	20.00	60.00	15.00	40.00	45.00
G. lucidum	44.00	53.00	80.00	15.00	50.00	68.00
S. hirsutum	35.00	50.00	55.00	6.00	15.00	20.00
***Cymbapogan citrates* (Nees) Stapf.**						
L. sterioides	53.94	100	100	48.36	69.93	84.96
T. pini	44.0	87.84	100	37.30	78.57	85.71
S. commune	18.75	53.75	97.65	34.67	48.38	58.06
G. lucidum	53.54	85.16	100	0	46.66	63.33
S. hirsutum	49.44	72.77	100	0	0	0
***Datura metel* L.**						
L. sterioides	74.25	100	100	15.55	33.33	76.66
T. pini	65.42	83.56	99.06	26.12	44.18	62.79
S. commune	35.27	70.83	100	6.06	22.72	65.15
G. lucidum	--	--	--	--	--	--
S. hirsutum	--	--	--	--	--	--
***Callistemon linearis* DC.**						
L. sterioides	73.41	89.24	100	-9.29	-16.90	-19.71
T. pini	45.52	80.95	100	19.40	38.80	67.14
S. commune	26.66	82.96	88.14	38.96	57.14	77.92

G. lucidum	--	--	--	--	--	--
S. hirsutum	--	--	--	--	--	--
***Parthenium hysterophorus* L.**						
L. sterioides	26.66	50.00	90.00	38.40	58.00	70.00
T. pini	15.00	59.00	88.78	20.00	75.80	100.00
S. commune	25.23	38.57	50.45	36.24	58.85	100.00
G. lucidum	--	--	--	--	--	--
S. hirsutum	--	--	--	--	--	--

It is evident from Table 36 that leaf extracts of 10 dicot, 1 monocot and 1 gymnospermous plant was tested against 5 wood degrading fungi *in vitro*. In most of the cases 25% methanolic extract was more effective than 5 and 10% concentrations. Plants used as botanical fungicides and their details are listed in Table 37. *L. sterioides* was completely inhibited by 5% leaf extract of *P. juliflora* and 10% leaf extracts of *Prosopis*, and *Cymbopogon* and 25% concentration of *Datura*. Leaf extracts of *Tagetes, Eucalyptus, Azadirachta,* and *Prosopis* controled all the 5 test fungi completely. Extracts of *P. juliflora* and *A. indica* were comparatively more effective than the other plants. Methanolic extract of *Cymbopogon* showed 100% inhibitin of *S. hirsutum*, while its aquous 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. Aquaeous extracts of *A. indica P. Juliflora* and *P. hysterophorus* may be further tried *in vivo* against all the 5 wood decay fungi. Extracts of *Tagetus, Eucalyptus, Azadirachta, Saraca, Cymbopogon, Datura* and *Callistemon* were 100% effective at 25% concentration. Arya (1988) found leaf extract of *E. globulus* against stylar end rot pathogen of guava (*Phomopsis psidii* Nagaraj and Ponappa). Pandey *et al.* (1983) reported control of *Pestalotia* rot of guava by application of Neem (*Azadirachta indica*) and Tulsi (*Ocimum sanctum*)

11.2. Use of different Oils

Use of fixed oils (Arya 1988) and essential oils (Balchin *et al.* 1996, Dixit *et al.* 1983, Mishra *et al.* 1997) is reported to control fungal growth. Volatile oils are sweet-smelling lipids synthesized and stored in various plant parts. These oils are essentially mixtures of two classes of terpenoids *i.e.* the monoterpenes and the sesquiterpenes, the former predominating in most cases. It has been found that D- limonene and Cineole present in *Cymbopogon martini* (Wilson *et al.* 1997) and Eugenol present in *Ocimum*

gratissimum inhibited the radial colony diameter of *Alternaria alternata, Rhizoctonia* sp. and *Sclerotium rolfsii* (Thakur *et al.* 1989).

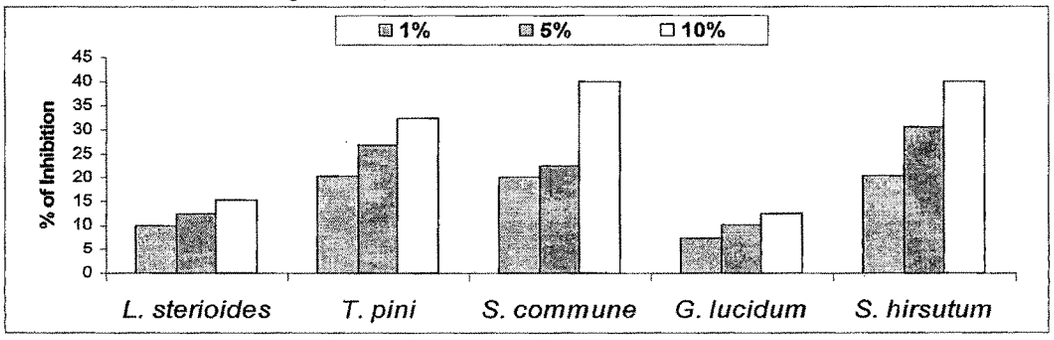
Garlic (*Allium sativum* L.) cloves and *Bignonia alliciverum* leaves may be effective due to the presence of sulphur compound Allicin in them (Arya *et al.* 1995). Leaf extract of *Strichnos nux vomica* was effective against *Phomopsis psidii* (Arya 1988).

Table 38 : Oils and gels used as antifungal agents to control fungal organisms

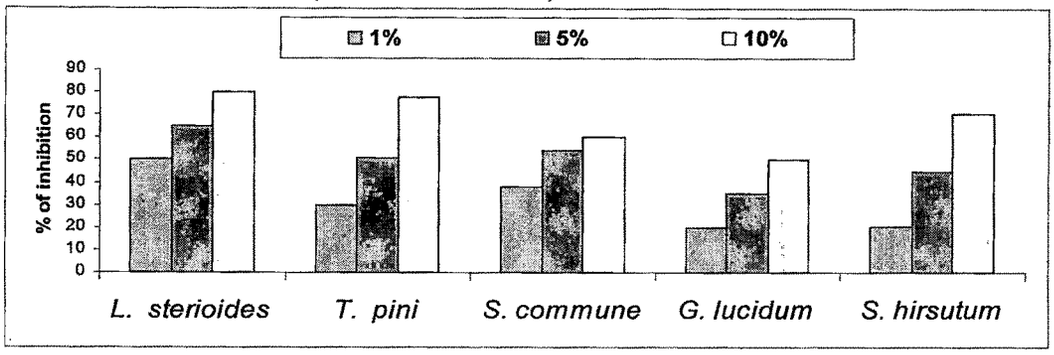
S. No.	Plant	Family	Active ingradients
1	***Cymbopogon citrates*** **(Lemon grass oil)**	Poaceae	Citral, citronellol, Geraniol and Myrcene
2	***Anacardium occidentale*** **L. (Cashwenut shell oil)**	Anacardiaceae	Anacardic acid and Cardol
3	***Gossypium barbadensis*** **L. (Cotton Seed oil)**	Malvaceae	Gossypol
4	***Linum usitatissimum*** **L. (Alsi oil)**	Linaceae	Cyanogenetic glycoside linamarin (used for making paints Varnishes) and Linoleum
5	***Aloe vera*** **L. Gel (Ghrat kumari)**	Liliaceae	Barbaloin, Iobarbaloin, Aloinoside
6	***Aloe ferox*** **Mill. (Gel)**	"	Lesser amount of Aloe-emodin Beta barbaloin

It is evident form Table 38, Histogram 24-27 that out of 4 oils tested 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.* Use of oils prevents infection in plants or wooden planks by making their surface water repellent. Earlier use of cashew nut oil has been found effective against members of mitosporic fungi (*Cochliobolus verruculosus, Aspergillus ustus* and *Pencillium citrinum*) (Shah and Arya 2000)

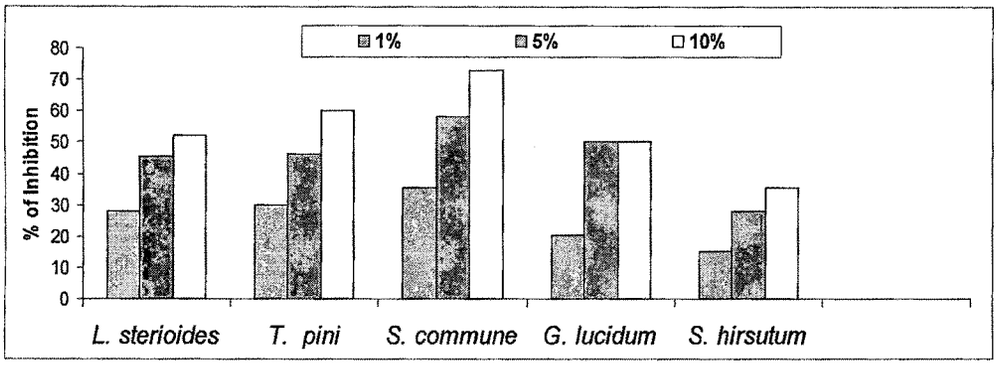
Histogram 24: Percentage inhibition of different wood rotting fungi by *Cymbopogon* (Lemmon grass oil)



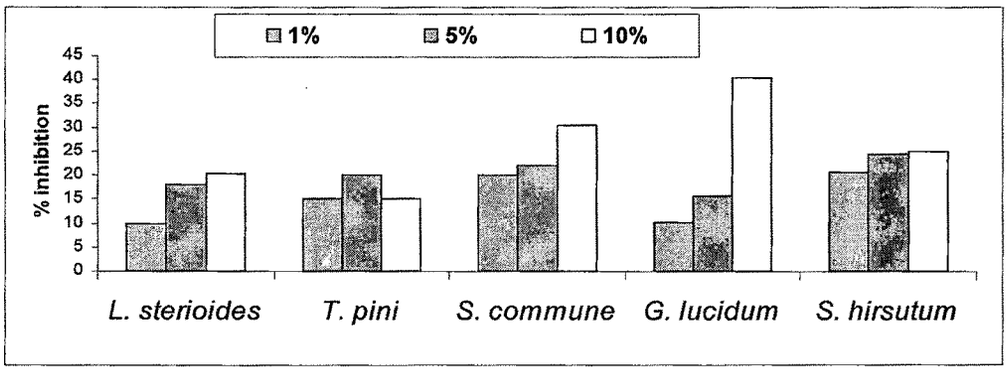
Histogram 25: Percentage inhibition of different wood rotting fungi by *Anacardium occidentalis* (Cashwenut shell oil)



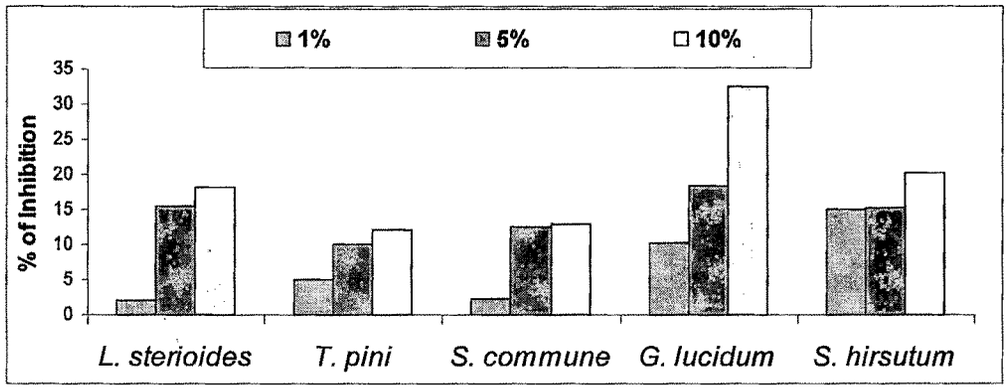
Histogram 26: Percentage inhibition of different wood rotting fungi by *Gossypium barbadensis* (Cotton Seed oil)



Histogram 27: Percentage inhibition of different wood rotting fungi by *Linum usitatissimum* L. (Alsi Oil)



Histogram 28: Percentage inhibition of different wood rotting fungi by *Aloe vera* Gel



Histogram 29: Percentage inhibition of different wood rotting fungi by *Aloe ferox* (Gel)