

Delignification of Valuable Timbers decayed by India Lignicolous fungi

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ABSTRACT

Wood degrading capacity of lignicolous fungi was studied by decay test. In which two methods were followed, i) wood chips method ii) wood block method. Eight timbers infected by six fungi were selected for studying percentage of decay and biochemical test was done to know delignification. After 12 months, 90 % of wood block of *T. arjuna* was decayed by *L. stereoides*. In teak wood 16.82 % of decay was due to *H. apiaria* in 3 months. As the percentage of moisture was less, percentage of weight loss was also less; this indicated that decay capacity of fungi will depends on % moisture content in wood. The percentage loss in hot water soluble substrates was more in case of *T. crenulata* due to *L. stereoides* for 5 months, whereas lowest in case of teak wood decayed by *H. apiaria* for 5 months. The percentage loss in ethanol benzene soluble substrate was more in case of Adina wood decayed by *C. versicolor* for 5 months, whereas lowest in case of teak wood infected with *L. stereoides* for 3 months. As the incubation period increases, percentage loss in acid soluble lignin was more in case of infected woods. *L. stereoides*, *C. versicolor*, and *H. apiaria* showed selective delignification in all infected woods, whereas *T. pini* showed simultaneous degradation of lignin in all woods tested. The valuable timber like teak wood was not resistant to wood decay because they loss 50% of lignin. The in vitro wood decay test can't be taken as absolute evidence for wood decay behavior of lignin-degrading fungi, so we should conform decay of wood by consider biochemical test. For rapid evaluation of wood decay the wood chip method was best suitable. For the first time the wood decay and biochemical test of 8 wood samples infected by white rot fungi like *S. commune*, *L. stereoides*, *H. apiaria*, *C. versicolor*, *T. pini* and soft rot fungi like *T. viride* was studied.

Keywords: Biochemical; Delignification; Lignicolous fungi; Wood decay; Teak

1. INTRODUCTION

Wood was a very important byproduct, produced by different biological processes in tree. It was an important natural resource from forests which can contribute greatly to climate change and biodiversity conservation (Kaimowitz D. 2003). Based on the FAO definition

there are around 3.7 billion ha of forests in the world. Majority of forest was natural forests, in which more than 50 % in South America and Europe, while plantations cover only 187 million ha, representing 5 % of the total forested area (FAO 2007). Global wood consumption was increasing but at a relatively low pace. In the last 20 years the average global consumption of wood increased on average only 0.3 % per year, and the estimated annual wood consumption was now around 3.5 billion cubic meters (ITTO 2006). Out of this total volume approximately 50 % are classified as industrial logs. The global consumption of industrial roundwood would achieve around 1.9 billion by the year 2010 (FAO 2007).

The total forest cover in India according to the latest State of Forest Report 2011 is 78.29 million ha and this constitutes 23.81 % of the geographic area. The state of Gujarat was one of the progressive states in western part of India, with an area of 78,687 sq mi (203,800 km²). While recorded forest area is 18,962 sq. km. which was 10 % of total geographical area. The production of fuel and timber was much less than the demand. The forest area which produces timber and fuel wood was only 63.5 % of the recorded area. Lignicolous fungi belonging to Aphyllorphorales were economically important, as many of these were pathogens of forest trees and cause serious damage (Natarajan K. and Kolandavelu K. 1998). The forest wood cell wall was composed of cellulose, hemicelluloses and lignin. Cellulose was most consistent of structural components varying minimally between wood species. Lignin and hemicelluloses, however, vary both in composition and amounts not only between hard woods and conifers but also among hardwoods (Timells T. 1967). Cellulose was a long chain polymer of glucose anhydride units joined by β 1-4 linkages. In general, cellulose components of wood are light in colour, have strong affinity for water and were soft and tough. Hemicelluloses consist of similar polymers of glucose joined by other linkages or polymers of monosaccharide's other than glucose. Lignin was quite different from celluloses and hemicelluloses and was also most resistant to biodegradation. It was three dimensional amorphous, branched polymers of phenyl-propane units joined by a variety of inter-units linkages (Alder E. 1977).

Delignification was deterioration of timber brought about by chemical breakdown and separation of the cell walls of timber. It was also known as "Defibration of Timber". Timber decay was caused by primarily enzymatic activities of microorganisms. Lignicolous fungi were responsible for decay in timber and those fungi that feed on the cell contents, causing stains. These fungi seriously weather timber, ultimately rendering it valueless by consuming cell wall constituents and lead to the disintegration of wood tissue (Desch, H.E. and Dinwoodie, J. M. 1996). Three general types of decay were recognized (Blanchette, R.A. 1991, Eaton, R. A., and Hale M. D. C. 1993, Zabel, R. A., and Morrell J. J. 1992). In white rot, all cell-wall constituents was degraded. Two forms of white rot were distinguished. In selective delignification, polyoses (=hemicelluloses) and lignin were preferentially attacked, especially in early stages. In simultaneous white rot, carbohydrates and lignin were attacked more or less uniformly (Blanchette, R.A. 1991). In brown rot, carbohydrates were extensively removed, but lignin was degraded only to a limited extent (Wilcox, W. W.1968). Soft rot, most recently described type of wood decay, has proven difficult to define and differentiate from other decays. It was caused by Ascomycetes and Deuteromycetes, All cell wall constituents may be degraded during soft rot, but there was usually a preference for carbohydrates, especially in hardwoods (Esllyn, W. E., Kirk T. K., and Effland M. J. 1975, Nilsson, T. Daniel T. G., Kirk T. K., and Obst J. R. 1989).

A better understanding of diverse kinds of lignicolous fungi and their decay types will support efforts to prevent and control wood decay as well as recent efforts to find biotechnological applications of such fungi in the pulp and paper and other industries (Kirk,

T. K., and H.M. Chang. 1990). Blanchette (1984) mentioned about lignicolous fungi that remove lignin selectively without appreciable losses of cellulose were extremely attractive for use in biological pulping processes. Such knowledge may also provide perspective in considering evolution of wood-decay capability in various groups of fungi. Our objectives were to 1) survey lesser-known and previously neglected lignicolous fungi for the ability to cause wood decay; 2) elucidate the wood decay and biochemical features of decayed wood by such fungi in comparison with known decay types; 3) decay classification of lignicolous fungi, and; 4) Quick identification of wood decay by different test. In the present paper wood degrading capacity of lignicolous fungi was studied by decay test like wood chip and wood block method. Wood of *Tectona grandis*, *Terminalia arjuna*, *T. bellerica*, *T. crenulata*, *Adina cordifolia*, *Dalbergia sissoo*, *Pinus longifolia*, and *Acacia arabica* were selected on the value of timber. Lignicolous fungi like *S. commune*, *L. stereoides*, *H. apiaria*, *C. versicolor*, *T. pini* and soft rot fungi like *T. viride* were used to infect wood chips, blocks and logs of above timbers and these were chemically analyzed.

2. MATERIALS AND METHODS

Wood degrading capacity of lignicolous fungi was studied by decay test. In which two methods were followed, i) wood chips method ii) wood block method. In biochemical analyses the degraded wood samples were analyzed for water content, pH of samples, solubility in hot water, and ethanol-benzene, acid insoluble lignin and chlorite holocellulose (Dill, I.; Kraepelin, G. 1986).

2. 1. Wooden chips method

A survey was undertaken in forests and sawmills of Gujarat, India, during January 2007 to July 2011, to find out occurrence of lignicolous fungi. These fungi were isolated by PDA medium from sporophore and decayed wood and were grown on 2 % malt extract agar in petriplates for 7d prior to inoculation in decay chambers. Seven woods like *T. grandis*, *T. arjuna*, *T. bellerica*, *A. cordifolia*, *D. sissoo*, *P. longifolia*, and *A. arabica* wooden chips (0.5g) were added to decay chamber containing Modified Asthana and Hawker's medium 'A'. The composition of medium was 10 g of D – glucose, 3.5 g of KNO₃, 1.75 g of KH₂PO₄, 0.75 g of MgSO₄·7H₂O and 20 g of Agar. The decay chambers were sterilized at 121 °C for 1 h. The decay chambers were inoculated with Lignicolous fungi like *S. commune*, *L. stereoides*, *H. apiaria* and soft rot fungi like *T. viride*. Three decay chambers were used for each isolated lignicolous fungi per wood and the fungus inoculated in decay chamber without wooden chips served as control. Assembled decay chambers were incubated in the dark for 20 and 40 d at 27 ±1 °C. The wooden chips were filtered, oven dried, and weighed. Percent weight loss was determined as follows:

$$\text{Percent weight loss} = \frac{\text{Weight loss of oven – dried wood after incubation}}{\text{Weight of oven – dried original wood}} \times 100$$

Each set of treatment was run in triplicates and average weight loss was always taken as standard value for comparison of wood decay. The weight loss results were statistically analyzed by using the MS office Excel software and the significant values were taken for study.

2. 2. Wooden block method

Timber decay caused by Lignicolous fungi like *S. commune*, *L. stereoides*, *H. apiaria*, *C. versicolor* and soft rot fungi like *T. viride* was observed in wood blocks of *T. grandis* and *T. arjuna* and *T. bellerica* for 20 days to 1 year. These fungi were grown on 2 % malt extract agar for 7 d prior to inoculation. The PDA plates were prepared by inoculating each isolate of above fungi and incubated for 7-10 d.

Totally 12 wooden blocks (1 x 1 x 1 cm) per wood per each lignicolous fungi were cut from the respective logs and soaked in distilled water for 30 min. These wooden blocks were autoclaved. After completely spreading of above fungus, four blocks of each wood were placed on the medium per plate and incubated for 3, 6 and 12 months at 27 ± 1 °C. To maintain moisture in test plates two layers of Whatmen filter paper No 1 were placed on the surface of blocks. The sterile distilled water was added to it regularly. The un-inoculated wooden blocks acted as control. After completion of incubation period each block was cleaned (of the mycelium), oven dried and weighed. The percentage weight loses was calculated as described above.

2. 3. Spawn preparation

For spawn preparation a media used for developing sporophores of wood rot fungi as suggested by Etter (1929) was used. The spawn preparation medium consisted of 48 g of corn-meal, 16 g of corn-starch and 8 g of powdered wood. The spawn was taken in a polypropylene bag and 2.5 % malt extract was added. The bag was closed by putting the moist cotton swab to maintain moisture level inside the bag; such bags were sterilized at 121 °C for 1 h and inoculated with four test fungi like *L. stereoides*, *C. versicolor*, *H. apiaria*, *T. pini*. The bags were incubated in dark for 15d at 27 ± 1 °C. The fully-grown spawn was used for artificial inoculation in wooden logs.

2. 4. Wooden log preparation

The wood log of *T. grandis*, *A. cordifolia*, *T. crenulata* and *T. arujna* were infected with above lignicolous fungi and used for biochemical analysis. The average size of (4) wood plank used was 2 x 2 x 30 cm length. The bark was not removed. It helps to maintain moisture and keeps away the foreign fungi. A 5/16" drill bit was used to make holes in the logs for insertion of spawn as diamond drilling pattern. After drilling the entire log was autoclaved for 1 h. After autoclaving spawn was inoculated into the holes. It was then sealed off with paraffin wax. Logs were covered with cheese cloth to maintain moisture, packed in polythene bags and incubated in dark for 12 months.

2. 5. Bio – chemical analysis of decayed wood

The chemical composition of sound and decayed wood as determined by previously described technique (Dill, I.; Kraepelin, G.1986). Decayed wood was dried at 105 °C and then ground to pass through a 60 µ mesh screen. It was used for further analysis.

2. 5. 1. Water content

To obtain water present in the sample decayed wood (3 g) was dried at 105 °C for about 48 h, cooled in a desiccator and weighed. The difference in two weights gave the water content in milligrams.

2. 5. 2. pH of samples

The pH was determined potentiometrically after suspension of the samples in distilled water for about 30 to 45 min. Analysis of decayed wood was made with a few grams of fresh material and that of corresponding sound wood was made with 1 g of dry wood meal.

2. 5. 3. Solubility in hot water

One gram of dry wood meal was placed in a 250 ml Erlenmeyer flask. After addition of 100 ml of distilled water, the mixture was slowly stirred at 80 °C for 3 h. The samples were then filtered by using Whatman filter paper No 1, washed with hot water, dried at 105 °C for about 24 h, cooled in desiccator and weighed.

2. 5. 4. Solubility in ethanol - benzene

About 1.5 g of dry wood meal was extracted with ethanol – benzene (1:2 v/v) for 4 h in a Soxhlet extractor, keeping the liquid boiling briskly. Each extracted sample was washed with 50 to 100 ml of ethanol and dried at 105°C. After evaporation of solvent, each extract was dried at 105 °C for 24 h, cooled in desiccator and weighed.

2. 5. 5. Acid insoluble lignin (klason lignin)

Flasks containing 1 g of ethanol - benzene extracted wood meal and 20 ml of H₂SO₄ (72 %) were gently shaken in a water bath at 30 °C for 1 h. The acid was then diluted with H₂O to 4 % (wt/vol), and the samples were autoclaved at 121 °C for 30 min. The lignin that settled overnight was quantitatively collected by filtration through a Whatman filter paper No. 1, washed free of acid with hot water, and dried. The lignin content was calculated as a percentage of oven-dried, non-extracted wood meal.

2. 5. 6. Chlorite holocellulose (CHC)

Chlorite holocellulose was also determined as described by Seifert (1983). Extracted wood samples of approximately 400 mg were placed in 50 ml Erlenmeyer flasks. Seven milliliters of buffer solution consisting of 60 ml glacial acetic acid and 1.3 g sodium hydroxide per 1000 ml distilled water was added to each flask. Three milliliters of 20 % (w/w) aqueous solution of sodium chlorite was immediately added and the flasks were sealed with paraffin wax and aluminum foil. The flasks were placed in an orbital shaker at 110 rpm at 45 °C for 36 to 40 h. After incubation period; flasks were placed in ice bath to stop the reaction. The contents were then transferred to pre weighed Whatman filter paper No. 1 using 100 ml of 1 % acetic acid. The holocellulose was washed with 5 ml of acetone three times and oven dried at 105 °C for 4 to 6 h before weighing.

3. RESULTS AND DISCUSSION

3. 1. Wooden chips method

Percentage decay of seven different woods caused by *S. commune*, *L. stereoides*, *H. apiaria* (White rot fungi) and *T. viride* (soft rot fungi) was observed. As compared to other white rot and soft rot fungi, teak and sissoo wood was efficiently degraded by *L. stereoides*, where the percentage weight loss was 34.6 and 44.6 % after 40 days. Whereas in case of *T. arjuna*, *T. bellerica*, *A. cordifolia*, *A. arabica*, and *P. longifolia* 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 wood decayed after 40 days of incubation, *S. commune* showed 29.1 % decay in *A. arabica*, whereas the *L. stereoides* 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 1 and Histogram 1).

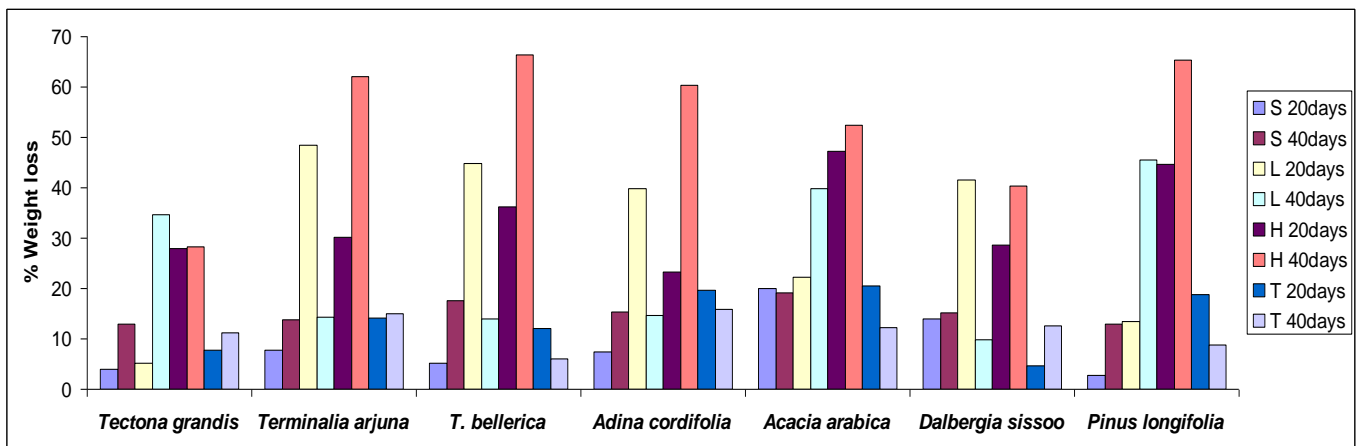
Table 1. Percentage weight loss of wood chips of seven woods by four Lignicolous fungi.

Percentage weight loss																
	<i>Schizophyllum commune</i>				<i>Lenzites stereoides</i>				<i>Hexagonia apiaria</i>				<i>Trichoderma viride</i>			
	*20 days		pH		*20 days		pH		*20 days		pH		*20 days		pH	
<i>Dalbergia sissoo</i>	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
<i>Adina cordifolia</i>	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
<i>Acacia arabica</i>	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

<i>T. bellerica</i>	<i>Terminalia arjuna</i>	<i>Tectona grandis</i>	<i>Pinus longifolia</i>
5.2±1.6	7.8±2.5	3.9±0.9	2.8±1.0
5.97	6.39	5.36	5.46
17.6±1.5	13.8±1.8	12.9±2.5	13.0±2.3
5.8	6.6	5.2	5.4
44.9±3.8	48.4±3.2	5.1±2.5	13.4±2.4
4.4	5.0	4.9	4.2
54.0±4.3	54.3±3.2	34.6±2.5	45.5±4.5
4.3	5.1	4.0	3.9
36.2±1.8	30.2±4.9	27.9±2.4	44.7±4.8
4.35	4.72	4.25	4.62
66.4±5.6	62.0±5.1	28.3±2.3	65.4±5.3
4.26	5.50	5.20	5.26
12.1±2.1	14.2±1.7	7.7±1.8	14.8±2.4
7.04	8.86	3.34	9.04
16.0±2.5	15.0±3.4	11.2±1.8	18.8±2.6
8.24	8.97	9.00	10.0

* 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 1. The % weight loss in different woods chips infected with four lignicolous fungi.



According to ASTM (1969) method of classification of decay resistant classes, teak wood was very resistant in case of *T. viride*, and *Lenzites* sp. up to 3 weeks of incubation. Moderate resistance was observed in case of *H. apiaria* and *L. stereoides* (Nagadesi P. K., Arya A., Albert S. 2013). 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 teak wood was resistant to moderately resistant when different wood rotting fungi were tested in wood chip method.

The Basidiomycetes plus composite inocula caused significantly more weight loss of inoculated wood chips than members of Basidiomycetes alone after 5 months (Blanchette R.A. and Shaw C.G. 1978). In the present study the lignicolous fungi like *S. commune*, *L. stereoides*, *H. apiaria* and soft rot fungi like *T. viride* alone showed significant decay in wood chips after 40 days. Wood chips from slash less than 1 and 2 year old were approx. 40 % decayed by the brown rot fungus *P. placenta* for 5 months. The decay caused by the white rot fungus, *C. versicolor* was approximately half than the *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 (Blanchette R.A. and Shaw C.G. 1978). In the present study the increase in weight loss by *H. apiaria* 66.4 % in *T. bellerica* for 40 days. Whereas soft rot fungi like *T. viride* showed 22.3 % weight loss in *A. arabica* for 40 days.

The wood chips of ten-year old plantation of *Pinus caribaea* (morelet) were inoculated separately with two species of white-rot fungi; *Coriolopsis polyzona* and *Pleurotus squarrosulus*, and two species of brown rot fungi; *Lentinus lepideus* and *Gleophyllum striatum*. Wood weight loss due to biodegradation varied from 1.5-48.1 % for *Coriolopsis 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, E. A., Ekeke, B. A. and Oyebade, B. A. 2008). In the present study the weight loss due to degradation varied from one to 13% for *S. commune*, 1 to 45.5 % for *L. stereoides*, 1-65.4 % for *H. apiaria* and 1-18.8% for *T. viride* in 40days. The highest decay was shown by *H. apiaria* with 27.9 % weight loss in 20 days. The lowest decay was showed in case of *T. viride* with 4.7 % weight loss in 20 days (Nagadesi P. K., Arya A., Albert S. 2013). In the present study teak and sissou woods were efficiently degraded by *L. stereoides*, whereas, the percentage weight loss was 34.6 and 44.6 in 40 days and *T. arjuna*, *T. bellerica*, *A. cordifolia*, *A. arabica*, and *P. longifolia* these woods were efficiently degraded by *H. apiaria* whereas the percentage weight loss was 62.0, 66.4, 60.4, 52.5 and 65.4 % respectively in 40 days only observed.

The molecular structure of wood suggests that cellulose a structural component thereof could be bio-recycled into glucose, a fermentable sugar (Reddy N. and Yang Y. 2009). Cellulase, a multi-component enzyme system produced by soft rot fungi such as *T. viride* and *Aspergillus niger* exhibits the ability to saccharify cellulose. These enzymes have been proved to be effective in the bioconversion of wood products such as wastepaper into fermentable sugars (van Wyk J. P. H. 2001). In the present paper the ability of soft rot fungi to degrade lignin was proved by wood chip test. The various sawdust wood samples were exposed to *T. viride* cellulase action with the delignified cellulose component bio-converted into fermentable sugars such as glucose (Bohdan V, Yaser D 2011). In the present study the *T. viride* showed significant weight loss in seven woods so it may be used for saccharify cellulose.

3. 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. stereoides* 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, 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 lignicolous fungi depends on the % moisture content in wood (Table 2 and Histogram 2).

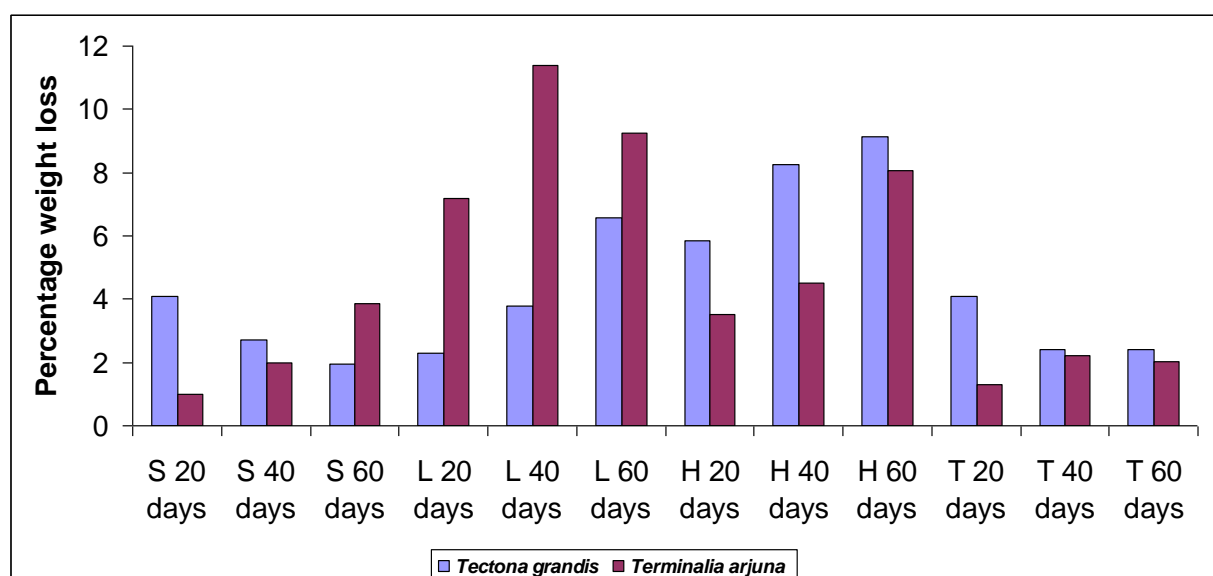
Table 2. Moisture loss and weight loss of two different wood blocks caused by three lignicolous fungi.

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

Histogram 2. The % weight loss of two wood blocks infected with lignicolous fungi.



Percentage wood decay of three different woods *T. grandis*, *T. bellerica* and *T. arjuna* was infected by white rot fungi like *L. stereoides*, *C. versicolor* and *H. apiaria* for 3, 6, 12 months. After 12 months 90 % of wood was decayed in *T. arjuna* due to *L. stereoides*, 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 3 and Histogram 3).

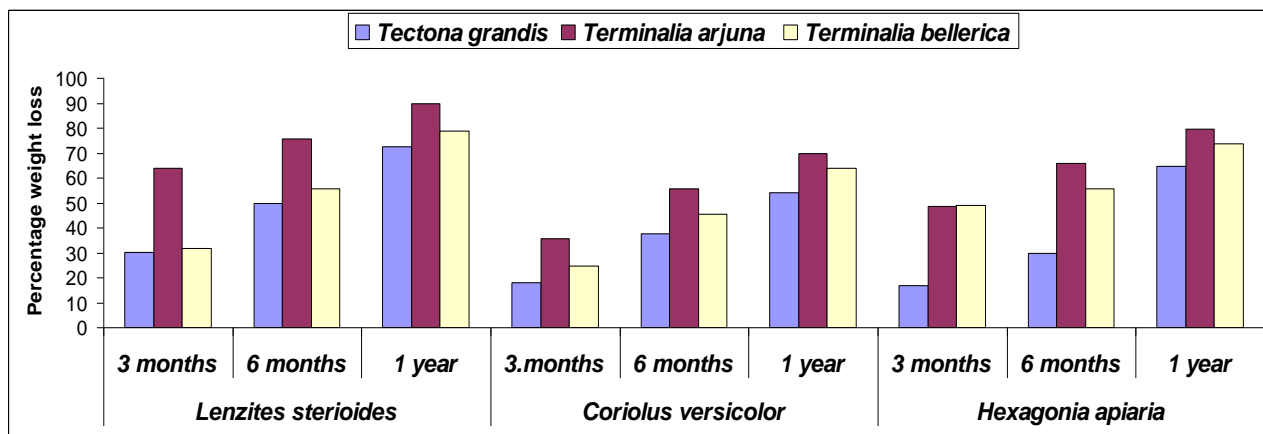
Table 3. The % weight loss in three wood blocks caused by three lignicolous fungi.

Wood	% Weight loss								
	<i>Lenzites stereoides</i>			<i>Coriolus versicolor</i>			<i>Hexagonia apiaria</i>		
	*3 months	*6 months	*1 year	*3 months	*6 months	*1 year	*3 months	*6 months	*1 year
<i>T. grandis</i>	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
<i>T. arjuna</i>	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
<i>T. bellerica</i>	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 3. The % weight loss in 3 wood blocks infected with lignicolous fungi.



Biological agar block method allowed wood samples to be evaluated and monitored in terms of colonization and development of decay by *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*. There was a high correlation between the mass loss and apparent damage. Therefore biological evaluation of wood regarding biodegradation and selection of wood types for various applications will be of high priority (Olfat A.M., Karimi A.N., and Parsapajouh D. 2007). In the present study also there was a correlation between the weight loss and damage, so different biochemical test were studied to conform that biological evaluation of wood was necessary.

The Basidiomycetes, *Poria carbonica* and *C. versicolor*, caused substantial wood weight loss over the test period. These results were usually obtained by soil or vermiculite burial methods (Morrell J.J. and Zabel R.A. 1989). In the present study the wood chip and block method showed significant weight loss in seven woods infected by white rot and soft rot fungi. The decreasing weight in studied samples showed that *C. versicolor* can grow quickly and may rapidly affect the appearance and degrade the wood (Olfat A. M., and Karimi A.N., 2005). In the present study the growth of *C. versicolor* was quick to degrade the wood very fast when compared to other lignicolous fungi. 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 A. M., and Karimi A.N., 2005). In the present study also *C. versicolor* degrade *T. arjuna* wood very fast.

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 (Guilley et al. 2004). In the present study the heart wood of teak showed 72.68 %, 54.15 %, 64.88 % of weight loss by *L. sterioides*, *C. versicolor* and *H. apiaria* respectively. Based on percentage weight loss, the American Society for Testing Materials ASTM (1969) classified 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 and terminalia wood was moderately resistant to non resistant when infected with different lignicolous fungi in wood block test. The *in vitro* decay of five commercial woods by *C. versicolor* was studied by the agar block method showed strong resistance of *U. glabra* and lowest resistance in *F. orientalis* (Olfat A.M., Karimi A.N., and Parsapajouh D. 2007). In the present study teak wood infected with *C. versicolor* showed resistant to non resistant. Where as in terminalia wood infected with same fungi showed moderately resistant to nonresistant. Twelve hundred samples from 31 trees were exposed to four fungi: *Pycnoporus sanguineus*, *Antrodia* sp., *Gloeophyllum 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 teak wood infected with *L. stereoides* showed moderately resistant to nonresistant, with *C. versicolor* and *H. apiaria* showed resistant to non resistant.

So, the *in vitro* wood decay test cannot be taken as absolute evidence for the behavior of lignicolous fungi, they were useful to determine their wood-degrading 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 A. and Wolcott M. P. 2005). In the present study the most effective method was agar block method when compared to wood chip method.

3. 3. Biochemical analysis

3. 3. 1. Artificially inoculated wood blocks

The physicochemical analysis of teak, and pine woods infected with wood decay fungi was done. The details were recorded in Table 4 and 5.

Table 4. Physico-chemical analysis of sound wood of *Tectona grandis*, *Adina cordifolia* and *Terminalia bellerica*.

Plants	*Moisture %	pH	*Swelling capacity %	% of dry weight				
				*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 5. Chemical analysis of decayed woods of *Tectona grandis* and *Pinus longifolia* by 4 lignicolous 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
	<i>Lenzites sterioides 2</i>	20	5.9±0.4	5.57	4.3±0.4	8.2±1.8	11.6±1.2	7.5±1.5
	<i>L. sterioides 1</i>	20	5.7±0.2	5.16	3.8±0.8	7.1±1.6	25.6±1.8	10.0±1.3

	<i>Schizophyllum commune</i>	20	4.5±0.6	5.30	4.7±0.5	7.3±2.4	19.6±1.6	5.0±1.8
	<i>Trichoderma viride</i>	20	6.2±1.2	6.05	3.5±0.4	7.1±2.6	26.0±2.3	11.5±1.4
	<i>H. apiaria</i>	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
	<i>L. stereoides 2</i>	20	5.9±0.7	6.01	4.9±0.3	13.8±1.8	13.0±1.5	19.5±1.4
	<i>L. stereoides 1</i>	20	5.5±0.6	5.05	4.5±1.5	12.2±1.4	26.8±1.4	10.5±1.8
	<i>S. commune</i>	20	5.9±0.1	5.70	5.9±1.8	17.4±2.3	29.2±1.8	19.0±2.0
	<i>T. viride</i>	20	6.2±1.5	6.90	4.6±0.4	14.0±2.5	31.0±1.6	14.0±2.5
	<i>H. apiaria</i>	20	5.3±1.8	4.89	5.5±0.7	15.6±1.8	18.8±1.9	7.0±1.0
	<i>H. apiaria</i>	40	9.6±2.5	5.27	16.0±2.3	18.4±1.5	41.4±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.

Highest percentage of moisture was shown by teak wood infected with *H. apiaria* whereas it was lowest in case of pine wood infected with *S. commune*. High acidic nature was shown 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 substrates was more in case of teak wood decayed by *H. apiaria* for 40 days, whereas, lowest was observed in pine wood decayed by *L. stereoides 1* and *T. viride*. The highest percentage loss of acid insoluble lignin was observed in case of teak wood decayed by *H. apiaria* for 40 days, whereas, lowest in case of teak wood inoculated with *L. stereoides 2*. Where as highest percentage loss of Acid insoluble lignin was observed incase of pine woods decayed by *H. apiaria* and lowest incase of pine wood inoculated with *L. stereoides 2*. The percentage loss of holocellulose was more in case of teak wood infected with *L. stereoides 2*, whereas, lowest in case of wood decayed by *H. apiaria*. The highest loss of holocellulose in pine wood was observed by *H. apiaria* decay and lowest in case of *S. commune* decay

Three white rot fungi *Daedalea elegans*, *Polyporus glaganetus*, and *L. betulina* were screened for their lignin degrading abilities on rice straw, maize 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 *D. elegans* after 90 days (Adejoye O.D. and Fasidi I.O. 2009). But in the present study only 26 % of reduction in lignin was observed in pine and teak wood blocks infected by *L. stereoides*, 30 % loss in pine and 18 % loss of lignin in teak woods infected by *H. apiaria*, 31 % loss in teak and 26 % loss of lignin in pine woods infected by *T. viride*

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, S.; Wajid, S.A.; Padmanabhan, S. 1978). 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* (Padhiar A., Albert S., Nagadesi P. K. and

Arya A. 2010). But in the present study the maximum lignin loss was recorded in teak than in pine by *L. stereoides*. The loss of lignin in teak and pine wood infected by *S. commune* was 29.2 % and 19.6 % respectively.

3. 3. 2. Artificially infected wood Planks

The artificially infected wood log of *Tectona*, *Adina*, *T. crenulata* and *T. arujna* were chemically analyzed. The percentage of moisture loss was highest in case of *T. crenulata* wood decayed by *L. stereoides*, 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. stereoides*. The percentage loss in hot water soluble substrates was more in case of *T. crenulata* due to *L. stereoides* 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 5 months. Whereas, lowest in case of teak wood infected with *L. stereoides* for 3 months. The percentage loss of acid soluble lignin was more in case of *T. crenulata* wood decayed by *L. stereoides* for 5 months, whereas, lowest in case of teak wood decayed by *L. stereoides* for 3 months. The percentage loss in holocellulose was more in case of *Adina* wood decayed by *C. versicolor*, whereas, least in case of *T. crenulata* wood infected with *C. versicolor* for 5 months and *T. pini* for 5 months period (Table 6 and Histogram 4).

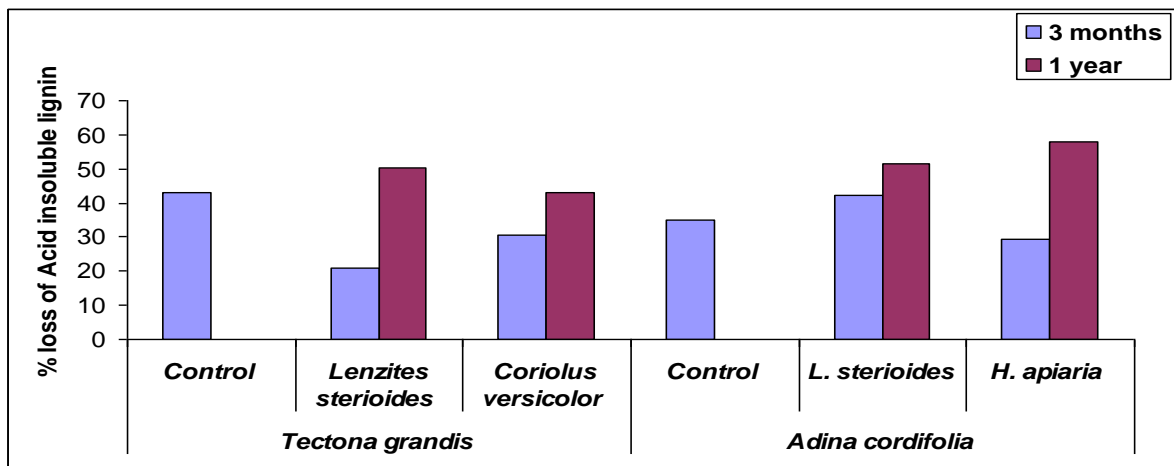
Table 6. Chemical analysis of three woods decayed by lignicolous for 5 months.

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

Histogram 4. The % loss of Acid insoluble lignin in 2 woods infected with lignicolous fungi.



When *S. commune* was grown on liquid media containing ¹⁴C-lignin-labeled wood, the degradation of lignin was low and variable (Boyle, C.D.; Kropp, B.R.; and Reid, I.D. 1992). *S. commune* has ability to produce lignin degrading enzymes for degradation of lignocellulosic materials (Padhiar A., Albert S., Nagadesi P. K. and Arya A. 2010). In the present study as the incubation period was increase the loss of lignin also increase. After five months, the highest lignin loss was observed in *T. crenulata* infected with *L. stereoides*.

The chemical analysis of artificially inoculated wood blocks for 1 year was studied (Table 7). 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 5).

Histogram 5. The % loss of Acid insoluble lignin in woods infected with lignicolous fungi.

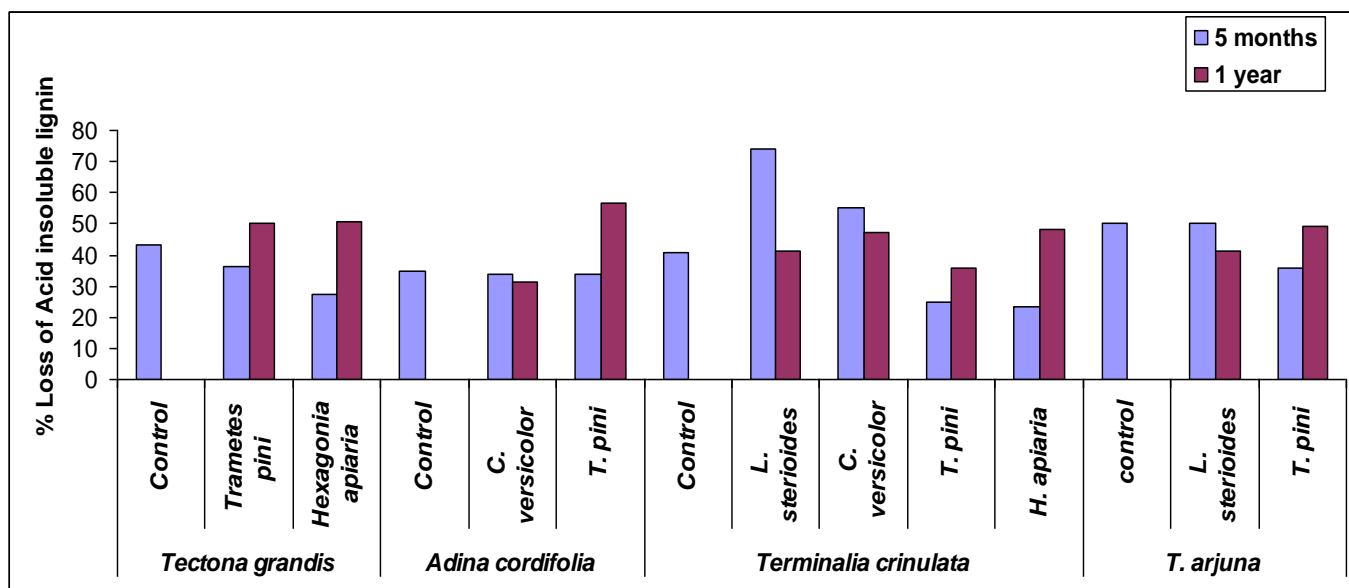


Table 7. Chemical analysis of four woods decayed by lignicolous fungi for one year.

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

The highest percentage of hot water soluble substrates in teak wood was 10 % when infected with *L. stereoides* whereas lowest incase of *T. pini* infected wood (6.0 %). The highest percentage of hot water soluble substrates in Adina wood was 39.6 % when infected with *C. versicolor* whereas lowest incase of *L. stereoides* infected wood (12.8). The highest percentage of hot water soluble substrates in *T. crenulata* wood was 19.8 % when infected with *T. pini* whereas lowest incase of *L. stereoides* infected wood (13.4 %). The highest percentage of hot water soluble substrates in *T. arjuna* wood was 13.8 % when infected with *L. stereoides*. The highest percentage of ethanol- benzene soluble substrate in teak wood was 18.6 % when infected with *T. pini* whereas lowest incase of *H. apiaria* infected wood (15.2 %). The highest percentage of ethanol- benzene soluble substrate in Adina wood was 28.5 % when infected with *C. versicolor* whereas lowest incase of *H. apiaria* infected wood (7.2 %). The highest percentage of ethanol- benzene soluble substrate in *T. cenulata* wood was

19.2 % when infected with *C. versicolor* whereas lowest incase of *H. apiaria* infected wood (11.8 %). The highest percentage of ethanol- benzene soluble substrate in *T. arjuna* wood was 28.73 % when infected with *T. pini*. The highest percentage loss of lignin in teak wood was 54.8 % when infected with *H. apiaria*; whereas lowest in case of *C. versicolor* infected wood (43.0 %).

The highest percentage loss of lignin in Adina wood was 57.8 % when infected with *H. apiaria*, whereas lowest incase of *C. versicolor* infected wood (31.2 %). The highest percentage loss of lignin in *T. crenulata* wood was 48.2 % when infected with *H. apiaria*, whereas lowest incase of *T. pini* infected wood (35.8 %). The highest percentage loss of lignin in *T. arjuna* wood was 49.0 % when infected with *T. pini*. The highest percentage loss of holocellulose in teak wood was 16.5 % when infected with *T. pini*. whereas lowest incase of *L. stereoides* infected wood (8.0 %). The highest percentage loss of holocellulose in Adina wood was 46.2 % when infected with *C. versicolor*, whereas lowest in case of *H. apiaria* infected wood (14.0 %). The highest percentage loss of holocellulose in *T. crenulata* wood was 28.5 % when infected with *C. versicolor*. whereas lowest incase of *T. pini* infected wood (6.0 %). The highest percentage loss of holocellulose in *T. arjuna* wood was 16.5 % when infected with *L. stereoides*.

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* (Padhiar A., Albert S., Nagadesi P. K. and Arya A. 2010). In the present study the *L. stereoides*, *C. versicolor*, and *H. apiaria* showed the selective delignification in all artificially inoculated woods, whereas, *T. pini* showed simultaneous degradation of lignin in all 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. (Padhiar A., Albert S., Nagadesi P. K. and Arya A. 2010). 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* (Padhiar A., Albert S., Nagadesi P. K. and Arya A. 2010).

In the present study the white rot fungi degraded highest amount of lignin in teak, adina, terminalia woods. The chemical analysis decayed wood showed highest delignification (i.e. Loss of Klason lignin) up to 84.71 % by *L. stereoides* in teak wood (20) but in the present study showed delignification up to 50.4 % only by *L. stereoides* in teak wood, but highest amount of delignification was shown by *H. apiaria*.

The maximum percentage of lignin loss by *D. confragosa* & *Phellinus pectinatus* was found to be in wood shavings Bamboo clum – PDA + Hydrofluoric acid (41.66 % & 33.33 % respectively) (Albert S. and Padhiar A. 2012). The highest percentage loss of lignin in teak, Adina, *T. crenulata* wood was 54.8 %, 57.8 %, 48.2 % respectively when infected with *H. apiaria* where as in *T. arjuna* wood was 49.0 % when infected with *T. pini*.

4. CONCLUSIONS

The wood degrading capacity of lignicolous fungi was studied by decay test. In which two methods were followed, i) wood chips method ii) wood block method. For rapid detection of decay in wood, the wood chips method was best. When we compare Soil Block method, Agar Block method and wood chip method, the agar block method was best to study decay pattern and Biochemical changes in wood. As the fungi required suitable conditions like suitable temperature, moisture, pH for better growth and also for decay of wood. In the present paper in initial stages of decay the percentage of moisture was more, whereas in advanced stages of decay the % moisture was less. As the percentage moisture was less percentage weight loss was also less, this indicates that the decay capacity of fungi depend on % moisture content in wood. Based on the weight loss studies different scientists have explained that the woods were resistant to a particular fungi. But in present study it was found that in case of lesser weight loss also wood was severely degraded by the lignicolous fungi. Therefore, on the basis of weight loss studies alone the type of wood decay can not be certainly decided. As the incubation period increased the percentage loss in acid soluble lignin was more in case of all infected woods. *L. stereoides*, *C. versicolor*, and *H. apiaria* showed the selective delignification in all infected woods, whereas, *T. pini* showed simultaneous degradation of lignin in all woods tested. The valuable timber like teak wood was not resistant to wood decay by lignicolous fungi used because they utilized 50% of lignin. For the second time the ability of wood decay by soft rot fungi like *T. viride* was described. For the first time the biochemical changes like loss of klason lignin and holocellulose from *T. viride* infected woods was described. For the first time the wood decay and biochemical changes in *T. crenulata*, *T. bellerica*, and *T. arjuna* woods infected by *L. stereoides*, *C. versicolor* *H. apiaria* *T. pini*, *S. commune*, and *T. viride* was described.

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