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Differential Changes in Structural Constituents of Heart woods and Sap woods by Brown-Rot Fungi

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Chemical changes in woods of *Shorea robusta*, *Ficus bengalensis* and *Dalbergia sissoo* decayed by three brown-rot fungi, viz., *Trametes persoonii* Fr., *Polyporus ostreiformis* Berk. and *Trametes cubensis* (Mont.) Sacc. respectively, were studied. Considerable loss of cell wall components of wood in respect of total carbohydrate, cellulose, hemicelluloses and lignin was recorded after 4 and 8 months of decay. Quantitative and qualitative analyses of cellulases corroborated the observations of chemical studies and indicated that the fungi mainly consumed the cellulose and hemicelluloses along with lignin but to a much lesser extent. Different fungus-host combinations exhibited differential loss of wood components which increased with time.

Key words : Wood decay, Carbohydrate content, Lignin content, Holocellulose content, Alpha-, Beta- and Gamma- cellulose content, *S. robusta*, *F. bengalensis*, *D. sissoo*, *T. persoonii*, *P. ostreiformis*, *.T cubensis*

INTRODUCTION

Brown rot fungi cause wood damage primarily by depolymerizing the cellulose (Cowling, 1961) followed by decay of depolymerized cellulose as well as the hemicelluloses (Kirk, 1975). This is often accompanied by a slight depletion of lignin (Kirk, 1973) thus leaving mainly the degraded lignin in a thoroughly brown rotted wood (Highley *et al.*, 1985). Analysis of different wood fungus combinations have revealed selective removal of cell wall components (Kirk and Highley, 1973 ; Blanchette *et al.*, 1985 ; Santra and Nandi, 1975, 1976, 1977).

The objective of the present study is to make a detailed comparison of the variations in the amount of celluloses, hemicelluloses and lignin in three hard wood species decayed by three brown-rot fungi.

MATERIALS AND METHODS

Wood samples and decay tests

Pure cultures were made from fructifications of *Trametes persoonii* Fr., *Polyporus ostreiformis* Berk. and *Trametes cubensis* (Mont.) Sacc., growing luxuriantly on the logs of *Shorea robusta*, *Ficus bengalensis* and *Dalbergia sissoo* respectively in and around Burdwan. Blocks of 2 cm × 2 cm × 1 cm (in the small dimension in the fibre direction) were cut from heartwood and sapwood of the species. Small test blocks were used rather than large blocks because of more uniform decay likely to occur throughout, particularly in early stages. The blocks were numbered, sterilized, conditioned to constant weight at 27°C and 80% relative humidity and then weighed. The blocks were subjected to decay by mycelia of the above three brown rot species of family Polyporaceae by agar-block method. The agar-block tests, used in this study, were designed not only to provide favourable conditions for decay but also to prevent contamination of the blocks by foreign nutrient material and leaching of degradation product from the blocks. The test fungi were grown in Kolle-flasks containing 2.5% nutrient agar medium. After the fungi covered the agar surface 10 blocks were exposed to the mycelium for different lengths of time (4 to 8 months) to obtain samples at different stages of decay. Following incubation the blocks were taken out, surface mycelia carefully removed, reconditioned, weighed and their weight losses were calculated. Non-inoculated blocks served as controls.

Analytical techniques

Sound and decayed wood blocks were ground to 40 mesh and the meal dried thoroughly at 45°C. The samples were analysed for lignin, carbohydrate, holocellulose and nitrogen. Losses of each due to decay were calculated.
For quantitalive estimation of lignin in wood, the method proposed by Saeman *et al.*, (1954) was mainly followed. The lignin was condensed to an insoluble residue by hydrolysis in H_2SO_4 and was then determined quantitatively.

Total carbohydrate was estimated quantitatively following the colorimetric method of Viles and Silverman (1949).

For the estimation of cellulose in wood, methods of TAPPI standard (1954) and Cowling (1961) were mainly followed. Holocelluloses was taken as residue remaining upon successive pre-extraction of wood meal with ethanol-benzene, ethanol and hot water to remove extraneous substances, followed by a succession of chlorination and mono-ethanol-amine extraction to remove lignin. The isolated holocellulose was treated with 17.5% aqueous NaOH when hemicellulose was dissolved while alphacellulose fraction remained insoluble and was separated. The beta-cellulose was precipitated on acidification of the alkaline hemicellulose solution while gamma-cellulose remained in the acidified solution solution. The percentage yield of alpha-and beta-cellulose was determined by dry weight method. The gammacellulose portion was determined by substracting the percentage of alpha-and beta-cellulose from the percentage of holocellulose in the original moisture free sample.

Total nitrogen was estimated colorimetrically following mainly the method of the Vogel (1961).

RESULTS

Analytical values of lignin, total carbohydrate, holocellulose and fractions of cellulose in the decayed heart- and sap-wood are illustrated graphically in the Figs 1 and 2 as loss expressed in precent of the original amount each. The amount of nitrogen in the decayed wood have been shown in Table 1.

Removal of cell-wall components by the three brown rotters was more or less similar. Distinct variations were, however, seen in the relative rates of removal of the components mostly from heart- and sap-wood of a wood species by the different fungi.

Typical of brown rot decay the polysaccharides were utilized by all test fungi earlier than the lignin and holocellulose depletion. The values for carbohydrate utilization during 4 months decay varied in different host-fungus combinations and with the longer treatment, its utilization increased. It reached 10 to 3.5% in heart wood and more than 13% in sap wood in each case.

The values in lignin removal increased and was between 5-9% in heart wood and 10-12% in sap wood of the host species.

The three brown-rot fungi showed different rates of degradation of holocellulose after 4 months which increased considerably further after 8 months of

decay. The values for cellulose degradation were 9-12% in heart-wood and 10.8 to 13.5% in sap-wood after 8 months of decay. The relative rate of holocellulos degradation to total carbohydrate was less after 8 months than after 4 mohths.

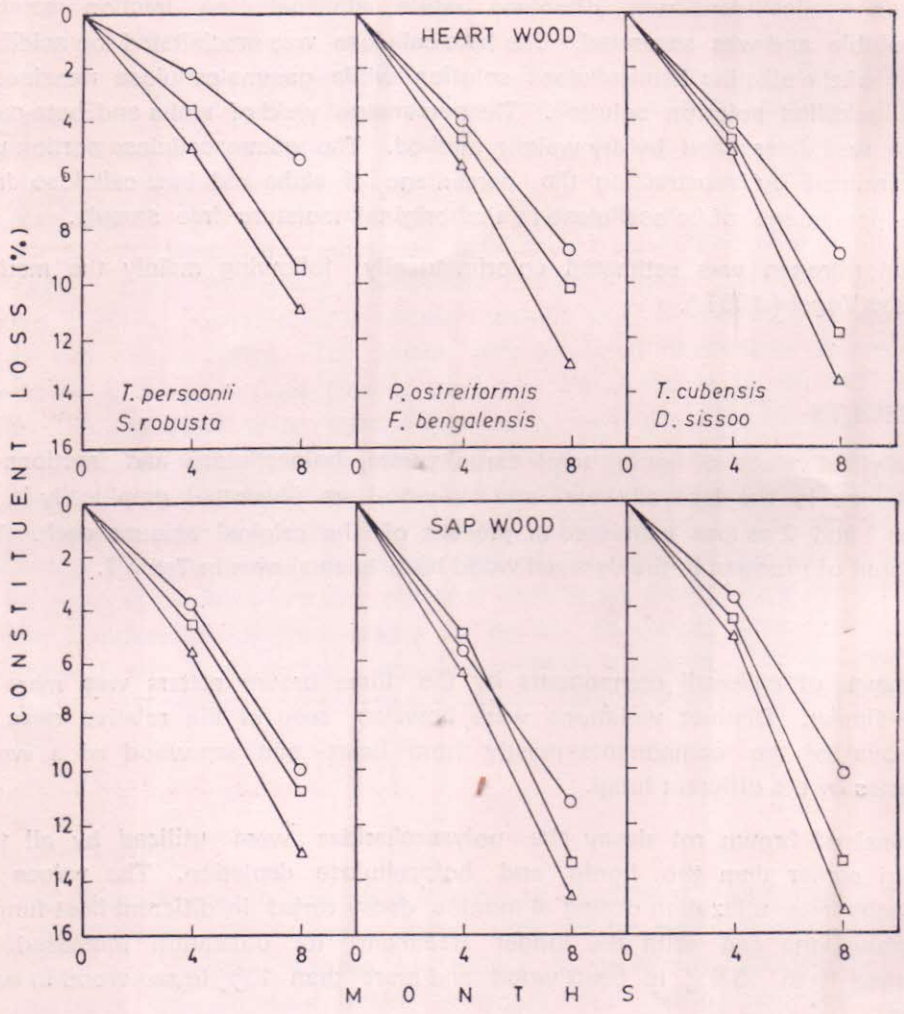

Fig. 1 : Changes in total cdrbohydrate (△), lignin (₀) and holocellulose (□) in decayed heartwood and sapwood of *S. robuta*, *F. bengalensis* and *D. sissoo* by *T. persoo iii*, *P. ostreiformis* and *T. cubensis* respectively

The fractions of holocellulose was considerably higher in sap-wood than those in heart-wood. Loss of the fractians were also higher in sap-wood than heart-

wood. Maximum loss of alpha-fraction was noted by *T. cubensis*, beta and gamma fractions by *P. ostreiformis* on their hosts.

Nitrogen content in wood increased with increase in period of decay. It increased from 0.327 to 0.634% and 0.525 to 1.25% after 4 and 8 months of decay respectively. Nitrogen content increased more in heart-wood than in sap-wood.

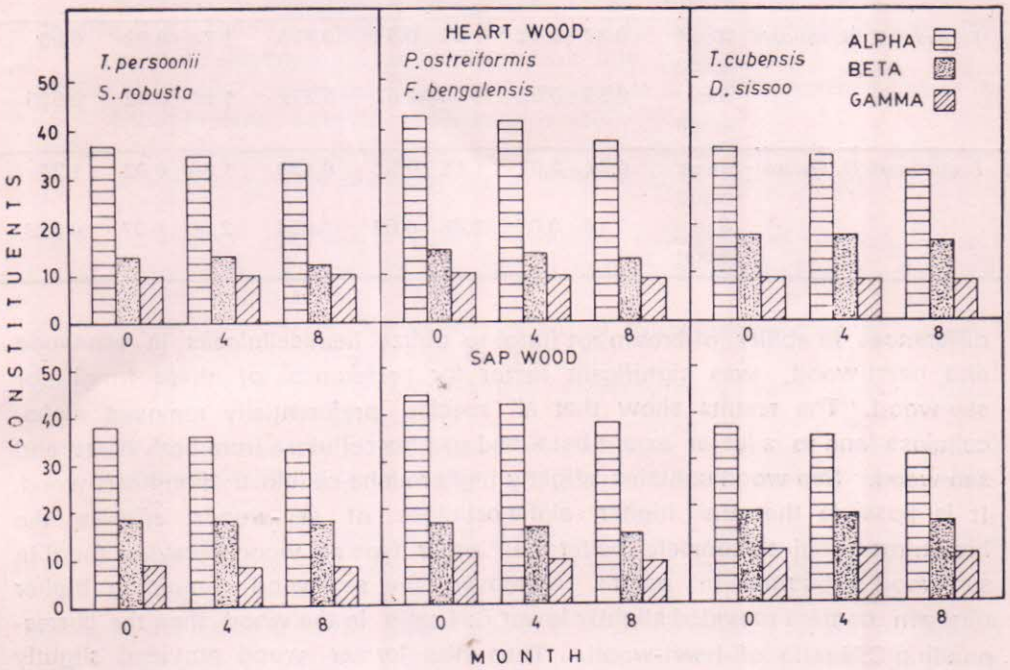

Fig. 2 : Changes in alpha, beta and gamma cellulose in sound and decayed heartwood and sapwood of *S. robusta*, *F. bengalensis* and *D. sissoo* by *T. persoonii*, *P. ostreiformis* and *T. cubensis* respectively after different periods of incubation.

DISCUSSION

In nature, brown-rot fungi are known to attack and grow preferentially on sap-wood. In the present study, the brown-rot fungi were effective in decaying heart- and sap-wood under the laboratory conditions. The test fungi showed distinct differences in utilizing the different holo-and hemicelluloses in both type of woods in their respective host-fungus combinations. This is in conformity with the earlier report of Keilich *et al.*, (1970) who suggested that the

Table 3. Changes in nitrogen content of sound and decayed wood of *F. bengalensis*, *S. robusta* and *D. sissoo* by *P. ostreiformis*, *T. persoonii* and *T. cubensis* respectively after different periods of incubation

Organisms	Hosts	Type of wood	Sound wood Mean*(%)	Decayed wood (4 M) Mean*(%)	Increase (%)	Decayed wood(8 M) Mean*(%)	Increase (%)
P. ostreiformis	*F bengalensis*	*Heart* wood	1.07±0.8	1.42±0.8	0.327	1.21±0.8	0.758
		Sap woods	1.25±0.34	1.68±0.34	0.344	1.94±0.34	0.552
T. persoonii	*S. robusta*	Heart wood	0.87±0.52	1.24±0.52	0.425	1.74±0.52	0.90
		Sap wood	0.99±0.02	1.26±0.02	0.272	1.51±0.02	0.525
T. cubensis	*D. sissoo*	Heart wood	0.82±0.02	1.18±0.02	0.439	1.86±0.02	1.26
		Sap wood	1.15±0.07	1.88±0.07	0.634	2.00±0.07	0.826

differences in ability of brown-rot fungi to utilize hemicelluloses in sap-wood and heart-wood, was significant factor for preference of these fungi for sap-wood. The results show that all species preferentially removed alpha-cellulose and to a lesser extent beta- and gamma-cellulose from both heart- and sap-wood. Sap-wood contains slightly higher alpha-cellulose than heart-wood. It is possible that the higher alpha-cellulose of sap-woods enables the brown-rot fungi to compete batter than other type of wood decaying fungi in sap-wood substrates in nature. Moreover, the sap-wood containing higher nitrogen content provided slightly lower C:N ratio in the wood than the corresponding C:N ratio of heart-wood. Thus, the former wood provided slightly more favourable condition for growth of the fungi.

Hemicellulose is known to form an encrusting envelop around the cellulose microfibrils, thus protecting them from cellulolytic attack. The hemicellulose in the present study showed slower loss after 4 months which increased considerably after 8 months. The varying degrees of loss of the cellulose component in the different host fungus combinations at the end of the experimental period could be explained from the differential removal of protective barrier of the hemicelluloses by the fungus concerned.

Along with the decay of cellulose these brown-rot fungi was capable of also decaying lignin but to a lesser extent.

Although the three brown-rot fungi were more or less similar in decaying the wood components, *Polyporus ostreiformis* was to some extent a faster decayer

than the others. Cellulase production ability was higher in this species than the other (unpuble.) and thus decayed more cellulose.

The results suggest that the hemicellulose utilization may be a critical initial step in establishment of the brown-rot fungi in woods (of. Highley, 1987).

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