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Scanning Electron Microscopic Analysis of Decayed Wood Elements of *Dalbergia sissoo* and Control of Biodeterioration of Wood

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The wood blocks of *Dalbergia sissoo* Roxb. exposed to *Pycnoporus sanguineus* for 16 weeks at 28-30°C under diffused light showed swelling of cell walls at several places, distinct bore holes in the walls, web-like hyphal network in vessels (70-80%) and ramification of hyphae in different wood elements. Besides, thinning, rupturing and depletion of lignin (42% in relation to control) in the cell walls was also observed under SEM. To study the efficacy in suppressing the wood decay, five water soluble and two water repellent preservatives were tested against three fungi, namely *P. sanguineus*, *Coriolus hirsutus*, and *Flavodon flavus* both *in vitro* and *in vivo*. ASCU-A (CCA) and creosote were found to be the most effective preservatives. Retention capacity of wood for ASCU-A, ASCU-B, $ZnCl_2$, sodium pentachlorophenate. Arsenic trioxide was 10.4 kg/m^3, 14.4 kg/m^3, 6.4 kg/m^3, 5.6 kg/m^3, 1.6 kg/m^3 respectively. The amount of retention of creosote and PS-2 (both water repellent) in *Dalbergia* wood was 64 kg/m^3 and 36 kg/m^3 respectively after 1 hr impregnation treatment.

Key Words: ***Dalbergia*, SEM, Wood preservative, *Flavodon*, *Pyenoporus*, *Coriolus***

Introduction

Dalbergia sissoo Roxb, an important timber plant, is prone to attack by a number of wood-rotting polypores, resulting in a large quantity of wood degradation annually. Resistance of sissoo wood to fungal decay was studied earlier by Bakshi et al. (1961) and Puri and Khan (1968). Subsequently, Pattanayak and Purkayastha (1990) estimated the amount of cellulose and lignin degraded by three wood-rotting fungi (viz. *Pycnoporus sanguineus*, *Flavodon flavus* and *Coriolus hirsutus*). However, no work has been reported so far regarding SEM analysis of decayed wood elements of *Dalbergia* and control of fungal decay of its wood. It appears from the literature that *P. sanguineus* caused more than 75% decay of sapwood within 4 months' time under laboratory conditions followed by *F. flavus* and *C. hirsutus* (Pattanayak & Purkayastha 1990). The paper deals with SEM analysis of sound and decayed wood elements of *D. sissoo* and control of wood biodegradation.

Materials and Methods

Preparation of Wood Sections for SEM Studies

Pycnoporus sanguineus was grown on wood blocks (5 × 2.5 × 1.25cm) in metal screw capped glass jars containing 125g of sterilized loam soil (moisture content about 60%) for 16 weeks. The remaining procedure was as described by Pattanayak and Purkayastha (1990). Longitudinal and transverse sections were prepared from sound and artificially de-

cayed wood blocks for Scanning Electron Microscopy (SEM). Best section was fixed on stud by adhesive and then placed in IB-2 ion coater (Hitachi) for gold coating. This coating is done by sputtering process after thorough evacuation. The thickness of gold coating was 300A. The studs carrying the section were placed in the observation chamber of SEM (Hitachi S-415A) and subjected to an electron energy of 15KV. Surface morphology of the section was observed under suitable magnification on the fluorescent screen of the microscope. Electron micrographs of the desired portions of the section were taken.

Evaluation of Efficacy of Wood Preservatives ***in vitro***

The method of Da Costa and Osborne (1968) was followed with modifications for evaluation of efficacy of wood preservatives. Sapwood blocks of *D. sissoo* were dipped in preservative solution for 1 hr and then allowed to dry. These blocks were dried at 50°C for two consecutive days to remove excess solvent, weighed, soaked in distilled water for 10 min and sterilized by propylene oxide. When the odour of propylene oxide completely faded away the blocks were taken out and placed on feeder strips containing polysporous mycelia of desired fungus in a moist soil jar. The rest of the procedure was as reported earlier by Pattanayak and Purkayastha (1990).

Field test: The field test is often referred to as the "Graveyard test" (Cartwright & Findlay 1946). In this case "billets" ($20 \times 2.5 \times 2.5$cm) were prepared from the sapwood of *D. sissoo*. Fifty per cent of the billets were impregnated with wood preservative for 1 hr while the control billets were treated with distilled water for a similar period. Subsequently, billets were allowed to dry for two days. Each billet was inoculated at the upper end with one feeder strip containing mycelia of the test fungus and covered with sterilized moist cotton and a piece of polythene sheet. Lower end of each inoculated billet was partially embedded (about 8cm) in the ground and examined after an interval of 32 weeks. Finally, billets were taken out, cleaned, dried at 60°C up to constant weight and the percentage of weight loss was calculated.

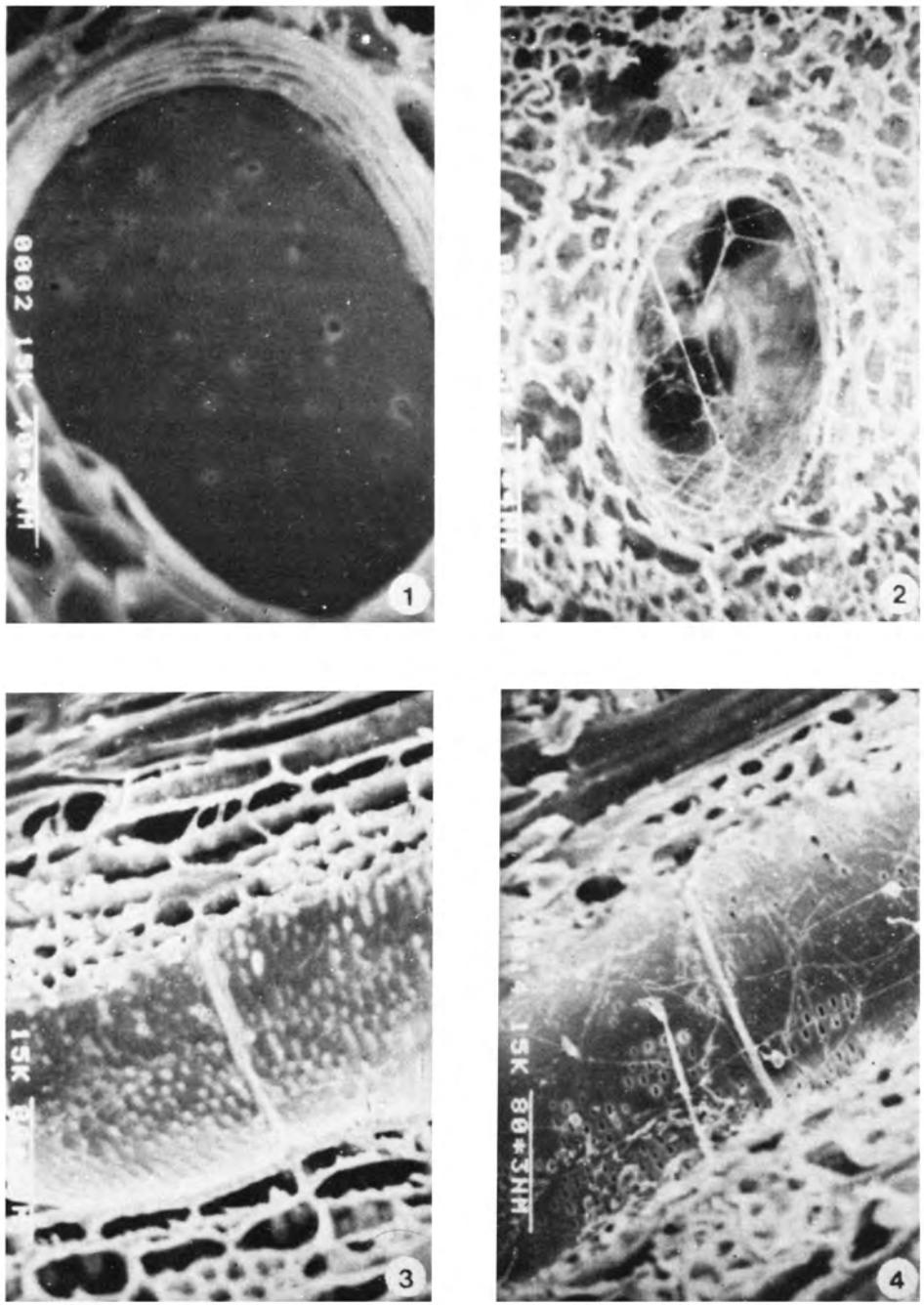
Results and Discussion

SEM Studies

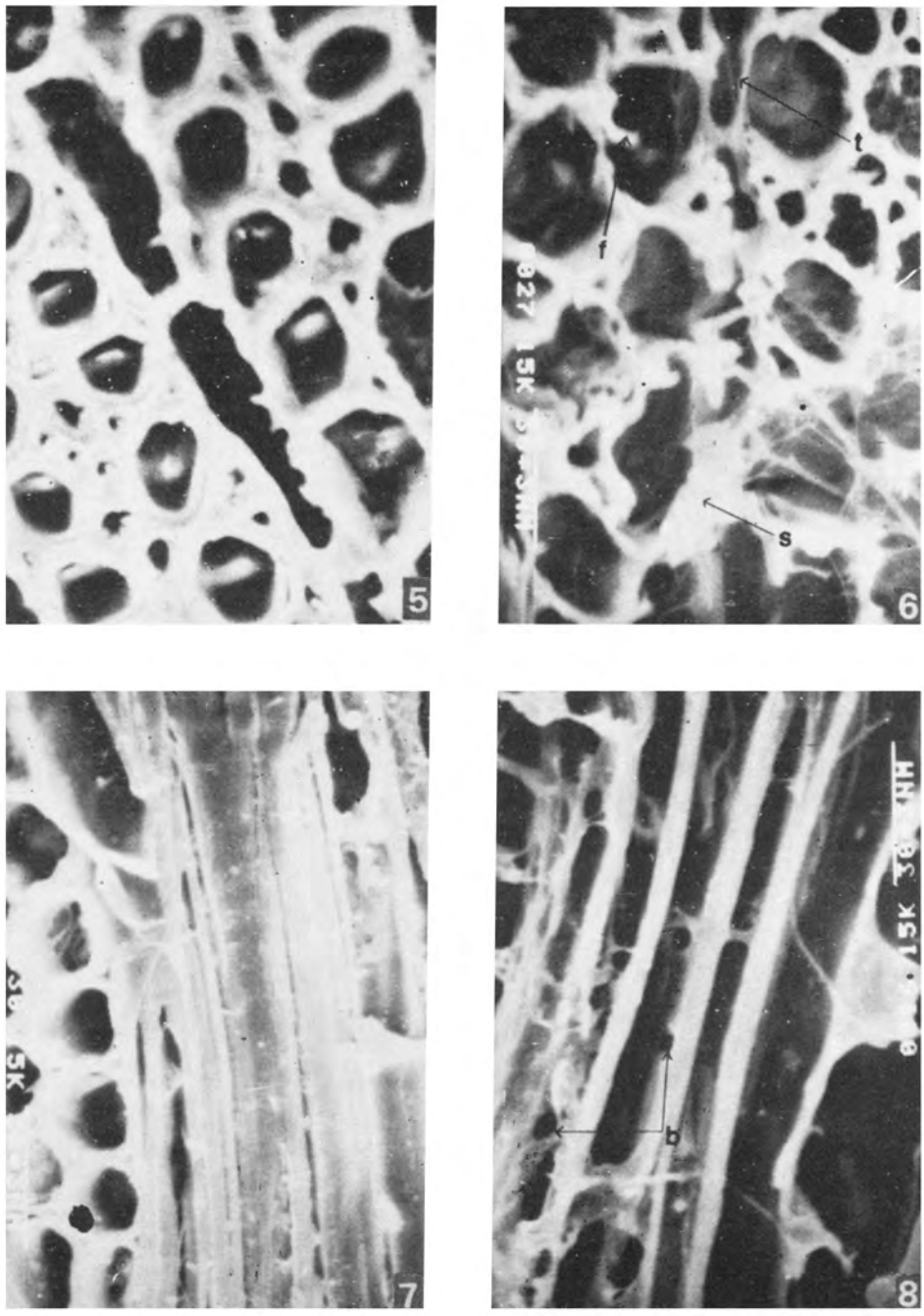
SEM analysis of sound and artificially decayed (16 weeks exposure) wood elements revealed localised thinning swelling and fragmentation of cell walls (figures 2 & 4) and depletion of lignin (42%). Distribution of hyphae in wood elements and bore holes in the walls were of common occurrence in longitudinal section (figure 4). Web-like hyphal network was prominent in the vessels (70-80%) (figure 6). No such structures were observed in sections of sound wood (figures 5 & 7). Sachs et al. (1989) examined the Aspen chips under SEM after 3 weeks' exposure to *Phanerochaete chrysosporium* (strain BKM-F-1767) and observed a strong web-like network over the exterior surfaces of chips and fungal bore holes in the wood cell. Similarly, Blanchette et al. (1988) carried out SEM studies on decayed wood blocks of *Acer saccharum*. The wood blocks were exposed to *Dichumitus squalens* for 12 weeks. They reported complete degradation of fibres and selective attack on ray parenchyma and voids among the wood elements. It thus appears that the nature of degradation of wood elements by white rot causing fungi is similar in many respects which is also evident from SEM analysis.

Effect of Wood Preservatives on Fungal Decay

Three common destructive wood-rotting polypores [*Pycnoporus sanguineus* L. ex. Fries, *Coriolus hirsutus* (Wulf ex Fr.) Que'l and *Flavodon flavus* (Kl) Ryv.] were chosen for the study. Five water soluble (sodium pentachlorophenate, zinc chloride, arsenic trioxide, ASCU-A and ASCU-B) and two water



Figures 1–4 Scanning electron micrographs of sound and decayed sapwood of **D. sissoo**; **1**, TS of sound sapwood showing thick-walled, rigid cell wall structures; **2**, TS of decayed wood showing enzymatic softening of cell walls of fibres resulting in partial collapse of wall structures. Localised thinning, swelling and fragmentation of cell walls and clear distribution of hyphae in some wood elements; **3**, LS of sound sapwood showing medullary ray cells and fibres; **4**, LS of decayed sapwood showing dissolution of medullary ray cells, thinning of cell walls in some places and clear bore holes on wall structure (*b*, bore hole; *f*, fragmentation; *s*, swelling; and *t*, thinning).



Figures 5-8 Scanning electron micrographs of sound and decayed sapwood of **D. sissoo**; **5**, TS of sound sapwood showing a thick-walled vessel (× 750); **6**, TS of decayed sapwood showing a vessel, wood parenchyma and fibres. Thinning and fragmentation of cell walls of different wood elements and web-like hyphal network prominent in vessel (× 250); **7**, LS of sound sapwood showing vessel, wood parenchyma and fibres (× 380); **8**, LS of decayed wood showing mycelial wefts in vessel, thinning of cell walls of fibres and parenchyma cells (× 380)

Table 1 *Evaluation of wood preservatives* ***in vitro***

Treatment	% loss in dry weight of sapwood block with S.E.			Overall effect due to treatment
	P. sanguineus	*C. hirsutus*	*F. flavus*	
Control	74.49±2.57	67.28±2.53	49.02±2.54	63.60
Sodium pentachlorophenate	62.01±2.81	53.28±2.83	35.83±2.05	15.33
Zinc chloride	47.75±3.34	42.10±3.31	30.01±2.75	18.32
Arsenic trioxide	29.81±2.52	25.27±2.72	16.39±2.83	50.37
ASCU-B	24.57±2.94	16.44±2.42	13.96±2.13	23.82
ASCU-A	22.92±3.00	13.60±2.51	9.47±1.93	12.46
PS-2	15.61±2.30	12.76±2.31	9.01±2.09	39.95
Creosote	8.19±1.72	4.09±0.87	2.74±0.67	5.00
Overall effect on fungus	20.80	29.35	35.67	

2% concentration in all cases except PS-2 and creosote
Temperature = 28-30°C; Incubation period = 16 weeks
3 replicates/treatment
*No weight loss was observed in case of non-inoculated non-treated blocks

CD values for comparison of the averages

CD at levels	*Fungus*	*Treatment*	*Interaction*
5%	2.46	4.02	6.96
2%	3.24	5.29	9.16

repellent preservatives, namely, PS-2 and creosote were tested.

Among the 5 water soluble wood preservatives. ASCU-A(CCA) was most effective in controlling decay caused by the three fungi studied. It contains copper, arsenic and chromium salts in definite proportion. ASCU-A (2%) gave maximum protection (>80% reduction in decay) against *F. flavus* and minimum (69%) against *P. sanguineus* although the amount of retention of ASCU-A was less than that of ASCU-B (table 1). Variable performance of wood preservatives may be explained to some extent on the basis of microdistribution theory which suggests that some preservatives are distributed poorly in the wood, both at the macroscopic and microscopic levels within cell walls (Nicholas & Preston 1984). Da Costa and Kerruish (1967) reported that *Poria monticola* was highly tolerant to copper chromate but rather sensitive to copper chrome arsenate preservatives. Creosote was much more sensitive than two water soluble wood preservatives, namely,

Table 2 *Effect of creosote on the decay resistance of billets of* ***D. sissoo*** *to 3 wood-rotting polypores*

Treatment	% loss in dry weight of billets±SE		
	P. sanguineus	*C. hirsutus*	*F. flavus*
Treated	20.86±5.25	13.97±4.35	8.37±0.78
Untreated (Control)	51.45±3.60	44.74±4.02	24.64±2.63
% reduction in relation to control	40.54	31.22	33.96

Experimental period = 32 weeks.
3 replicates/treatment

Table 3 *Analysis of variance of data presented in table* 2 (*treated, untreated and combined*)

Source of variation	DF	Sum of squares	Mean sum of squares	'F' value value	CD at levels	
					5%	1%
Fungi	2	1194.970	597.485	22.34**		
Treatment	1	3013.725	3013.725	112.63**	6.51	9.12
Fungus × Treatment interaction	2	207.477	103.739	3.89^{ns}		
Error	12	320.981	26.748	—	—	—
Total	17	4737.153	—	—	—	—

**P < 0.01
^{ns}Not significant (P > 0.05)

ASCU-A and ASCU-B. About 90% reduction in decay was recorded when wood blocks were treated with creosote for 1 hr and then exposed separately to the three test fungi for 16 weeks (table 1). The greater efficacy of creosote than that of PS-2 was probably due to greater amount of retention of creosote by the wood substrate and also greater toxicity of the same. Effectiveness of creosote was also tested in the field following impregnation method. This wood preservative effectively controlled fungal decay (30-40% reduction in decay in relation to control) of wood (billets) (tables 2 & 3). The efficacy of a number of wood preservatives was tested against three wood-rotting fungi, but ASCU-A and creosote were undoubtedly the best and hence these could be recommended for commercial use.

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