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EVALUATION OF BIFENTHRIN, FIPRONIL AND DDAC FOR PROTECTION OF RUBBER WOOD VENEER AGAINST SAP-STAIN FUNGI AND SOLID WOOD AGAINST DECAY BY *TRAMETES HIRSUTA*

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Abstract: Wood deterioration caused by sap-stain and decay fungi reduces the aesthetic, structural, and commercial value of wood products. This study evaluated the efficacy of bifenthrin (3%), fipronil (3%), and Didecyldimethylammonium chloride (DDAC, 3%) in protecting rubber wood (*Hevea brasiliensis*) veneer and solid wood. Specimens were dip-treated for 24 and 48 h, and preservative retention was determined according to IS 4873 (Part I): 2008. Sap-stain resistance was assessed under accelerated humidity-chamber conditions, while decay resistance against *Trametes hirsuta* was evaluated using the Kolle flask method. DDAC-treated veneer showed the highest preservative retention (0.84-0.93 kg m^{-3}) and the lowest fungal colonization (approximately 8-9%), providing more than 85% protection over untreated controls. Fipronil exhibited moderate-to-high resistance, restricting colonization to 24-29%, whereas bifenthrin was comparatively ineffective (approx. 74% colonization). Untreated wood recorded the greatest mean weight loss (34.75 ± 11.04%), while DDAC-treated wood showed the lowest losses (8.12-8.46%), followed by bifenthrin after 48 h of dipping (8.80%). Treatment effects were highly significant ($P < 0.001$). Retention showed a negligible relationship with weight loss ($R^2 = 0.0007$; $n = 36$), indicating that preservative chemistry largely determined antifungal performance. Overall, DDAC was the most effective preservative for rubber wood protection.

Keywords: Bifenthrin, DDAC, Fipronil, Sap-stain fungi, *Trametes hirsuta*, Wood preservation.

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INTRODUCTION

Indeed, wood is a renewable, biodegradable, and environmentally sustainable material widely used in construction, furniture, packaging, and engineered wood products because of its high strength-to-weight ratio, ease of processing, and aesthetic appeal. Despite these advantages, wood are highly susceptible to bio deterioration by fungi, insects, and other biological agents, particularly under warm and humid conditions. Among these, fungal deterioration is one of the major causes of economic losses in the timber industry because it adversely affects both the appearance and structural integrity of wood products (Rowell, 2013; Reinprecht, 2016). Sap-stain fungi primarily colonize freshly sawn timber and veneer, causing dark pigmentation that lowers the commercial value of wood without significantly affecting its mechanical properties. In contrast, wood-decaying fungi, particularly white-rot fungi such as *Trametes hirsuta*, degrade cellulose, hemicellulose, and lignin, resulting in substantial reductions in wood strength and service life (Schmidt, 2006).

Application of chemical preservatives remains the most effective strategy for protecting wood against biological deterioration. Modern wood preservatives are expected to provide broad-spectrum protection while exhibiting an improved environmental compatibility compared with conventional heavy metal-based formulations. Among the commercially available preservatives, bifenthrin, fipronil, and didecyldimethylammonium chloride (DDAC) have gained considerable attention because of their effectiveness against wood-destroying organisms and their compatibility with industrial wood treatment processes (Reinprecht, 2016). Bifenthrin and fipronil are primarily used for termite and wood-boring insect management, whereas DDAC, a quaternary ammonium compound, possesses broad-spectrum antimicrobial and antifungal activity and is widely incorporated into modern wood preservative formulations (Boyce, 2023).

The effectiveness of a preservative depends not only on the amount retained within the wood but also on its penetration, fixation, and anatomical structure of the wood, the treatability, and the penetration index (Tarmian *et al*. 2020). Higher preservative retention does not necessarily translate into improved resistance if the active ingredient lacks sufficient antifungal efficacy or is poorly distributed within the wood matrix (Ganguly *et al.,* 2026). Therefore, simultaneous evaluation of preservative retention and biological performance is essential for selecting suitable preservative systems for rubber wood protection.

A number of studies have examined the efficacy of individual wood preservatives, comparative information on bifenthrin (Kelkar and Shukla, 2023), fipronil (Przewloka *et al,* 2007), and DDAC (Kartal *et al.,* 2005) against both sap-stain fungi and wood-decaying fungi. However, studies evaluating these preservatives against wood-destroying fungi under Indian conditions remain limited. The present study was therefore undertaken to evaluate the retention characteristics and biological efficacy of these three preservatives in rubber wood veneer and solid wood. The preservatives were assessed for their ability to suppress sap-stain fungal colonization under accelerated humidity chamber conditions and to improve resistance against the white-rot fungus *T. hirsuta* using the Kolle flask method. In addition, the relationship between preservative retention and fungal resistance was examined to determine whether preservative uptake alone is a reliable indicator of biological performance.

MATERIALS AND METHODS

Study material

The investigation was conducted at the Forest Protection Division, ICFRE-Institute of Wood Science and Technology (IWST), Bengaluru, India. Healthy, defect-free rubber wood (*Hevea brasiliensis* Muell. Arg.) veneer and solid wood specimens were used for evaluating the efficacy of selected wood preservative chemicals against sap-stain fungi and the white-rot fungus *T. hirsuta*. Veneer specimens measuring 15 × 15 × 1.2 cm were prepared for sap-stain studies, whereas solid wood blocks measuring 2.5 × 5 × 5 cm were prepared for decay resistance studies. Prior to preservative treatment, all specimens were conditioned under laboratory conditions to attain uniform moisture content, labelled individually, and weighed using an analytical balance.

Preservative treatments

Three wood preservatives, namely bifenthrin (3%), fipronil (3%), and DDAC (3%), were evaluated. Untreated specimens served as the control. Veneer and solid wood specimens were immersed separately in each preservative solution for 24 h and 48 h under ambient laboratory conditions. After treatment, excess preservative was allowed to drain, and the specimens were air-dried for 24 h before further experimentation. The treatments employed in the investigation are presented in Table 1. The experiment was conducted in a Completely Randomized Design (CRD) with six replications for each treatment.

Table 1: Experimental treatments evaluated in the study.

Treatment	Preservative	Dipping duration
T_1	Untreated control	—
T_2	Bifenthrin (3%)	24 h
T_3	Bifenthrin (3%)	48 h
T_4	Fipronil (3%)	24 h
T_5	Fipronil (3%)	48 h
T_6	DDAC (3%)	24 h
T_7	DDAC (3%)	48 h

Determination of preservative retention

Preservative retention was determined according to IS 4873 (Part I):2008. Individual specimens were weighed before treatment (W_1) and immediately after dipping and draining (W_2). Actual specimen dimensions were measured using a digital Vernier caliper to calculate specimen volume. Retention values were expressed as kilograms of preservative retained per cubic metre of wood (kg m^{-3}). For each treatment, six specimens were evaluated, and the mean preservative retention was calculated separately

for veneer and solid wood. Preservative retention (R) was calculated according to IS 4873 (Part I):2008 using the equation:

$$\text{Retention } R = \frac{G \times C \times 10}{V}$$

Where R is preservative retention (kg m^{-3}), G is the amount of preservative solution absorbed by the specimen (g), calculated as W2−W1, C is the concentration of the preservative solution (%), and V is the volume of the specimen (cm^3). Retention values were initially calculated in g cm^{-3} and subsequently converted to kg m^{-3} for reporting.

Evaluation against sap-stain fungi

The efficacy of the preservatives against sap-stain fungi was evaluated using a mold fungal inoculation chamber developed from a glass aquarium. Sterile distilled water inoculated with sap-stain fungal cultures was maintained at the bottom of the chamber to maintain relative humidity above 75%, while the temperature was maintained at 27 ± 2°C. An aluminium rack positioned above the water surface supported the test specimens and prevented direct contact with water. Naturally infected rubber wood blocks bearing sapstain fungi were placed within the chamber to serve as a continuous inoculum source. Preservative-treated and untreated veneer specimens were arranged randomly on the rack and incubated for 45 days. At the end of the incubation period, fungal colonization was assessed visually as the percentage of surface area colonized. Disease reactions were classified according to the rating scale shown in Table 2.

Table 2: Disease rating scale used for sap-stain fungal colonization.

Disease grade	Surface area colonized (%)	Reaction
0	0	Highly resistant
1	1-25	Resistant
2	26-50	Moderately resistant
3	51-75	Moderately susceptible
4	76-100	Susceptible

Percentage protection over the untreated control was calculated as:

$$\text{Percentage Protection (\%)} = [(C - T)/C] \times 100$$

Where,

C = Mean fungal colonization observed in untreated control specimens.

T = Mean fungal colonization observed in preservative-treated specimens.

Evaluation against Trametes hirsuta

Resistance against the white-rot fungus *T.hirsuta* was determined using the Kolle flask method following IS 4873 (Part I):2008.Pure cultures of *T. hirsute* were maintained on potato dextrose agar (PDA). The medium was sterilized by autoclaving at 121°C and 15 psi for 20 min, dispensed into sterile Kolle flasks, and inoculated aseptically. After seven days of incubation at 27 ± 2°C, actively growing fungal cultures completely covered the medium surface. Prior to fungal exposure, all treated and untreated wood blocks were oven dried at 103 ± 2°C to constant weight and their initial oven-dry weights were recorded. Individual blocks were then placed on the fungal mat in separate Kolle flasks and incubated for 45 days under controlled laboratory conditions. Following incubation, fungal mycelia were carefully removed from the specimens, which were again oven dried to constant weight. Percentage weight loss was calculated as:

$$\text{Percentage Weight Loss (\%)} = [(W_1 - W_2)/W_1] \times 100$$

Where,

- W_1 = Initial oven-dry weight of the wood specimen before fungal exposure (g)
- W_2 = Final oven-dry weight of the wood specimen after fungal exposure (g)

Lower weight loss indicated greater preservative efficacy.

Statistical analysis

Data were analyzed using Microsoft Excel. Mean ± SD were calculated for each treatment. One-way analysis of variance (ANOVA) was used to assess differences among treatments.Pearson correlation analysis and simple linear regression were performed using individual solid-wood preservative retention values paired with corresponding absolute fungal weight-loss values from six replications of each preservative × dipping-duration treatment (n = 36). For correlation analysis, absolute weight loss (g) was used as the response variable, whereas percentage weight loss (%) was used for comparative assessment of fungal decay among treatments. Statistical significance was evaluated at $P < 0.05$.

RESULTS AND DISCUSSION

1. Preservative retention in rubber wood veneer and solid wood

The preservative retention varied among the three formulations and between veneer and solid wood specimens (Table 3). When the dipping duration was increased from 24 to 48 h, preservative retention in

bifenthrin- and fipronil-treated specimens increased, whereas DDAC showed consistently high veneer retention but some variation in solid-wood retention between dipping durations. Among the veneer specimens, DDAC recorded the highest retention (0.84-0.93 kg m^{-3}), followed by fipronil (0.53-0.57 kg m^{-3}) and bifenthrin (0.48-0.53 kg m^{-3}). In solid wood, the highest retention was observed in fipronil-treated specimens after 48 h (6.82 kg m^{-3}), followed by bifenthrin (6.76 kg m^{-3}) and DDAC (6.66 kg m^{-3}).

The differences in preservative retention can be attributed to variations in preservative chemistry, wood permeability, and diffusion characteristics. The differences in retention between veneer and solid wood may be related to differences in specimen geometry, exposed surface area, wood structure, and preservative chemistry and treatment conditions. However, because penetration depth was not directly measured, differences in penetration rate cannot be inferred from the present retention data alone. Although fipronil exhibited the highest retention in solid wood, it did not provide the greatest biological protection, indicating that preservative loading alone is insufficient to explain treatment efficacy (Przewloka *et al.*, 2007; Tarmian *et al.* 2020). In copper-based wood preservatives also similar observations have been reported by Freeman and McIntyre (2008) and Reinprecht (2016), who emphasized that preservative fixation, distribution within the wood matrix, and intrinsic antifungal toxicity are more important determinants of wood durability than chemical loading alone.

The comparatively high retention of DDAC may be related to its physicochemical properties, interaction with the wood matrix, and treatment conditions and broad-spectrum antimicrobial activity (Freeman and McIntyre, 2008). In contrast, the lower retention observed in bifenthrin- and fipronil-treated specimens may reflect differences in formulation, solubility, and affinity for the wood matrix. Bifenthrin is relatively hydrophobic, whereas fipronil uptake can be influenced by its formulation, solvent system, and treatment conditions (Kartal *et al.* 2006; Tomlin, 2009). Rubber wood is relatively permeable to preservatives because of its vessel structure and comparatively low extractive content, which favours preservative uptake (Sreeja and Edwin, 2013). However, the present results indicate that permeability alone did not determine biological performance. Rather, preservative chemistry, distribution within the wood matrix, and compatibility with the substrate appear to have played a greater role in determining retention and antifungal efficacy.

Table 3: Mean preservative retention values (kg m^{-3}) of preservative-treated rubber wood veneer and solid wood specimens subjected to different treatment durations.

Preservative	Treatment duration	Veneer retention (kg m^{-3})	Solid wood retention (kg m^{-3})
Bifenthrin (3%)	24 h	0.48	5.19
Bifenthrin (3%)	48 h	0.53	6.76
Fipronil (3%)	24 h	0.53	5.48
Fipronil (3%)	48 h	0.57	6.82
DDAC (3%)	24 h	0.84	6.66
DDAC (3%)	48 h	0.93	5.89

2. Resistance against sap-stain fungi

Marked differences were observed among preservative treatments in suppressing sap-stain fungal colonization after 45 days of incubation (Table 4). Untreated control specimens exhibited approximately 80% surface colonization and were classified as susceptible. DDAC was the most effective treatment, restricting fungal colonization to approximately 8-9% after both dipping durations and producing resistant reactions. Fipronil provided moderate protection, reducing colonization to approximately 24-29%, whereas bifenthrin was comparatively ineffective, with nearly 74% of the veneer surface colonized irrespective of dipping duration. The strong performance of DDAC is consistent with reports describing quaternary ammonium compounds as effective antisap-stain and mould-control agents (Boyce, 2023). Conversely, the limited efficacy of bifenthrin against fungal colonization agrees with observations that this pyrethroid provides little improvement in fungal decay resistance when used alone (Kelkar and Shukla, 2023).

Untreated and bifenthrin-treated specimens showed extensive dark fungal discoloration, whereas DDAC-treated veneers remained almost free of visible fungal growth throughout the incubation period. Fipronil-treated specimens exhibited intermediate levels of

fungal development. The superior performance of DDAC can be attributed to its quaternary ammonium chemistry, which exerts broad-spectrum antimicrobial activity by disrupting fungal cell membranes and interfering with spore germination and mycelial development (Hwang *et al.,* 2007; Reinprecht, 2016). Kartal *et al*. (2006) also reported comparable protection of hardwoods against mould and sap-stain fungi using DDAC-based formulations.

The comparatively lower antifungal efficacy of fipronil and bifenthrin may be related to their primary use as insecticides rather than fungicides. Fipronilis principally used for the management of termites and other wood-damaging insects, and its moderate reduction in fungal colonization in the present study may therefore reflect limited or indirect antifungal activity rather than a primary fungicidal effect (Simon-Delso *et al.,* 2015). Bifenthrin, a pyrethroid insecticide, showed the poorest suppression of sap-stain fungi, with colonization remaining comparable to the untreated control. Thus, its usefulness in wood protection is likely to be greater when combined with an effective fungicidal component rather than when applied alone. Overall, the sap-stain results clearly demonstrate that insecticidal activity does not necessarily translate into antifungal protection and that DDAC was substantially more effective than fipronil and bifenthrin under the present experimental conditions.

Table 4: Effect of preservative treatments on sap-stain fungal colonization of rubber wood veneer under accelerated fungal exposure conditions after 45 days.

Preservative type	Dipping duration	Mean % area colonized	Reaction
Bifenthrin (3%)	24 h	~74	Moderately susceptible
Bifenthrin (3%)	48 h	~74	Moderately susceptible
Fipronil (3%)	24 h	~29	Moderately resistant
Fipronil (3%)	48 h	~24	Resistant
DDAC (3%)	24 h	~9	Resistant
DDAC (3%)	48 h	~8	Resistant
Control	–	~80	Susceptible

3. Resistance against *Trametes hirsuta*

Significant differences in fungal decay resistance were observed among preservative treatments (Table 5). Untreated control specimens recorded the highest mean weight loss (34.75 ± 11.04%), confirming severe degradation by *T. hirsuta*. Preservative treatment significantly affected fungal weight loss ($P < 0.001$), with all treated groups showing numerically lower weight loss than the untreated control. DDAC provided the greatest protection. Mean weight losses of DDAC-treated specimens were only 8.46 ± 2.44% and 8.12 ± 1.79% after 24 and 48 h dipping, respectively. The bifenthrin showed improved performance following prolonged treatment, reducing weight loss from 16.67 ± 7.47% to 8.80 ± 3.47%, whereas fipronil recorded comparatively higher weight losses (19.31–21.15%) (Fig.1), despite exhibiting relatively high preservative retention. One-way ANOVA confirmed a highly significant effect of preservative treatment on fungal decay ($P < 0.001$).

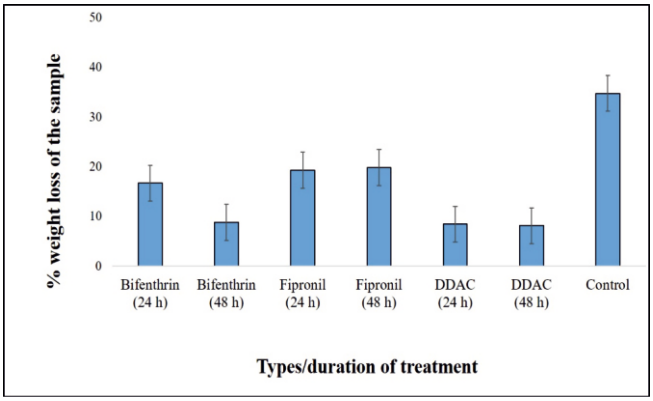

Fig. 1: Influence of preservative type and dipping duration on fungal weight loss.

The lower weight loss recorded in DDAC-treated specimens therefore indicates reduced fungal decay and associated wood mass loss. White-rot fungi cause wood deterioration through extracellular oxidative and hydrolytic enzymes that degrade lignin, cellulose, and hemicellulose (Schmidt, 2006). Terzi *et al*. (2011) and Reinprecht (2016) have reported similar reductions in fungal deterioration following DDAC treatment.

Exposure to *T. hirsuta* further demonstrated differences in treatment performance. DDAC-treated specimens recorded the lowest weight loss at both dipping durations, while untreated specimens suffered the greatest deterioration. Bifenthrin showed

Table 5: Effect of preservative treatments on fungal decay of wood specimens.

Treatment	Weight loss (%; Mean ± SD)	Reduction over control (%)
Bifenthrin (24 h)	16.67 ± 7.47	52.0
Bifenthrin (48 h)	8.80 ± 3.47	74.7
Fipronil (24 h)	19.31 ± 8.44	44.4
Fipronil (48 h)	21.15 ± 11.93	39.1
DDAC (24 h)	8.46 ± 2.44	75.7
DDAC (48 h)	8.12 ± 1.79	76.6
Control	34.75 ± 11.04	

Table 6: One-way ANOVA for percentage weight loss of wood specimens subjected to different preservative treatments.

Source of variation	Sum of Squares (SS)	df	Mean Square (MS)	F	P-value
Between treatments	2788.79	6	464.80	9.44	<0.001
Within treatments (Error)	1723.49	35	49.24		
Total	**4512.28**	**41**			

improved protection with prolonged dipping, whereas fipronil remained comparatively less effective despite its relatively high retention. This contrast indicates that preservative retention alone is not a reliable indicator of fungal protection, and that biological efficacy depends on factors such as preservative chemistry and activity against the target organism. Thus, under the present laboratory conditions, DDAC provided the most consistent protection against fungal deterioration, while the performance of bifenthrin was influenced by treatment duration and fipronil was comparatively limited. Preservative selection should therefore be based on demonstrated efficacy against the intended biological hazard rather than chemical loading alone (Khademibami and Bobadilha, 2022).

4. Relationship between preservative retention and fungal resistance

Comparison of preservative retention with fungal weight loss demonstrated (Fig. 2.) that higher retention did not necessarily correspond to greater decay resistance. Although fipronil-treated solid wood exhibited the highest retention values, these specimens sustained comparatively higher fungal degradation. In contrast, DDAC provided the lowest weight losses despite retention values comparable to those of the other preservatives.Pearson correlation indicated a negligible relationship between preservative retention and absolute fungal weight loss ($r = 0.026$, $P > 0.05$), while simple linear regression gave $R^2 = 0.0007$ ($n = 36$).

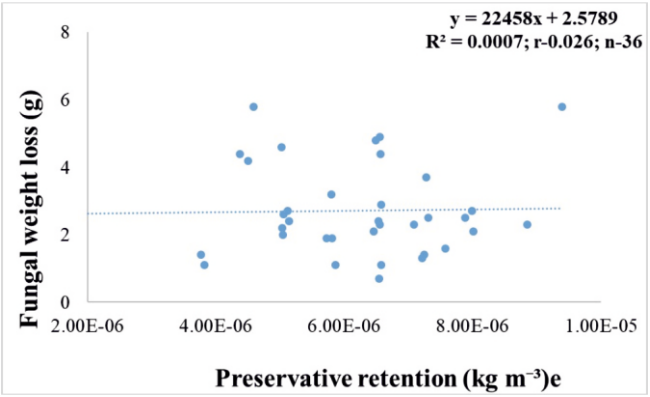

Fig. 2: Relationship between preservative retention and absolute fungal weight loss of treated rubber wood specimens. The regression was based on individual observations from six replications of six preservative × dipping-duration treatments (n = 36).

The weak relationship between preservative retention and fungal resistance suggests that factors beyond preservative loading, including preservative chemistry and biological activity, may contribute substantially to fungal resistance. Similar conclusions have been drawn by Freeman and McIntyre (2008), who reported that copper-based wood preservatives distribution and fixation largely determine long-term biological performance, and by Reinprecht (2016), who emphasized that optimization of preservative formulations, should focus on biological efficacy rather than maximizing chemical retention. The regression analysis indicates that preservative retention alone was not a reliable predictor of fungal

weight loss, suggesting that preservative chemistry and intrinsic antifungal activity also contributed substantially to biological performance.

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