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Study on Fungi Inhabiting Indoor Woods and their Eco-Friendly Management

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Abstract. Biodeterioration of indoor wood and microbial pollution inside buildings is one of important problem in humid areas. Number of fungi are associated with indoor wood and many of them are responsible for its deterioration by causing decay and stain. Some of them may release mycotoxins, which have hazardous impact on human health. In present study, fifteen fungi associated with wood surface were isolated and out of which genus *Aspergillus* dominate with four species. *A. flavus* was recorded from all locations. The potential of leaf extracts of nine tree species on the growth of *A. flavus* was evaluated by amending culture media. Considerable growth inhibition of *A. flavus* in the range of 1.14-45.45% was recorded on solid media and 9.37-86.66% in liquid media. Amendment of culture media @ 30% concentration of the leaf extract of *Corymbia torelliana* have recorded maximum growth inhibition irrespective of the media used.

Introduction

Wood is important forest product being used from time immemorial in house construction as well for furniture and pilings. Under prolonged exposed environmental conditions, it also deteriorates like other organic matter, due to variety of biotic and abiotic causes. The major biotic agents responsible for wood deterioration in buildings are insects, bacteria and fungi. In this paper, the fungi associated with indoor wood were identified and their eco-friendly management has been discussed. The timber available in market is generally pretreated with fungicides to avoid any kind of stain, mold, and decay fungi. The fungal propagules can remain dormant in the timber for years until they get the favourable conditions like oxygen, moisture and nutrients. About 30% moisture is considered as critical requirement for colonisation by wood fungi [1]. The spore germination and hyphal growth of wood decay fungi do not occur if the moisture content of the wood is much below fiber saturation. The optimum temperature for growth of most wood-decay fungi is between 21°-32°C [2]. The decay fungi cause distinct morphological and chemical changes in the infected wood, which may result in significant loss in the strength of the wood, if the conditions persist for longer period. The fungi secrete variety of extracellular enzymes that break down the wood into usable food. The major constituents of wood are cellulose, hemicelluloses and lignin. The cellulose forms the framework, hemicelluloses form the matrix and lignin is glue-like substance which holds and binds the cells together. On the basis of physical and chemical changes produced in decaying wood, the decay fungi are classified as brown rots, white rots, and soft rots. Brown-rot and white-rot fungi mostly belong to Basidiomycetes [3]. While most of soft-rot fungi belongs to Ascomycetes and Deuteromycetes and can be distinguished from other decay fungi by their decay patterns [4,5]. White rot fungi breaks down all major wood components (cellulose, hemicelluloses and lignin) and the rotted wood appear soft, spongy and white bleached. The strength of the wood infected with white rot fungi decreases gradually and the wood generally does not crack but shrink and collapse in case of severe infection. The brown rot fungi primarily decay the cellulose and hemicelluloses in wood, leaving a brown residue of lignin. Wood affected by brown rot, usually appears dry and

fragile, which gradually crack into cubes. Soft rot fungi degrade only the cellulose and hemicelluloses of the wood especially having high water and nitrogen content. The degradation is generally slower as compare to white and brown rots. They are mostly affecting the window frames, wet floor boards and fence posts, etc.

Besides the wood decay fungi, numerous molds and stain fungi grow on the surface of wood. They have little influence on the strength of wood, but their presence is a good indicator of high moisture level in the wood to support the growth of decay fungi. Molds can amplify the capacity of wood to absorb moisture and make it susceptible for wood decay fungi. Under favorable conditions the mold can even grow on inorganic materials like glass, metal, concrete or painted surface by getting organic nutrient from dust and soil particles [6]. Mold and stain fungi primarily colonize the sapwood and differ from each other. The mold fungi have colorless hyphae, while stain fungi have pigmented hyphae and are responsible staining of affected sapwood [2]. Mold and stain fungi derive their food from the materials stored in cell cavities or from nutrients available on the wood surface. Mold and mold spores are omnipresent and are always part of our environment. Some molds are known as 'toxic-molds' because under certain conditions, they can produce mycotoxins. Mycotoxins are the compounds produced by fungi which are toxic to humans or animals [7]. Mycotoxins are secondary metabolites. This means, the mold does not need to produce mycotoxins to grow or survive [6]. The most common fungal genera associated with mold infection are *Aspergillus*, *Cladosporium* and *Penicillium* [8]. Pure microbial toxins, such as the products of *Fusarium* (fumonisin B1, deoxynivalenol), *Stachybotrys* (satratoxin G), *Aspergillus* (ochratoxin A) and *Penicillium* (ochratoxin A, verrucosidin), have been reported as neurotoxic *in vitro* and *in vivo* trials [9-13].

It is very important to put a control on indoor molds because of their negative impact on human health. Application of chemicals for the management of indoor molds should be discouraged due to their toxic nature, high cost and they are also not eco-friendly solutions. Nature has provided complete package of remedies to cure all sort of ailments of mankind. Their easy availability, low cost, negligible side effects have made natural products popular worldwide. Several plants are available in nature which have potential to restrict the growth of wide spectra of organisms. Antimycotic activity of plant extract to restrict the growth of phytopathogenic fungi has been reported by number of researchers [14-16]. Plants contain variety of phytochemicals like phenols, tannins, flavanoids, carotenoids, vitamins, carbohydrates, glycosides, proteins and terpenoids [17-19]. These compounds may be responsible for the antimicrobial potential of plant species. In present study, the fungi associated with wood surface in damp condition were isolated and crude plant extracts were evaluated against dominant fungal species under laboratory conditions.

Materials and Methods

Sample collection: The wood located in humid parts of building were wiped with autoclaved cotton and filter papers for the isolation of associated surface mycobiota. Leaves of nine tree species were collected from the campus of Forest Research Institute, Dehradun and their crude extracts were evaluated for antimycotic activity against dominant fungal species.

Isolation and identification of mycobiota associated with wood surface: The cotton and filter paper containing surface molds were washed with sterilized distilled water and diluted samples were inoculated on potato dextrose agar (PDA) medium on Petri plates under aseptic conditions. The media was supplemented with antibiotic to avoid bacterial growth. The Petri plates were incubated at 25 ±2°C and observed for the fungal growth. Individual colonies were sub cultured on PDA plates and pure cultures were obtained by subsequent sub culturing. Pure cultures were maintained on PDA for further experiments. Temporary mounts were made in lactophenol cotton-blue and observed under high magnification (10x45 lenses). The fungal species were identified by following standard taxonomical manuals and utilizing expertise of Forest Pathology Division, FRI Dehradun.

Extract preparation of plant material (botanical): Fresh leaves of nine healthy plants (*Azadiracta indica, Chukrasia tabularis, Corymbia citriodora, Corymbia torelliana, Dalbergia sissoo, Melia azedarach, Populus deltoides, Santalum album* and *Tectona grandis*) were collected and washed thoroughly with tap water and air dried. One hundred grams of leaf sample was ground using pestle and mortar by adding equal amount (100 ml) of sterilized distilled water (1: 1, w/v). The pulverized mass was squeezed through the cheese cloth and the extracts were centrifuged at 10000 rpm for 5-10 minutes. The supernatant was filtered through Millipore filters (45µm) using vacuum pump assembly under aseptic conditions. Freshly collected filtrate was utilized to amend the culture media.

Antifungal Activity Assay

Screening of botanicals against *A. flavus* on solid media: Poisoned food technique [20] was followed to evaluate the effect of crude extracts of selected tree species on the growth *A. flavus.* PDA was amended with crude filtrate to get desired concentration (10, 20 and 30%) in media and the non-amended PDA served as control. The plates were inoculated with *A. flavus* and radial growth was measured after five days of incubation and compared.

Screening of botanicals against *A. flavus* in liquid media: Potato dextrose broth (PDB) was poured in different flasks and amended with crude extract to get required concentration (10, 20 and 30%) and the media not amended with leaf extract served as control. The flasks were inoculated with 5 mm discs of *A. flavus* and after five days of incubation, broth was filtered through Whatman-I filter paper and mycelia were oven dried to get constant weight and compared.

The percent inhibition of mycelial growth was calculated using the formula:

$$I = (C-T)/C \times 100$$

where, I = Per cent growth inhibition, C = Colony diameter (dry mycelial weight) in control, T = Colony diameter (dry mycelial weight) in treatment.

Results

Diversity of mycobiota associated with wood surface: Fifteen fungal species were isolated and identified from the samples collected from three different locations of building (Table-1). Maximum nine fungal species were recorded from location-I, followed by seven species from location -II and minimum six species from location -III. *Aspergillus flavus* was recorded from all locations, whereas *Alternaria triticina, Aspergillus fumigatus, Curvularia* species were recovered from two locations (I & II), while *Verticillum* was present in location II and III. The species of *Absidia, Cladosporium, Macrophoma, Mucor, Periconia* were found in location I only, while *Gliocladium, Aspergillus* species were recorded in location II and *Aspergillus niger, Penicillium, Trichoderma* species were collected from location III only. Among the fifteen identified fungi, *A. flavus* recorded in all sites with maximum frequency of occurrence. *Aspergillus* species are well known cellulase producing fungi and also reported to cause diseases in human beings also. Therefore, *A. flavus* was selected as representative species to conduct *in vitro* trials for management of wood associated fungi by applying botanicals.

Growth inhibitions of *A. flavus* by plant extracts on solid media: PDA was amended with crude extract of selected tree species to get the desired concentration of 10, 20 and 30 per cent. The results revealed 1.14-45.45% growth inhibition of *A. flavus* in amended media as compared to control (Table-2). The rate of growth inhibition invariably increased with increase in the concentration of plant extract in the media. When PDA was amended with 30% crude extracts of plant material, maximum radial growth inhibition of *A. flavus* was recorded by *Corymbia torelliana* (45.45%), followed by *Melia azedarach* (42.56%), *Corymbia citriodora* (40.96%) and minimum 4.94% by *Santalum album*. The growth inhibition by rest of species was in between 23.57-35.81%.

Growth inhibitions of *A. flavus* by plant extracts in liquid media: Irrespective of the plant species used, the growth inhibition of *A. flavus* was increased with increase in the concentration of

crude extract in PDB (Table-3). The growth inhibition was recorded in the range of 9.37-86.66%. When the PDB was amended @ 30% extract of plant material, maximum inhibition was recorded by *Corymbia torelliana* (86.66%) followed by *Populus deltoides* (76.66%), *Corymbia citriodora* (73.33%) and minimum (28.12 %) by the extract of *Chukrasia tabularis*. The leaf extract of rest of species exerted the growth inhibition between 23.25-63.33%.

Table 1. Fungal diversity associated with wood surface in three different location of the building

S. No	Fungi	Location-I	Location-II	Location-III
1.	*Absidia*	+	-	-
2.	*Alternaria triticina*	+	+	-
3.	*Aspergillus flavus*	+	+	+
4.	*Aspergillus fumigates*	+	+	-
5.	*Aspergillus niger*	-	-	+
6.	*Aspergillus sp.*	-	+	-
7.	*Cladosporium*	+	-	+
8.	*Curvularia*	+	+	-
9.	*Gliocladium*	-	+	-
10.	*Macrophoma*	+	-	-
11.	*Mucor*	+	-	-
12.	*Penicillium*	-	-	+
13.	*Periconia*	+	-	-
14.	*Trichoderma*	-	-	+
15.	*Verticillum*	-	+	+

(+ sign indicate the presence of particular fungi)

Table 2. Growth inhibitions of *A. flavus* on PDA amended with different concentrations of crude leaf extracts

S. No.	Plant material	Growth inhibition (%) by crude leaf extract at different concentrations		
		10 %	20 %	30 %
1.	*Azadiracta indica*	18.91	22.29	30.40
2.	*Chukrasia tabularis*	17.87	23.57	30.41
3.	*Corymbia citriodora*	30.32	35.16	40.96
4.	*Corymbia torelliana*	13.63	22.72	45.45
5.	*Dalbergia sissoo*	8.74	12.54	23.57
6.	*Melia azedarach*	30.40	31.41	42.56
7.	*Populus deltoides*	12.90	22.58	32.25
8.	*Santalum album*	1.14	1.14	4.94
9.	*Tectona grandis*	23.64	27.36	35.81

Table 3. Growth inhibitions of *A. flavus* on PDB amended with different concentrations of crude leaf extracts

S. No.	Source of Botanical	Growth inhibition (%) by crude leaf extract at different concentrations		
		10 %	**20 %**	**30 %**
1.	*Azadiracta indica*	12.50	25.00	31.25
2.	*Chukrasia tabularis*	21.87	25.00	28.12
3.	*Corymbia citriodora*	60.00	63.33	73.33
4.	*Corymbia torelliana*	80.00	83.33	86.66
5.	*Dalbergia sissoo*	26.66	43.33	63.33
6.	*Melia azedarach*	9.37	15.62	40.62
7.	*Populus deltoides*	40.00	60.00	76.66
8.	*Santalum album*	26.66	43.33	63.33
9.	*Tectona grandis*	12.50	25.00	31.25

Discussion

Since centuries, human is using timber for construction of buildings and furniture requirement. Wood is environmental friendly and biodegradable material. Variety of wood decay fungi, besides other biotic agents like bacteria, insects, termites are causing huge loss to the woods being used inside the buildings. The indoor molds can grow on anything in humid condition, if get suitable food source. Most molds reproduce through the production of spores that float continuously in the indoor and outdoor air [21]. In present study, fifteen such molds were isolated from the surface of wooden material fitted in the humid conditions. Out of these, four species belong to the genus *Aspergillus.* Earlier research work also revealed the presence of these species in indoor environment [22-26]. Many of the indoor fungi cause indoor microbial pollution and also known to produce mycotoxins. Their exposure to human cause allergic reaction and common symptoms include sneezing, runny nose, eye irritation, coughing, congestion, aggravation of asthma, and skin rash [27].

A. flavus selected in present study for *in-vitro* management trials, is one of important indoor mycotoxin producing fungus. Mycotoxins produced by genus *Aspergillus* have a negative impact on human health. The major mycotoxins produced by *Aspergillus* species include aflatoxins, sterigmatocystin, ochratoxins, fumonisins, patulin, gliotoxin and cyclopiazonic acid [28]. Due to known toxic effects of fungicides on human health, their application for the management of molds, affecting the indoor wood is not advisable. Therefore, natural products seem to be a viable solution to the environmental and health problems caused by the synthetic pesticides. The plant based pesticides are cheap, locally available, non-toxic and easily biodegradable. In this context, crude extracts of leaf samples belonging to nine tree species were evaluated against *A. flavus* under laboratory conditions. On tested concentrations of leaf extract, a considerable growth inhibition of test fungus was observed on solid as well in liquid media. The presence of antifungal compounds in higher plants has long been recognized as an important factor in disease resistance [29]. There are evidences from earlier works that several plant species possess antifungal and antibacterial properties [15,16,30,31. Although the most of literature for screening plant material for the antifungal activity is available on the plant disease management and least on the management of indoor wood decay fungi. The rate of growth inhibition of *A. flavus* was higher in liquid culture experiment, this may be due to easy diffusion of antifungal compounds in the solution as compare to solid media. Plants are reservoir of variety of metabolites; common chemical groups are like

flavonoides and isoflavonoides, saponins, steroids, tannins, phenolic and phenolic acids, coumarins and pyrones, which are responsible for antimicrobial activity [17]. Out of nine selected tree species, the extracts of five species were able to restrict the growth of *A. flavus* with an efficiency of more than 60%, when tested in liquid media. Maximum 86.66% antifungal activity was recorded by the extracts of *Corymbia torelliana*, this may suggest their potential for future formulation into products for managing indoor microbial pollution. Their phytochemical characterization and *in-vivo* application needs further investigation.

Conclusions

The crude leaf extracts of the selected plant species are quite effective to restrict the growth of *A. flavus* in laboratory conditions. The subject needs further research on isolation and identification of bioactive compounds responsible for growth inhibition and their *in-vivo* applications.

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