Azole-based antimycotic agents inhibit mold on unseasoned pine ☆

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Abstract

Inhibiting the growth of mold fungi on cellulose-based building materials may be achievable through the use of azole-based antimycotics. Azoles were variably effective against mold fungi that are frequently found on wood and wood products. Unseasoned southern yellow pine specimens that were dip-treated with varying concentrations of eight azoles were evaluated for their ability to resist mold infestation when challenged with *Aspergillus niger, Penicillium chrysogenum,* and *Trichoderma viride* spores. Minimal fungicidal concentrations (MFC$_{90}$) were determined to be 0.016% for thiabendazole and 0.043% for voriconazole, the most efficacious azoles against the challenge fungi. We conclude that thiabendazole or voriconazole may be used alone or in combination to inhibit mold fungi on unseasoned pine.

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Keywords: Mold fungi; Mildewcide; *Aspergillus niger* ; *Penicillium chrysogenum* ; *Trichoderma viride*

1. Introduction

A number of factors can account for the recent increased incidence of mold infestations and the accompanying decline in indoor air quality (IAQ). Architectural design, condensation, improper construction practices, inadequate site drainage, water leaks, inadequate insulation, and improper ventilation can all lead to increased indoor humidity (Clausen, 2002). Both new and existing structures are equally susceptible to mold infestation in the absence of moisture management. Development of nontoxic, nonvolatile fungicides that could be surface-applied or incorporated within wood products in service would add a level of protection against mold infestation. Utilizing pharmaceutical or agricultural antimycotics to protect wood from indoor mold infestations is one strategy to address this problem.

Azoles constitute a major category of antifungal agents in clinical use. In general, they target inhibition of ergosterol synthesis. Like mammalian cells, fungi are eukaryotes, so agents that affect protein or nucleic acid biosynthesis are likely to display general eukaryotic toxicity (Gupte et al., 2002). Ergosterol, the predominant component of fungal cell membranes, is therefore an obvious and specific target for fungal inhibition. The first reports of antifungal properties for imidazoles were published in the late 1960s (Holt, 1980; Sheehan et al., 1999). Triazoles and imidazoles, first approved for use in the late 1980s and early 1990s, were considered a major advance in the treatment of systemic fungal infections in humans. The original azoles approved for clinical use—miconazole, econazole, and ketoconazole—have a complicated mode of action that involves inhibition of several membrane-bound enzymes as well as membrane lipid biosynthesis (Ghannoum and Rice, 1999). The next generation of azoles, which includes fluconazole, itraconazole, and the most recent addition, voriconazole,

act in part by inhibiting cytochome P-450-dependent 14α-sterol demethylase (P-450$_{DM}$) (Sanati et al., 1997; Johnson and Kauffman, 2003).

Voriconazole has been demonstrated to be about 250 times more active against fungal P-450$_{DM}$ than against mammalian P-450$_{DM}$. Based on the fact that mammalian cholesterol synthesis is inhibited by azoles at the 14α demethylation stage, Hitchcock et al. (1995) showed that the 50% inhibitory concentration of voriconazole was 7.4 µM against P-450$_{DM}$ of rat liver cholesterol but only 0.03 µM against fungal P-450$_{DM}$. This indicates fungal-specific inhibition. As a result of this characteristic, not only is voriconazole less toxic to humans than are other azoles, but it has a stronger avidity for 14α-lanosterol demethylation found in mold fungi. Therefore, voriconazole appears to be broadly fungicidal against common mold fungi, unlike some other azoles.

Clotrimazole (an imidazole) and miconazole are used clinically to treat several genera of pathogenic dermatophytes and *Candida,* while itraconazole is active against fungi responsible for pulmonary disease, such as *Blastomyces, Histoplasma,* and *Aspergillus fumigatus,* as well as dermatophytes and yeasts. Voriconazole more specifically inhibits *Aspergillus* spp., *Fusarium* spp., and *Scedosporium* spp.

A relatively small percentage of the approximately 250,000 species of fungi are classified as mold fungi and are all members of the Deuteromycotina and Ascomycotina. Of the true mold fungi, about 30 genera are associated with wood-based building products. Although these fungi do little to wood structurally, their appearance and odor are unpleasant, and they all have the potential to cause allergic or asthmatic symptoms. A few genera encountered on cellulose (*Penicillium, Alternaria, Fusarium, Trichoderma,* and *Stachybotrys*) produce mycotoxins, but only two species of *Aspergillus* are considered to be human pathogens. Many fungi can cause serious health effects in persons with suppressed immune systems.

Strategies for indoor mold inhibition include designing protective agents that exhibit low mammalian toxicity, are nonvolatile, and are environmentally benign. The objective of this study was to evaluate mildewcidal properties of azole compounds on unseasoned southern yellow pine and to establish minimal fungicidal concentrations for azoles that effectively control three mold fungi commonly found on cellulose-based building materials.

2. Materials and methods

2.1. Test fungi

Aspergillus niger 2.242, *Penicillium chrysogenum* PH02, and *Trichoderma viride* ATCC 20476 were maintained on 2% malt agar (Difco, Detroit, Michigan). Spore inocula were prepared according to ASTM standard D4445–91 (American Society of Testing and Materials, 1998) by washing the surface of individual 2-week-old cultures of each fungus with 10 mL sterile deionized (DI) water and transferring the liquid spore suspension into a spray bottle. Each spore suspension was diluted to 100 mL with sterile DI water. The spray bottle was adjusted to deliver 1 mL inoculum/spray.

2.2. Test chemicals

Triazole (3%), sodium triazole (3%), and difluconazole (2%) (Sigma–Aldrich Chemical, St. Louis, MO), voriconazole (Pfizer Inc., New York, NY), and itraconazole (10%) (Janssen Pharmaceutica Products, Titusville, NJ) were prepared in DI water. A single concentration of miconazole nitrate (2%) (Pharmacia Upjohn, Kalamazoo, MI) was tested as a result of limited product availability. A single concentration of clotrimazole (TARO Pharmaceuticals, Bramalea, Ontario), available as a 1% suspension in polyethylene glycol, was tested. Because of low solubility, thiabendazole (Sigma–Aldrich Chemical) dilutions were prepared in 70% ethanol.

2.3. Test method and apparatus

Wood specimens (7 by 20 mm cross section by 7 cm long) were cut from unseasoned southern yellow pine mill ends from a Mississippi sawmill and stored at 0 °C. Average moisture content of the specimens ($n = 3$) was 48% by weight. Seven random replicate specimens were dip-treated for 15 s in varying concentrations of each test chemical and held for 24 h in a covered test apparatus, according to a modification of ASTM standard test method D4445–91 (ASTM, 1998). Each test apparatus was assembled by placing 7–10 layers of 125-mm filter paper (Whatman, Hillsboro, OR) in a sterile disposable Petri dish (150 by 25 mm) (B-D Falcon, Los Angeles, California). A polyethylene mesh spacer was placed on top of the filter paper stack, which was saturated with 30 mL DI water. Each apparatus contained seven specimens treated with a single concentration of test solution per test fungus. Controls consisted of untreated specimens or control specimens dipped in 70% ethanol. Specimens were sprayed with 1 mL of fungal spore inoculum and Petri dish lids were replaced. Inoculated test apparatuses were sealed in polyethylene bags to prevent drying and incubated at 27 °C and 70% relative humidity (RH) for 4 weeks. Following incubation, specimens were individually rated for mold growth on a scale of 0–5 with 0 representing samples free from mold growth and 5 representing heavy mold growth covering 100% of the specimen sides and surface.

2.4. Statistical analysis

The minimum concentration of fungicide that resulted in effective resistance to mold growth (0 rating) was statistically estimated as the minimal fungicidal concentration (MFC_{90}) that will give at least a 90% probability of a 0 rating. Using SAS V8.2 (SAS Institute, Inc., 1999), ratings were modeled as ordinal responses in cumulative complementary log–log models, which modeled the probability of ratings as functions of the logarithm of fungicidal concentrations.

3. Results and discussion

At a high moisture content ($> 20\%$), mold can be established on wood in 24–48 h if temperature and humidity conditions are optimal. The high moisture content of the test specimens used in this study (48%) provided moisture levels adequate for mold establishment on untreated wood in 24–48 h. The ASTM (1998) standard for testing the ability of fungicides to control mold growth on unseasoned wood challenges dip-treated specimens under optimal laboratory conditions for 4 weeks, mimicking moisture conditions that one might see within a structure from chronic high humidity, leaks, or condensation (Clausen, 2002).

Average test assessment scores for each treatment and fungus are shown in Table 1. The MFC_{90} values are given for voriconazole and thiabendazole, the two compounds that showed excellent protection against test organisms. Miconazole nitrate completely inhibited all test fungi at 2%, but because of limited product availability, an MFC_{90} value could not be determined. The MFC_{90} values for thiabendazole and voriconazole were 0.016% and 0.043%, respectively (Table 1). Clotrimazole failed to substantially inhibit the test fungi at the single maximum test concentration of 1%. Difluconazole and the triazoles provided no protection to the wood at concentrations of 2% and 3%, respectively, while itraconazole failed to substantially inhibit the test fungi, even at 10% concentration.

Voriconazole, newly approved for treatment of aspergillosis, exhibits low mammalian toxicity and low volatility, is odorless, and is soluble in water (Sanati et al., 1997; Johnson and Kauffman, 2003). Thiabendazole, which is used as a fungicide for controlling spoilage on citrus fruit, also exhibits low mammalian toxicity and low volatility and is odorless, but it is only slightly soluble in alcohol (Merck, 1976). Insolubility in water can be viewed as either an advantage, because of treatment retention under conditions of high moisture, or as a disadvantage, because of difficulty in handling. Effective thiabendazole concentrations were low enough that solubility was not a concern, and ethanol controls demonstrated that the azole component was responsible for mold inhibition (Table 1).

The ability of cellulose-based building materials to resist mold growth directly affects IAQ in a structure. Laks et al. (2002) evaluated four types of common commercial untreated sheathing panels (southern yellow pine and Douglas-fir plywood, oriented strandboard, and gypsum board) for mold resistance. Their results showed that all of these products were prone to mold growth within 1 week of inoculation with mold spores and incubation at 27°C and 100% RH. In fact, all

Table 1
Relative resistance of azole-treated southern yellow pine specimens to growth of *P. chrysogenum, A. niger,* and *T. viride*

Azole compound	Treating solution (mg/mL)	Average rating[a]			Total score	Mold (%)	Rating[b]	MFC_{90} (%)
		P. chrysogenum	*A. niger*	*T. viride*				
Voriconazole	0.156	0	0	2.43	2.43	16		
	0.3125	0	0	1.29	1.29	9		
	0.625	0	0.14	0.43	0.57	4	Excellent	0.043
	1.25	0	0	0.07	0.07	0		
	2.5	0	0	0	0	0		
Thiabendzaole	0.0975	0	0	2.5	2.5	17		
	0.19	0	0	0	0	0	Excellent	0.016
	0.39	0	0	0	0	0		
	0.78	0	0	0	0	0		
	1.56	0	0	0	0	0		
Miconazole	20	0	0	0	0	0	Excellent	$\leqslant 2.0$
Itracoazole	100	1.9	0.3	4.1	6.3	42	Moderate	NA
Clotrimazole	10	3.4	5	3.7	12.1	81	None	NA
Difluconazole	20	4.7	5	5	14.7	98	None	NA
Triazole	30	5	5	5	15	100	None	NA
Na-triazole	30	5	5	5	15	100	None	NA
Control	—	5	5	5	15	—	—	—

[a] $n = 7$.
[b] Resistance ratings: Excellent, 0–25% mold; moderate, 26%–50% mold; none (no resistance), 76%–100% mold.

panels showed signs of mold after 1 week of preconditioning at 27°C and 100% RH prior to fungal inoculation. Clearly, surface treatment with a mildewcide or incorporating a mildewcide into these products would have an impact on IAQ. Further testing is necessary to demonstrate long-term efficacy.

4. Conclusions

Three of eight antifungal azole compounds tested were efficacious against *A. niger, P. chrysogenum,* and *T. viride* on dip-treated unseasoned southern yellow pine. Only thiabendazole and voriconazole provided complete protection against these mold fungi under the conditions of the study, and were efficacious at an MFC_{90} of 0.016% and 0.043%, respectively. These or similar antimycotic agents may be useful for inhibition of mold fungi in home environments.

Acknowledgement

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