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Wood Protecting Chemicals

Multifactorial Antimicrobial Wood Protectants

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ABSTRACT

It is unlikely that a single antimicrobial compound, whether synthetic or natural, will provide the ‘magic bullet’ for eliminating multiple biological agents affecting wood products. Development of synergistic combinations of selected compounds, especially those derived from natural sources, is recognized as a promising approach to improved wood protection. Recent adoption of effective fungicide technology in food sanitation and agriculture to applications in wood protection has led to the development of novel wood protectants. Specifically, formulations that incorporate low molecular weight monocarboxylic acids with selected adjuvants as potential synergists suggest use as fungal inhibitors and possible termiticides.

Keywords: Fatty acid, mould, synergist

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1. INTRODUCTION

Finding a single synthetic or natural antimicrobial compound, either newly recognized or already registered, to inhibit the immense variety of fungi capable of colonizing wood and wood products is highly unlikely. Likewise, biocide resistance that occurs frequently and to varying degrees in wood-inhabiting fungi increases the importance of co-biocide interaction for successful wood protection.

The development of antimicrobial wood protectants having multiple mechanisms of action holds more promise for success than single-mechanism antimicrobial compounds. Attempts at developing multi-action systems are becoming more popular (Brul *et al.* 2002, Green and Schultz 2003). Identification and development of synergistic combinations of selected compounds, preferably using those derived from natural sources, is generally recognized as the most promising approach for obtaining better antimicrobials (Marshall 2003, Dillon and Cook 1994, Ippolito and Nigro 2003, El Ghaouth *et al.* 2000). A number of effective combinations have been developed as general biocides such as nisin and garlic or phenolic compounds (Adams 2003), sorbates with vanillin or citral (Alzamora and Guerrero 2003), chitosan with an antagonistic yeast (El Ghaouth *et al.* 2000), antioxidants and/or metal chelators with an organic biocide (Schultz and Nicholas 2001), borates and quats with an azole (Clausen and Yang 2007), lactic and acetic acids (Adams 2003) and caprylic acid and glycolic acid (Coleman 2004).

Schmidt (1984) evaluated the influences of saturated fatty acids on spore germination of brown- and white-rot basidiomycetes and determined that caprylic or octanoic (C8), pelargonic or nonanoic (C9), and decanoic (C10) acids (100 ppm) destroyed spores of test fungi, whereas dodecanoic acid (C12) was effective against brown-rot but not white-rot fungal spores. Schmidt's findings suggested a high degree of specificity dependant on the fatty acid, concentration tested, and the test organism. For example, all concentrations of hexadecanoic acid (C16) tested were totally ineffective against spores of all test fungi, while pentanoic and hexanoic acid were effective against all test fungi but only at 10^3 ppm. Moreover, other researchers found that fungal spore germination is stimulated or inhibited depending on the particular acid and its concentration (Harman *et al.* 1980). Thus, some fatty acids at specific concentrations have potential use as wood protectants; however, use conditions relating to efficacy against a wide variety of ascomycetes, deuteromycetes, and basidiomycetes remains to be identified. One approach to enhance the performance of fatty acids as moldicides involves the use of proven adjuvants such as organic acids. To date, multi-factorial systems have not focused on combined organic and fatty acid chemistries applied specifically to control mould fungi for protection of wood.

Many organic acids, including acetic to decanoic acid and L-lactic, citric, malic, and glycolic acids are classified as GRAS (Generally Accepted As Safe) compounds by the U.S. Food and Drug Administration (FDA) and have common acceptance for use in the food industry as acidulants and flavor enhancers. The safety record of these compounds is a positive feature that addresses the need for development of antimicrobials based on "green chemistries." Certain organic acids such as acetic, citric, and tartaric are known to inhibit mould (Barbosa-Canovas *et al.* 1998), function as chelating agents and have been used to inhibit lipid oxidation and deter browning in food products (Doores 1993). Chelation may play a beneficial role in biocide function by altering availability of micronutrients necessary for germination and hyphal development.

The objective of this study was to evaluate combinations of selected fatty acids and specific organic acids and other adjuvants against mould growth on wood. Research findings demonstrate that certain adjuvants, even at low amounts, greatly enhance fatty acid activity against mould growth on southern pine.

2. EXPERIMENTAL METHODS

2.1 Test chemicals

Test chemicals evaluated in this study are summarized in Table 1. Chemicals were supplied by Summerdale, Inc., Verona, WI, USA. Emulsifiers and adjuvants used in this study are proprietary compounds. Experimental formulations consisted of a combination of 1 or more fatty acid, emulsifier or adjuvant component. Fatty acid concentrations in treating solutions ranged from 2.12 to 8% (v/v), emulsifier concentration ranged from 0.3 to 2.2% (v/v), and adjuvant concentration ranged from 0.24 to 1.0% (v/v).

Table 1: Classification of fatty acids, emulsifiers, and adjuvants

Fatty Acid		Emulsifier	Adjuvant
Propionic	C3	Emulsifier A	L-lactic acid
Butyric	C4	Emulsifier B	Adjuvant 1
Pentanoic	C5		Adjuvant 2
Caproic	C6		Adjuvant 3
Heptanoic	C7		
Caprylic	C8		
Pelargonic	C9		
Capric	C10		

2.2 Mould test

Specimens (7 × 20 mm cross section by 7 cm long) were cut from southern pine. The average moisture content of the specimens was 26.4% ($n=5$). Depending on the test, 10 to 18 replicate specimens were dip-treated for ~15 seconds in an individual biocide consisting of a fatty acid and emulsifier +/- adjuvant (Table 1) and held in a covered container overnight according to a modification of ASTM standard test method D4445-91 (1998). Specimens were arranged over 4 layers of blotting paper saturated with 30 mL of deionized (DI) water and a polyethylene mesh spacer in sterile disposable Petri dishes (150 × 25 mm) (B-D Falcon, Los Angeles, CA, USA). Untreated specimens dipped in DI water served as controls. Specimens were sprayed with 1 ml containing approximately 3×10^7 spores/mL of a mixed mould spore consortia consisting of *Aspergillus niger* 2.242, *Penicillium chrysogenum* PH02, and *Trichoderma viride* 20476, sealed in polyethylene bags to prevent drying and incubated at 27°C and 70% relative humidity (RH) up to 12 weeks. During incubation, individual specimens were periodically rated for mould growth on the following scale; 0 = no growth; 1 = 20%; 2 = 40%; 3 = 60%; 4 = 80%; 5 = 100% coverage with mould.

3. RESULTS AND DISCUSSION

Seven candidate fatty acids were evaluated in formulations for mould inhibition on wood products. At 6% concentrations, propionic (C3) and pentanoic (C5) acids were effective inhibitors of test fungi (Fig. 1). Butyric acid did not significantly inhibit the test fungi. Emulsifier A and B (Fig. 1) were compared for their ability to provide emulsion stability for fatty acids C5 to C9 in water. Emulsion stability is particularly important for fatty acid formulations used at relatively high concentrations ($>3.0\%$ v/v) as aqueous, mould-inhibitory solutions for treating wood products. Emulsifiers, especially emulsifier A, may possess some ability to independently inhibit mould fungi and/or enhance the bioactivity of a fatty acid as shown in Fig. 1. While both C6 emulsions were equally effective at inhibiting test fungi, results show that C7-C9 fatty acids emulsified with emulsifier A provided greater mould inhibition than formulations containing emulsifier B. However, emulsifier B provided superior emulsification properties compared to emulsifier A; i.e., good stability of water emulsions after storage for 30 min (data not shown).

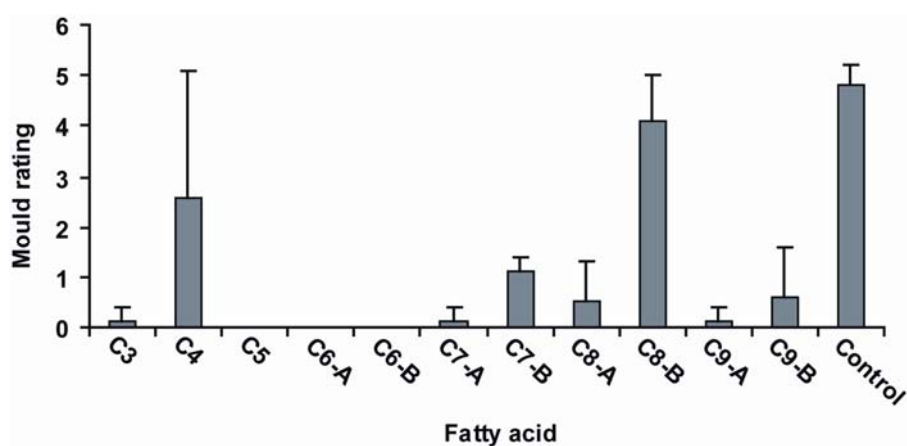


Figure 1: Effectiveness of 6% fatty acids against mould fungi on southern pine for 6 weeks; $n = 10$. A and B designate emulsifier A or B.

Therefore, a dual emulsification system was evaluated to assess the potential benefit of incorporating both emulsifiers. Fatty acid formulations were compared at relatively low application rates ($<3.0\%$ v/v) to distinguish treatment effects as mould inhibitors. Formulations including both emulsifier A and emulsifier B, compared with formulations containing emulsifier A alone, suggest that dual emulsification improves both emulsification and mould inhibitory activity (Fig. 2). The sole exception was pelargonic acid; all other dual emulsified fatty acid formulations exhibited better anti-mould properties than single emulsified formulations. All dual-emulsified fatty acid formulations diluted in water were very stable whereas pentanoic, hexanoic, and heptanoic formulations emulsified only with emulsifier A were less stable at 3.0% (v/v) in water (data not shown).

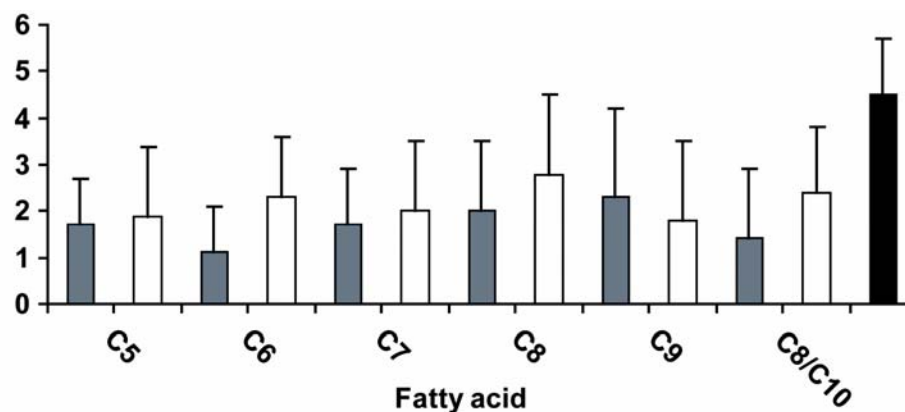


Figure 2: Effect of 2.1% fatty acid on southern pine comparing single with dual emulsification systems. Gray bars represent dual emulsification (0.6% A, 0.3% B); white bars represent 0.9% emulsifier A; n = 12.

Fatty acid formulations complemented with selected adjuvants as candidate mould inhibitors were evaluated (Table 2). Southern pine dip-treated with formulations with and without adjuvants were exposed to test fungi and rated periodically for 8 - 12 weeks. Early test results using L-lactic acid and two other adjuvants (adjuvant 1, 2) were very encouraging; i.e., butyric, pentanoic, heptanoic, and caprylic/capric formulations including adjuvant amendments maintained a high degree of inhibition for the duration of the test (Table 2). All fatty acid formulations benefitted substantially by the presence of an adjuvant.

Table 2: Effect of select adjuvants on mould inhibitory properties of fatty acid formulations

Fatty Acid	[%]	Amendment [%]	Mould rating ^b
Butyric	6.0	-	2.1 (1.8)
"	5.4	L-lactic [0.6]	0.2 (0.4)
Pentanoic	6.0	-	3.0 (2.5)
"	5.4	L-lactic [0.6]	0.3 (0.5)
Caprylic/capric	4.2	A [1.8]	2.6 (1.2)
"	4.2	A [1.5]; L-lactic [0.3]	1.3 (1.1)
Heptanoic	5.6	B [2.4]	3.1 (0.9)
"	5.6	B [2.0]; L-lactic [0.4]	1.3 (1.6)
"	5.6	B [2.16]; Adj. 1 [0.24]	2.1 (1.2)
"	5.6	B [2.16]; Adj. 2 [0.24]	0.1 (0.2)
Control	-	-	3.9 (1.2)

^aA and B designate emulsifier A or B.

^bAverage rating (standard deviation), n = 12.

A fatty acid formulation containing pelargonic acid (C₉), dual emulsifiers (emulsifier A, B), and L-lactic acid (as a beneficial adjuvant) was compared with and without additional adjuvants (adjuvants 1, 3) (Table 3). Mould inhibition of the C₉/dual emulsifier/L-lactic acid formulation, at both 6.0 and 8.0% (v/v) application rates, was enhanced by supplementation with either adjuvant 1 or 3.

Table 3: Effect of adjuvants on dual-emulsified pelargonic acid formulations

Formulation composition [%] ^a	Treating solution [%]	Adjuvant	[%]	Mould rating ^b
C9 (70) + A (10) + B (10) + LA (10)	6	-	-	1.1 (1.4)
"	"	3	1.0	0.6 (1.2)
"	"	1	0.25	0.3 (0.9)
C9 (70) + A (10) + B (10) + LA (10)	8	-	-	1.0 (1.1)
"	"	3	1.0	0.4 (0.9)
"	"	1	0.25	0.2 (0.5)
Control	-	-	-	2.4 (1.4)

^aA and B designate emulsifier A or B; LA = L-lactic acid

^bAverage rating of 3 replicate tests (standard deviation), $n = 36$

Lactic acid, produced by lactic acid bacteria (LAB) is well recognized as a biopreservative of food (Yang and Clausen 2005). A study by Yang and Clausen on mould inhibition by *Lactobacillus* reported that lactic acid, a major metabolite from LAB, together with other cell-free metabolites caused 95–100% fungal biomass inhibition of mould fungi *in vitro*. Adding select organic acids, such as L-lactic acid, to the fatty acid emulsifications may increase the proportion of non-ionized fatty acids over ionized species, thereby promoting greater fatty acid penetration through cell membranes. Intracellular proton pump activity is increased and more energy is required by the cell for electrolyte balance, thus placing more stress on the cell (Coleman and Penner 2006, 2008). Higher intracellular concentrations of free fatty acids possibly results in damage to organelle membranes and protein structure (Barbosa-Canovas *et al.* 1998, Ecklund 1989). This is one possible hypothesis for the mode of action on vegetative mould hypha, but would not apply to spore coats. Further studies are needed to assess whether spores are killed by the caprylic and pelargonic acid formulations as suggested by Schmidt (1984) or if germination is simply being inhibited. Leaching studies, pressure treatability, and additional efficacy studies are under way.

4. CONCLUSIONS

Several fatty acid formulations inhibited mould test fungi as well as propionic acid. L-lactic acid and selected adjuvants appear to enhance the antifungal properties of the formulations. Multifactorial fatty acid emulsifications incorporating an appropriate adjuvant are promising for effective protection against numerous fungal species that affect wood products. Using this strategy, new “green” wood protection formulations are being developed and their application is likely to extend to other biodeteriorating agents such as termites and decay fungi. More work is needed to optimize efficacy and assess field performance of these new mould-inhibiting formulations.

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