



Effect of chitosan on the mold resistance of wood and its surface properties

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Abstract

In this study, the effect of soluble chitosan, thyme oil and linseed oil on the mold resistance of wood and its surface properties was investigated. Both low and medium molecular weight chitosan with concentration of 1 and 2% were used. Cypress wood samples with dimensions of 20 × 20 × 70 mm and moisture content of 12% were saturated by the preservatives. The results showed that the maximum absorption was for linseed oil and the lowest one for low molecular weight chitosan with concentration of 2%. The lowest and highest leaching values were observed for the samples treated by linseed oil and medium molecular weight chitosan with concentration of 2%, respectively, but these differences do not seem to be statistically different. After 4 weeks, thyme essential oil was able to completely prevent mold growth. Compared to chitosan, linseed oil had a greater inhibitory effect to the growth of mold. Antifungal effect of chitosan was increased by increasing its molecular weight. In contrast to thyme oil and linseed oil, the wood treatment by chitosan increased its wettability. There was no significant difference between the roughness of control and treated samples.

Keywords: Chitosan, Linseed oil, Mold resistance, Surface properties, Thyme oil.

1. Introduction

Wood is a versatile biomaterial with uses in a wide range of applications. However, when exposed to moist or humid conditions, it may be attacked by decay and mold microorganisms and thus needs protection for a desired service life. The traditional protection methods are based largely on the use of inorganic and synthetic chemicals. Although some chemical materials with low toxic effects have been used over the last few decades but the public concerns remain about the use of these chemicals. Therefore, the new trends in wood preservation focus on products and processes that utilize technologies and materials with no harmful effects on environment. Various plant materials are believed to have antifungal property and many essential oils have been reported to have antifungal activities. (Yang and Clausen, 2007; Matan and Matan, 2008; Singh and Chittenden, 2010; Clausen and Yang 2011). However, only a few reported studies are available on activity of essential oils against decaying fungi in wood (Wang et al., 2005). Linseed oil is a natural, organic and hydrophobic chemical that can be used as a wood preservative. Impregnation of wood with various oils, including linseed oil, coco oil, and tall oils has been reported to significantly increase its water repellence (Olsson et al., 2001 and 2003). Nowadays, chitosan has been considered as an interesting environmentally friendly material for wood preservation. It is used as a potential wood preservative alone or in combination with other biocides to control wood inhabiting fungi (Schmidt et al., 1995; Majety and Kumar, 2000; Frederiksen, 2001; Chittenden et al., 2003; Chittenden et al., 2004; Larnøy et al. 2005; Eikenes et al., 2005; Treu and Larnøy, 2005; Larnøy et al., 2006). The molecular size of chitosan has a great impact on the properties of chitosan treated wood. Eikenes et al. (2005) found that high molecular chitosan gave a better antifungal effect. In addition, the use of chitosan to enhance the efficacy or to reduce the leachability of other wood preservatives has been investigated (Kobayashi and Furukawa 1995a, b). Laflamme et al. (1999) demonstrated that chitosan is not only effective in reducing the radial growth of fungi, but also induces severe morphological and ultrastructural changes in the fungi. The objective of this study was to investigate the effect of chitosan with different molecular weight and concentration on the resistance of wood to mold fungi in comparison to that of linseed oil and essential oil of thyme.

2. Materials and Methods

2.1. Wood sampling and treatment

Sapwood specimens of redbark cypress (*Cupressus arizonica*.) with 0.435 gr/cm³ specific gravity obtained from cut trees in botanical garden of the University of Tehran, and prepared with dimensions of 20×20×70 mm and conditioned at 27°C

and 70% relative humidity (RH) to EMC reach 12% at the end of conditioning and were selected for the study. Ten random replicate specimens for each treatment were dipped for 1 h in individual test solutions (Table 1). Oil-treated samples were treated under same conditions as chitosan-treated samples. Five Dip-treated specimens were held in a covered container overnight according to ASTM D4445-10 standard test method to allow chemical to penetrate. All specimens were re-weighed to determine gross absorption and air-dried for 1 week prior to conditioning at 27°C and 70% RH. Other five replicate specimens for each treatment were used for leaching test. Total number of samples was used is 30 samples for chemical to penetrate and then mold test and 30 samples for leaching test. Five control samples for mold test were treated with water under same conditions.

2.2. Preparation of preservatives

Chitosan, linseed oil and essential oil of thyme were purchased from Sigma-Aldrich Co, giah esans of Gorgan and Part Shamim daru Co., respectively. Essential oil of thyme and Linseed oil was used in pure form. The low molecular weight chitosan, (products numbers: 448869) and the medium molecular weight chitosan (products numbers: 448877) with concentration of 1 and 2% were used, and this concentration were chosen for identifying that whether the minimum concentration of chitosan can inhibit mold growth or not. Higher concentration of chitosan may be commercially relevant. Chitosan was dissolved in water with addition of acetic acid to pH 5.0. The chitosan solutions were mixed well in a concrete blender, and when all chitosan was dissolved, a 4% (weight/volume) aqueous solution of potassium nitrite with ratio of 500: 10: 0.5 water: chitosan: potassium nitrite for 2% solution and 500: 5: 0.25 for 1% solution was added by spraying a fine mist onto the chitosan while continuously mixing at 30 °C (Table 1). After 5 hours, the solution was transferred to a barrel and stored at ambient temperature.

Table 1. Characteristics of prepared commercial chitosan.

Molecular weight	Concentration (%)	Potassium nitrite (g) pure amount addition	Degree of acetylation (%)	Viscosity (CPS)
Low	1	0.25	0 ≥ 75	20-300
Low	2	0.5	0 ≥ 75	20-300
Medium	1	0.25	75-85	200-800
Medium	2	0.5	75-85	200-800

2.3. Chemical leaching test

Leaching test was performed using the European standard method (EN 84). After conditioning to equilibrium moisture content and reaching to 12% EMC, 4 specimens from each treatment group were weighed and placed into individual 500-mL beakers and submerged each vessel with deionized (DI) water to a ratio of approximately five volumes of water to one volume of wood, and mild agitation for a total of 14 days. Control samples were not subjected to the same procedure. Leach water was replaced with an equal amount of fresh DI water after 6 h, and 1, 2, 4, 6, 8, 10, 12, and 14 days. Following leaching, specimens were dried at 40 °C for 3 days before reconditioning at 27 °C and 70% RH to equilibrium moisture content. They were again weighed to allow estimation of chemical loss from leaching. Absorption and leaching of each sample was calculated according to the equations 1 and 2:

$$(1) \quad \frac{\text{Initial weight of sample (kg)} - \text{sample weight after 1 night civering(kg)}}{\text{sample weight after 1 night civering(kg)}} / V \text{ (m3)} = \text{Absorption}$$

$$(2) \quad \frac{\text{sample weight before leaching (kg)} - \text{sample weight after leching (kg)}}{\text{sample weight after leching (kg)}} / V \text{ (m3)} = \text{Leaching}$$

2.4. Test fungi

Mold fungi, *Penicillium spp.* grown on molded wood, were grown on 2% malt extract agar (Difco, Detroit, MI) at 27° C, and 70% RH for 2 weeks. A mixed mold spore suspension was prepared by washing the agar surface of one Petri dish with 10 mL of sterile deionized water (DI) according to ASTM D4445-10. Spores were collected, counted and equal numbers of spores for each test organism were transferred to a spray bottle. The spore mixture was diluted with DI water to yield approximately 3×10^7 spores mL⁻¹, accounted with number of spore on the reticulate tablet in the mycology lab. The spray bottle was adjusted to deliver 1 mL inoculum per spray and was mixed frequently during inoculation to ensure homogeneous inocula.

2.5. Mold resistance test

Unbleached specimens were evaluated for resistance to the mixed mold spore suspension. The specimens were arranged over 4 layers of blotting paper that was saturated with 30 mL DI water and a polyethylene mesh spacer in sterile disposable Petri dishes (150 x 25 mm). Water-treated wood blocks served as a diluent control. After spraying with 1 mL mixed mold-spore inoculum, plates

containing unleached test specimens were sealed in polyethylene bags to prevent drying and incubated at 27 °C and 70% RH. Specimens were visually examined with surface covering of mold on specimens rated for mold growth after 4 weeks on a scale of 0–5 according to the method of Waals et al. (2003) with 0 indicating the specimen was completely free of mold growth and 5 indicating the specimen was completely covered with mold growth (Table 2).

Table 2. Assessment method used to determine the mold growth.

Scale	Coverage surface (%)
0	0 (no growth)
1	1-5
2	6-25
3	26-50
4	51-75
5	76-100

2.6. Contact angle and roughness measurement

The contact angle of treated and unleached samples was measured according to ASTM D5946. Control sample also included in the test and 5 replicates were examined for each treatment and 3 measurements were made for each sample. A Mitutoyo SurfTest SJ-201P instrument (Figure 1) was employed for the surface roughness measurement. The R_a (average roughness), R_z (mean peak-to-valley height), and R_q (root mean square roughness value) roughness parameters were measured to evaluate the surface roughness of the samples according to the DIN4768 standard. The roughness is important index for effects of preservatives on the surface quality of wood specimens. Contact angle is the representative of water absorption in wood.



Figure 1. Mitutoyo SurfTest SJ-201P instrument.

3. Results and Discussion

3.1. Preservative uptake and leaching

The results of 5 samples for each different treatments showed that the greatest uptake belong to linseed oil with 48.83 Kg/m^3 and the lowest uptake belong to the chitosan with low molecular weight and 2% of concentration with 19.54 Kg/m^3 uptake (Figure 2). On the whole, there was no significant difference in the uptake value among the four chitosan solutions. Difference between thyme oil and chitosan treatments, as well as between linseed oil and 1% LMWC was observed. The uptake of preservatives in this study compared to the references because the longer treatment time was very high. In Figure 3, the leaching values of chitosan with different molecular weights and concentrations compared to that of linseed oil and thyme are presented. Based on the average leaching values for each treatment, the minimum leaching was observed for Linseed oil with a value of 23.57 Kg/m^3 , medium molecular weight chitosan with concentration of 2% showed the highest leaching (59.55 Kg/m^3). At concentration of 2%, low molecular weight chitosan showed lower leaching value (44.82 Kg/m^3); however, no significant difference was observed between low and medium molecular weight chitosan solutions at 1% concentration. SPSS software was used for statistical tests and the difference of the results averages compared in 95% assurance level.

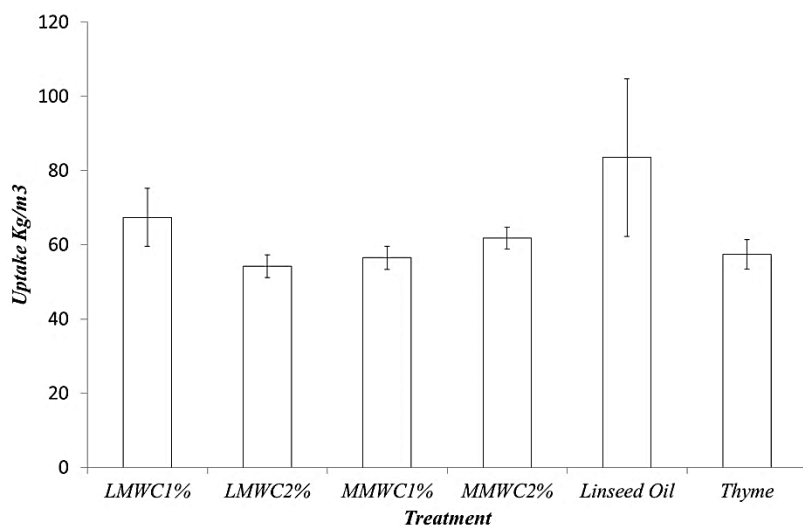


Figure 2. The uptake values of preservatives. LMWC: low molecular weight chitosan, MMWC: medium molecular weight chitosan.

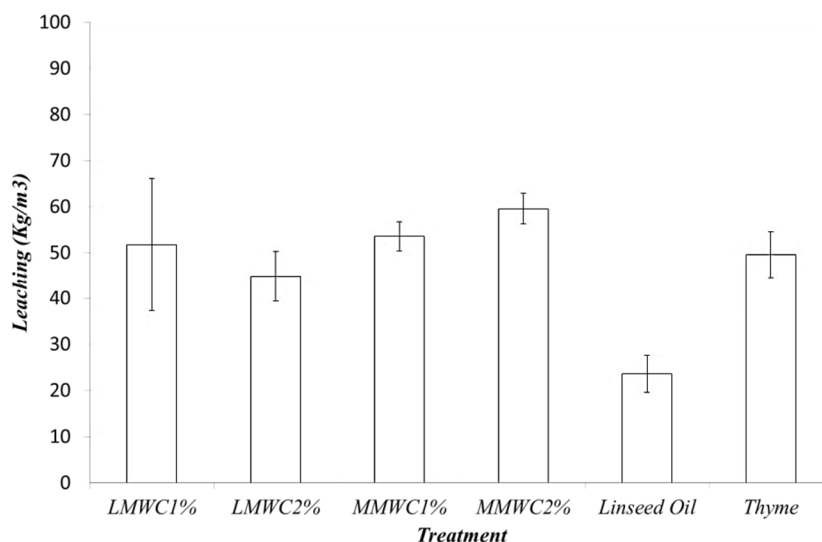


Figure 3. Leaching values of preservatives from wood specimens.

3.2. Molding

The results of the apparent mold contamination of samples with different treatments are shown in Figure 4. Based on the rating system (Table 2) and average mold covering for 5 replicates of each treatment, the samples treated with essential oil of Thyme did not show any infection, whereas water-treated control samples had the highest infection rate. Linseed oil showed better antifungal property than chitosan. In general, all used chitosan solutions improved the mold resistance of wood specimens except the LMWC2% and no difference in mold protection was noted between low and medium molecular weight (MW) chitosan at 1 % application. Medium molecular weight chitosan solutions with 2% concentration in compare to LMWC2% had better performance. Previous studies also confirmed the antifungal effect of chitosan (Schmidt et al., 1995; Chittenden et al., 2003; Alfredsen et al., 2004; Eikenes et al., 2005; Larnøy et al., 2006;) In contrast to low molecular weight chitosan, the antimold effect of medium molecular weight chitosan improved by increasing its concentration from 1 to 2%. Our results are in agreement with Yang and Clausen (2007) and, Clausen and Yang (2011) who found that thyme oil completely inhibited mold growth for 12 weeks.

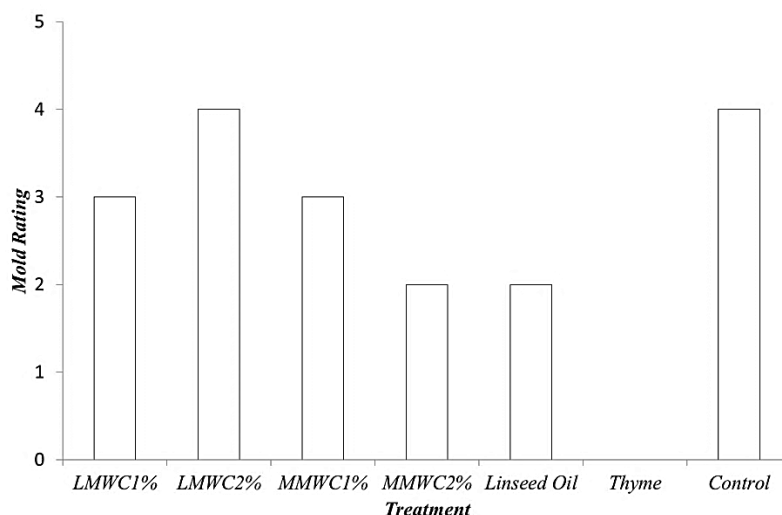


Figure 4. Average mold ratings for treated wood specimens after 4 weeks. Rating scale was 0-5 with 0 indicating specimens were completely free of mold growth and 5 indicating specimens were completely covered with mold growth.

3.3. Contact angle and surface roughness

The contact angles of samples measured after 1 and 10 seconds (Table 3). The results showed that the samples treated with linseed oil were more hydrophilic (contact angle 76° 1s and 67° 10s). The hydrophilic performance of samples treated with essential oil of thyme was close to that of the control samples (contact angle 93° 1s and 74° 10s). Wettability (contact angle) data for chitosan treatments have little to no difference from control samples. The contact angle of control samples were 103° in 1s and 90° . Compared to LMWC treated samples, more hydrophilic performance was observed for those treated by MMWC solution. The results showed that the contact angle in all samples after 10 seconds decreases. Table 4 shows the roughness parameters of samples (Ra, Rz and Rq). Ra parameter is important to indicate the surface roughness of samples. The results showed that all treated wood samples except the sample treated with medium molecular weight chitosan at 2% concentration had a roughness value less than the control sample. The sample treated with thyme oil had the lowest surface roughness.

Table 3. Average contact angle for treated wood specimens compared to control one.

Specimen	Contact Angle			
	1 s	(STDEV)	10 s	(STDEV)
LMWC1%	117	(8.4)	94.5	(11.95)
LMWC2%	89.5	(14.27)	80	(18.12)
MMWC1%	86.25	(22.06)	80.5	(21.48)
MMWC2%	99	(11.6)	95.25	(9.32)
Linseed Oil	75.75	(7.58)	66.75	(9.21)
Thyme	93.25	(21.23)	74.75	(13.45)
Control	98.25	(12.71)	89.75	(13.57)

STDEV= Standard Deviation

Table 4. Average surface roughness parameters for treated wood specimens compared to control one.

Treatment	Parameter		
	Rq (m μ)	Rz (m μ)	Ra (m μ)
LMWC1%	11.5	55.79	9.08
LMWC2%	11.38	53.97	9.05
MMWC1%	9.87	48.45	7.82
MMWC2%	15.35	75.07	12.18
Linseed Oil	10.51	49.91	8.45
Thyme	9.37	48.2	7.37
Control	11.45	55.45	9.11

4. Conclusions

Wood treatment by essential oil of thyme could completely prevent the mold growth after 4 weeks incubation. Compared to chitosan, linseed oil showed greater inhibitory effects against the growth of mold and thus it can be considered as the anti-mold fungal oil for wood treatment. In addition, linseed oil had the lowest value of leaching. Among four chitosan solutions used here, the medium molecular weight chitosan at 2% concentration had the greatest antifungal effect. Wettability (contact angle) data for chitosan showed little to no difference from control samples. However, all used preservatives had no effect on the wood roughness. Due to the preservatives leaching, further studies are recommended to fix them to wood for outdoor applications.

Acknowledgments

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