TOXICITY THRESHOLD OF CHITOSAN FOR THE SUBTERRANEAN TERMITE, *RETICULITERMES FLAVIPES*

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Abstract

Chitosan is a hydrophilic and biodegradable polysaccharide with antimicrobial properties. It has low toxicity to non-target organisms and is considered an environmentally friendly preservative. In this study, the efficacy of chitosan polymer (> 50 kDa) as a wood preservative was evaluated against the subterranean termites *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). The treatability and leachability of chitosan from wood was also studied. Visual ratings and mass losses were used to assess the degree of termite damage to the wood blocks, while percent termite mortality after two weeks was measured for determining the effect of chitosan on the termites. In addition, the mass of chitosan per gram of dried wood before and after leaching was used to determine the stability of the preservative for five different treatment concentrations. High termite mortality was observed in termites exposed to wood treated with 3%, 4% and 5% chitosan solution-treated samples, while only 10% mortality was shown in wood treated with 1% and 2% chitosan solutions. Mass loss of treated wood samples due to termite attack decreased with higher chitosan concentration. Leached samples did not contain enough chitosan to protect wood from termite attack. The results of this study indicate that treating solutions with chitosan concentrations greater than 3% could protect wood under dry conditions.

Background

Wood is considered to be the second-oldest construction material after stone, and over 90 percent of American homes are built with wood. It is also a major biodegradable and bio-renewable construction material, and as such, requires protection against wood degrading organisms. Worldwide wood degradation due to termites is estimated to cost 32 billion US dollars annually (Rust, 2014). The eastern subterranean termite, *Reticulitermes flavipes* (Kollar), is the most common termite species in the eastern region of the United States and the most devastating wood destroying insect in the United States. It is estimated that in high activity areas more than 1 in 5 homes have been or will be attacked (Smith & Johnston, 1970). *R. flavipes* survives in human domiciles due to favorable moist environments, optimal soil conditions, and the presence of food sources, i.e. wood substance (or any other cellulose containing material) (Emerson, 1952). *R. flavipes* is found near or below ground level of wooden structures.

Currently used wood protection agents contain heavy metals such as copper, which has a negative effect on the ecosystem (Kulikova et al., 2011; Mai et al., 2012; Martínez et al., 2014). Nowadays, when the general societal preference for sustainable solutions has become very clear, wood protection scientists are looking more closely into alternatives to traditionally used preservative systems. One alternative to copper treatment that has potential in the successful preservation of wood is chitosan (Liibert et al., 2011). Chitosan is produced by deacetylation of chitin, which is a structural polysaccharide from the exoskeleton of arthropods, such as shells of crustaceans and integument of insects (Shahidi & Synowiecki, 1991). The food industry produces chitin as a waste product in amounts that is not that less than the amount of cellulose (Roberts, 2008). Chitosan is used in agriculture and horticulture as a bio-pesticide. Several studies have shown the effectiveness of chitosan in preventing the proliferation of wood decaying fungi (Torr et al., 2005; Alfredsen et al. 2004), improving the mechanical and physical properties of wood (Basturk, 2012), and as an agricultural pesticide (Badawy & El-Aswad, 2012), but there is limited information on the effects of chitosan on termites.

In this study, the termiticidal efficacy of chitosan against *Reticulitermes flavipes* was evaluated. Low molecular weight chitosan (50-190 kDa) dissolved in 25% acetic acid was used for determining the toxicity threshold of chitosan to the termite at 1%, 2%, 3%, 4% and 5% concentration levels.

Materials and Methods

Materials

Low molecular weight chitosan powder (50-190 kDa), and acetic acid were purchased from Sigma-Aldrich Co. (St. Louis USA). A termite no-choice test (AWPA E1) was set up using play sand (Sakrete® Natural Play Sand) purchased at a local home improvement store. The termites used in this study were obtained from one population collected at Saucier, MS and kept in a metal bin at room temperature with sufficient wood material and moisture until they were utilized in the study. The termites were utilized within 6 months from collection.

Chitosan solution preparations

Using a 25% acetic acid solution, 1%, 2%, 3%, 4% and 5% (w/v) chitosan solutions were prepared. The solutions were stirred overnight in a fume hood until completely dissolved. Control wood samples were treated with solutions of distilled water and 25% acetic acid solution (v/v).

Wood treatment

Southern yellow pine sapwood samples were cut to 25 × 25 × 6 mm (tangential × radial × longitudinal) according to the AWPA E1 Standard (AWPA, 2014). Five end-matched replicates of oven-dried wood samples of known mass were vacuum-treated at 24 in Hg for 3 hours in each of the five chitosan solutions. Control samples were treated in the same manner in water and 25% acetic acid.

After treatment, the samples were allowed to equilibrate in solutions for approximately 24 hours and then air-dried and oven-dried at 50°C to constant mass. Treated weights were recorded and the retention or grams of chitosan per gram of oven-dried mass of wood was calculated as follows:

$$\textit{Retention (g/g)} = \frac{(m_{0t}-m_0)}{m_0}$$

Where:

m_{0t} = Oven-dry mass of samples post-treatment, g

m_0 = Oven-dry mass of samples pre-treatment, g

Termite exposure

In this study, the recommended protocol for laboratory evaluation to determine resistance to subterranean termites, AWPA E1 standard (AWPA, 2014), was used with one modification. Exposure time to the termites was reduced to 2 weeks, rather than 4 weeks, because the toxicity threshold was observed in the first week. The treated test blocks were placed individually in Qorpak round-bottom glass jars, containing autoclaved play sand and sterile distilled water. One gram of cleaned and healthy termites (about 355 individuals, including approximately 3 termite soldiers) was placed in each jar. Five replicates of water-treated control and five replicates of acetic acid-treated samples were included in the study. The jars were kept in an incubator at 28 ± 1°C for 2 weeks. Percent termite mortality and percent mass loss of test blocks were calculated as follows:

$$\textit{Mortality (\%)} = \frac{T_D}{T} \cdot 100$$

Where:

T = number of termites used

T_D = number of termites dead

$$\textit{Mass loss (\%)} = \frac{(m_{0t}-m_{0e})}{m_{0t}} \cdot 100$$

Where:

m_{0t} = Oven-dry mass of samples post-treatment, g

m_{0e} = Oven-dry mass of samples post-termite exposure, g

Leaching test

Leaching was performed according to a modified AWPA E11 Standard (AWPA, 2014). The modification consisted of an adjusted volume of water for the dimensions of the test samples. In short, five replicates of treated samples were submerged in 300 ml of deionized water in separate beakers per treatment group and then placed in a laboratory orbital shaker at 100 rpm for 6 hours. The leachate was then removed and replaced with fresh 150 ml of deionized water and shaken for an additional 24 hours. Subsequently, the leachate was removed and replaced with 150 ml of fresh deionized water and shaken for 48 hours. The previous step was repeated for a total period of 14 days.

After completion of the leachate collection, the treated blocks were air-dried, then oven-dried at 50°C to constant mass. The amount of chitosan remaining in the sample was corrected for the amount of lost extractives. The extractives were calculated from the difference between oven-dried masses of untreated samples and untreated leached control samples.

$$Retention_L\ (g/g) = \frac{(m_{0t}\text{-}m_{0c})}{m_0}$$

Where:

$Retention_L$ = g chitosan in leached samples per gram of oven-dried wood mass before treatment

m_{0c}=Oven-dry mass of samples post-leaching corrected for lost extractives, g

m_{0t} = Oven-dry mass of samples post-treatment, g

m_0 = Oven-dry mass of samples pre-treatment, g

$$m_{0c} = m_{0L} + m_{0e}$$

m_{0e} = mass of extractives lost, g

m_{0L} = oven-dry mass of samples post-leaching, g

Statistical analysis

Using the Statistical Analysis Software (SAS Institute), homogeneity of variance was tested within sample groups using the Levene's test, and Welch's ANOVA was used for establishing difference among samples for mortality, mass loss and chitosan retention levels upon leaching. Also, the LSMEANS statement was used to examine the differences between the means of the treatment groups. Welch's ANOVA does not assume equal variances within sample groups. The results are interpreted at 95% confidence interval (5% significance level).

Results and Discussions

Mass loss of wood blocks and termite mortality are displayed in Table 1. Acetic acid control samples showed higher mass loss compared to the water control samples. Modification of the wood by the acetic acid could have possibly rendered the wood softer and easier to chew for termites. The mass loss due to termite damage for the chitosan-treated samples generally decreased with higher chitosan concentration, although mass losses within the chitosan-treated groups were not significantly different. Treated samples, however, exhibited significantly lower mass loss compared to controls.

Treatments with chitosan concentration levels of 3% and above showed highest termite mortality (Table 1). Termites exposed to 1% chitosan solution did not show significant difference in mortality when compared to control samples. The 2% chitosan treatment mortality was intermediate and significantly different from the blocks treated with 1% and 3% chitosan concentrations. During the two weeks of the test, 100% mortality of termites was observed in the jars with 4% and 5% chitosan-treated wood samples, and 98.3% mortality in jars with 3% chitosan-treated wood samples.

Table 1: Retention, mass loss, and percent termite mortality of wood blocks treated with different percent chitosan solutions*

Treatment	**Retention (g chitosan/g oven-dried wood)** $\bar{x} \pm SD$	**Mass loss (g oven-dried wood)** $\bar{x} \pm SD$	**Mortality (%)** $\bar{x} \pm SD$
25% Acetic acid	0.009 ± 0.001 A	0.404 ± 0.062 A	0.0 ± 0.0 A
Water	0.000 ± 0.000 A	0.311 ± 0.050 B	0.2 ± 0.1 A
1% Chitosan	0.033 ± 0.005 B	0.192 ± 0.012 C	2.9 ± 1.5 A
2% Chitosan	0.059 ± 0.008 C	0.181 ± 0.019 C	6.2 ± 1.4 B
3% Chitosan	0.077 ± 0.010 D	0.141 ± 0.034 C	98.3 ± 2.3 C
4% Chitosan	0.088 ± 0.004 D	0.112 ± 0.025 C	100 ± 0.0 C
5% Chitosan	0.089 ± 0.014 D	0.121 ± 0.017 C	100 ± 0.0 C

* Means within the same column followed by different letters are significantly different ($p<0.05$).

Table 2: Effect of leaching on chitosan retention and percent chitosan leached

Treatment	**Pre-Leaching Retention (g chitosan/g oven-dried wood)** $\bar{x} \pm SD$	**Post-Leaching Retention (g chitosan/g oven-dried wood)** $\bar{x} \pm SD$	**Percent Chitosan Leached (%)** $\bar{x} \pm SD$
25% Acetic Acid	--	--	--
Water	--	--	--
1% Chitosan	0.024 ± 0.001 A	0.000 ± 0.002 A	100 ± 0.0 A
2% Chitosan	0.031 ± 0.003 A	0.023 ± 0.003 B	25.1 ± 4.6 B
3% Chitosan	0.070 ± 0.005 B	0.023 ± 0.004 BC	61.3 ± 3.9 C
4% Chitosan	0.083 ± 0.006 C	0.033 ± 0.003 C	60.4 ± 2.8 C
5% Chitosan	0.084 ± 0.008 C	0.030 ± 0.005 C	64.9 ± 3.5 C

* Means within the same column followed by different letters are significantly different (p<0.05).

Upon leaching, the amount of chitosan remaining in the wood varied among treatment groups. As shown in Table 2, 4% and 5% chitosan-treated samples showed the highest chitosan retentions before leaching. Samples treated with 3%, 4%, and 5% chitosan exhibited the highest chitosan retentions after leaching and exhibited the highest percentage of chitosan lost from leaching. ANOVA results indicated that the 1% and 2% chitosan-treated samples had similar retentions of chitosan upon treatment, but 2% treatments leached less. Unexpectedly, the amount of chitosan left in the samples treated with 3% chitosan solutions after leaching did not significantly differ from the amounts of chitosan remaining in the leached samples treated with 4% and 5% chitosan solutions.

However, none of the treated wood samples retained enough chitosan to provide protection against termites based on the toxicity results. As shown in Table 1, the 3% chitosan-treated samples retained approximately 0.077 g chitosan per gram of oven-dried wood and caused 98.3% mortality in a two-week period (Table 1). Upon leaching, although 4% and 5% chitosan solutions-treated samples showed the highest amounts of retained chitosan, levels dropped to 0.03 g chitosan per gram of oven-dried wood (Table 2). The latter retention was similar to the 0.03 g chitosan per gram of oven-dried wood found in the1% chitosan solution treatment, which caused only about 2.9% mortality in a two-week period (Table 1).

Chitosan has shown promise as an effective biocide against wood degrading fungi (Allan & Hadwiger, 1979). Chitosan has also shown to be effective against termites at the levels

comparable to acetylated wood (Imamura & Nishimoto, 1986; Wang et al., 2002); however, chitosan is below the threshold levels for protection post-leaching. Further investigations are needed to decrease chitosan leaching from wood without reducing its effectiveness. Also, it would be desirable to examine chitosan's termiticidal mechanism and the adaptability of termite hindgut flora to 1% and 2% chitosan concentration levels.

Conclusions

Novel preservatives effective for long-term protection of wood are being investigated as substitutes for traditional preservatives. Environmentally friendly preservatives are increasingly being studied due to the toxic effects of traditional preservatives on non-target organisms. Chitosan is an environmentally friendly, biodegradable alternative that could potentially increase the lifespan of wood and be safe for non-target organisms in the ecosystem. Chitosan is a non-toxic molecule with a median lethal dose (LD50) to salt and sugar (Arai et al. 1968) and has an LD50 of over 16 g/kg in mice (Hirano 1996). Results in this study show that chitosan has a potential termiticidal effect on *R. flavipes* when the termite workers are exposed to wood treated with solutions of chitosan above 3% concentration in dry conditions. In addition, retentions of 0.077 g chitosan per gram of oven-dried wood are estimated to be the toxic threshold for *R. flavipes*. Mass loss of the chitosan-treated samples was significantly reduced compared to the water and acetic acid-treated control groups. Leaching of chitosan-treated wood samples resulted in a decrease of chitosan retention to below the toxicity threshold level of 0.077 g chitosan per gram of oven-dried wood. The leaching results indicate that chitosan treatment is not appropriate for wood used in outdoor wet conditions.

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