

Antifungal Activity and Fire Resistance Properties of Nano-Chitosan Treated Wood

Laya Khademibami,^{a,*} H. Michael Barnes,^a Dragica Jeremic,^a Rubin Shmulsky,^a Keith Bourne,^b and Seyed Abolghasem Fatemi^c

Fungal decay and fire resistance properties of wood treated with nano-chitosan-TPP particles were investigated. Quaternized and non-quaternized nano-chitosan particles crosslinked with a commercial fire-retardant, tripolyphosphate, were prepared from low molecular weight chitosan (with a molecular weight of 50 to 190 kDa). Different treatments were performed on southern yellow pine wood samples *via* a vacuum impregnation process for both quaternized and non-quaternized nano-chitosan-TPP particles with a concentration ratio of 12% to 4.8% (nano-chitosan to TPP). Both the leached and unleached treated wood samples were exposed to brown rot (*Gloeophyllum trabeum*) and white rot (*Trametes versicolor*) fungi according to AWWA standard E10-16. The flammability test was performed with a cone calorimeter according to ASTM standard E1354-15. The heat release rate and the mass loss rate were measured. The results of the fungal tests indicated that the quaternization of the nano-chitosan particles resulted in a reduced mass loss in the pine samples when exposed to *Trametes versicolor* under leached conditions. Additionally, without the quaternization of the nano-chitosan particles, the mass loss in the pine samples was reduced when exposed to *Gloeophyllum trabeum* under unleached conditions. The production of nano-chitosan-TPP particles had a significant effect on the fire-retardant activity of the treated wood samples.

Keywords: Nano-chitosan particles; Fungal decay; Fire retardant; Southern yellow pine; Wood preservative

Contact information: a: Department of Sustainable Bioproducts, Mississippi State University, Box 9820, Mississippi State, MS 39762 USA; b: US Forest Service, Forest Products Laboratory, Madison, WI 53726 USA; c: Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762 USA; * Corresponding author: Lk475@msstate.edu

INTRODUCTION

Wood is degraded by microorganisms such as fungi, termites, and bacteria. Likewise, in the presence of heat and oxygen, wood is flammable, which limits its applications. A combination of fire retardants and antifungal preservatives provide resistance to both fire and fungi, which is favorable for many applications. Currently, there is an increased attention in the production of sustainable wood and paper-based goods due to environmental concerns and global environmental challenges. New trends in the wood industry include exploring more environmentally acceptable products (McIntrye *et al.* 2007). The industrial use of environmentally friendly wood preservative systems is becoming increasingly common. In addition, other wood preservatives, such as chromated copper arsenate (CCA) and organic copper mixtures, have been voluntarily withdrawn from certain markets; this withdrawal increases the need to improve wood preservation technology (McIntrye *et al.* 2007).

In the past decade, chitosan, a carbohydrate with antimicrobial properties, has drawn attention due to its beneficial characteristics (Eikenes *et al.* 2005; Torr *et al.* 2005; Hussain *et al.* 2012; Hussain *et al.* 2013). The distinctive chemical, physicochemical, and biological properties of chitosan, especially the presence of reactive functional groups, *e.g.*, amine and hydroxyl groups, enables it to be easily modified. In addition, chitosan is a nontoxic, biodegradable, and renewable material (Zhang *et al.* 2012; Varun *et al.* 2017), with antibacterial and antifungal characteristics (Seong *et al.* 1999; Hussein *et al.* 2012). Chitosan is readily available, inexpensive, and it is easy to prepare because it is made with chitin, which is a byproduct of crustacean shells, *e.g.*, shrimp, lobster, crawfish, and crab (Allan and Hadwiger 1979). The structure of chitin is similar to cellulose, and in the past two decades it has been used in agricultural, industrial, and medical fields (Kifune 1992). Due to the antimicrobial action of the amino group at the C-2 position found in chitosan, it is also known as an antibacterial and antifungal polysaccharide (Seong *et al.* 1999). Hussein *et al.* (2012) investigated the antifungal activity of chitosan against *Basidiomycetes* and reported that the growth of fungi was inhibited by the chitosan oligomers. This data indicated that the antifungal property of chitosan was shown to increase when the chitosan oligomers were used. The chitosan solution has hydrodynamic characteristic due to diffusion of solvent into chitosan resulted in aggregated polymer. This aggregated polymer is becoming enlarged into the solvent until it becomes bunches of entangled molecules called ‘hydrodynamic’ sphere or ellipsoid. (Tager 1972; Chattopadhyay and Inamdar 2010; Chattopadhyay and Inamdar 2012).

Sodium tripolyphosphate (TPP) has been used to scale the hydrodynamic volume of chitosan down to the nano level. Sodium tripolyphosphate (TPP) has been used to prepare a nano-scale product in combination with chitosan. It is possible, for a given molecular size of chitosan, to scale down the hydrodynamic volume to the nano level by means of ionotropic gelation using a suitable cross-linking agent, such as sodium tripolyphosphate (TPP) (Chattopadhyay and Inamdar 2010). It is well documented that TPP has shown positive effects on improvement in fire retardancy of cotton fabric, cotton textiles, cellulose, and cellulose derivatives in several field of studies (Kandola *et al.* 1996; Charuchinda *et al.* 2005; Khaled 2008).

As one of the determining factors of efficacy of a wood fungicide is its diffusion rate into the nanopores of wood cell walls, differing molecular weight (MW) chitosan samples were previously compared in terms of wood treatment efficacy. The initial penetrability of different MWs into the cell walls can be misinterpreted when the measured amount of chitosan in wood is estimated from the leached samples, because the low MW molecules diffuse out of the cell walls easier unless there is a strong interaction between the chitosan and the wood tissue. Hence, the penetration efficiency of the chitosan oligomers assessed *via* SEM for the unleached samples showed the presence of these small polysaccharides in the cell walls, as well as in the cell lumens (Singh *et al.* 2010). The effects of nano-chitosan-TPP particles on fungal and fire resistance has not been previously investigated. Therefore, the objective of this study was to determine the effects of nano-chitosan-TPP particles on the fungal and fire resistance of leached and unleached southern yellow pine wood.

EXPERIMENTAL

The methodology of this study focused on two main parts, the preparation of the nano-chitosan-TPP particles as a wood preservative and the treatment of the wood samples with either the nano-chitosan oligomers and N,N,N-trimethylchitosan (TMC) oligomers alone or in combination with TPP.

Treatment Layout and Sample Preparation

The preparation of the nano-chitosan-TPP particles was performed *via* the depolymerization of the chitosan into oligomers, the quaternization of the chitosan oligomers (preparation of N,N,N-trimethylchitosan), the binding of TPP to the nano-chitosan particles, the preparation of the wood samples, and the impregnation of the wood samples with nano-chitosan-TPP particles according to the method described by Khademibami *et al.* (2020). The 17 treatment combinations used in this study were presented in Table 1.

Table 1. Wood Treatments

	Main Treatments	Positive Control	Negative Control
Chemical	1: Nano-chitosan with TPP ¹ in 1% acetic acid	2: Nano-chitosan without TPP in 1% acetic acid	9: 1% acetic acid
	3: Nano-chitosan with TPP in 0.1 mol acetic acid + 0.2 mol NaCl	4: Nano-chitosan without TPP in 0.1 mol acetic acid + 0.2 mol NaCl	
	10: Quaternized-nano-chitosan with TPP in 1% acetic acid	11: Quaternized-nano-chitosan without TPP in 1% acetic acid	
	12: Quaternized-nano-chitosan with TPP in 0.1 mol acetic acid + 0.2 mol NaCl	13: Quaternized-nano-chitosan without TPP in 0.1 mol acetic acid + 0.2 mol NaCl	
Enzymatic	5: Nano-chitosan with TPP in 1% acetic acid + laccase ² (1 mg/mL) + HQ ³ (10 mmol) before ⁴	6: Nano-chitosan without TPP in 1% acetic acid + laccase (1 mg/mL) + HQ (10 mmol) before	
	7: Nano-chitosan with TPP in 1% acetic acid + laccase (1 mg/mL) + HQ (10 mmol) simultaneously ⁵	8: Nano-chitosan without TPP in 1% acetic acid + laccase (1 mg/mL) + HQ (10 mmol) simultaneously	
	14: Quaternized-nano-chitosan with TPP in 1% acetic acid + laccase (1 mg/mL) + HQ (10 mmol) before	15: Quaternized-nano-chitosan without TPP in 1% acetic acid + laccase (1 mg/mL) + HQ (10 mmol) before	
	16: Quaternized-nano-chitosan with TPP in 1% acetic acid + laccase(1mg/mL) + HQ (10 mmol) simultaneously	17: Quaternized-nano-chitosan without TPP in 1% acetic acid + laccase (1 mg/mL) + HQ (10 mmol) simultaneously	
¹ TPP: Tri poly phosphate, a commercial fire retardant ² Laccase: copper-containing oxidase enzymes found in many plants, fungi, and microorganisms ³ HQ: Hydroquinone, a mediator of laccase modification. A mediator is used to oxidize, and subsequently increase the reactivity of wood and promote the binding of chitosan to wood ⁴ Before the preservative treatment: first, the wood was treated with laccase and hydroquinone. Then, the wood was treated with nano-chitosan particles. ⁵ Simultaneously: The wood was treated with laccase + hydroquinone and nano-chitosan particles at the same time.			

Chitosan has been shown to be insoluble in water as well as other organic and inorganic solvents (Rinaudo 2006; Sankararamakrishnan and Sanghi 2006). It is well documented that acetic acid resulted in increased solubility of chitosan (Anthonsen and Smidsroed 1995; Rinaudo 2006; Sankararamakrishnan and Sanghi 2006). In the current study acetic acid was used in order to suspend the nano-chitosan in the solution to make nano-chitosan-TPP particles solutions to treat wood by vacuum impregnation process in all 17 treatments.

Beside the insolubility of chitosan oligomers, these oligomers have been shown to have polycationic characteristics, leading to being ionically crosslinked and aggregated with other polyelectrolytes (Berger *et al.* 2004b). Thus, ionically crosslinking of chitosan oligomers resulted in restriction in functionality of chitosan (Berger *et al.* 2004a; Correia *et al.* 2013). Monovalent salts including NaCl has been shown to improve the chitosan crosslinking processes, leading to stabilization of chitosan nanoparticles crosslinked with TPP (Jonassen *et al.* 2012).

Fungal Resistance of the Nano-chitosan-TPP Treated Wood

The wood samples were prepared for the leaching test as described by Khademibami *et al.* (2020) before being subjected to fungal activity. Six replicates per fungi species for both the leached and unleached treatments were prepared. The specimen dimensions were 14 mm x 14 mm x 14 mm. The specimens were then exposed to brown-rot fungus (*Gloeophyllum trabeum*) and white rot fungus (*Trametes versicolor*) in the soil block tests. For the testing procedure, 4.5 g of agar, 6.0 g of malt extract, 0.6 g of yeast extract (Sigma-Aldrich, St. Louis, MO), and 300 mL of deionized water were mixed to prepare fungal medium, and the fungal medium was poured into Petri dishes (150 mm × 20 mm). The dishes were inoculated with the test fungi and incubated at 27 °C and 80% relative humidity (RH) for 10 d. These fungi used for soil block tests were selected according to AWWPA standard E10-16 (2016). The soil was collected from a Dorman test site at Mississippi State University, in the John W. Starr Memorial Forest. After an incubation period of 8 weeks for the brown rot samples and 16 weeks for the white rot samples, the infected samples were weighed, oven-dried, and then reweighed to determine the dry mass loss (ML) according to Eq. 1,

$$M_L = \frac{W_i - W_d}{W_i} \cdot 100 \% \quad (1)$$

where M_L is the dry mass loss (%), W_i is the dry weight before decay (g), and W_d is the dry weight after decay (g).

Fire Resistance of the Nano-chitosan-TPP Treated Wood

The flammability of the treatment samples was examined *via* cone calorimetry performed at the Forest Product Laboratory (FPL) in Madison, WI. The oven-dried mass was measured *via* oven drying of the wood samples to a constant mass at 105 °C. Then, the samples were moved to a conditioning room at 21 °C and approximately 50% RH to achieve an equilibrium moisture content. The specimens tested in the cone calorimeter were nominally 100 mm x 100 mm, and could be no smaller than 98 mm x 98 mm. To meet the recommended sample size, 7 of the nominally 14 mm x 14 mm x 100 mm specimens were glued together to form a specimen that was 98 mm x 100 mm x 14 mm. Approximately, 1 g of phenol-resorcinol formaldehyde (PRF) adhesive (Hexion Corporation, Columbus, OH) was used per cone sample.

After the adhesive was cured, the specimens were tested in the cone calorimeter. Three specimens from each treatment group (as shown in Table 2) were tested in the calorimeter in a horizontal position at an irradiance of 50 kW/m² according to ASTM E1354-15 (2015). The average moisture content for the control samples was 6.7%, while the samples treated with nanochitosan-TPP had an average moisture content of 6.3% before performing the fire test. The heat release rate (HRR), mass loss rate (MLR), and time to ignition were measured. The cone calorimeter test data was obtained as a function of time.

Table 2. Treatments for Fire Test

No	Treatments	
1	Control	1% Acetic Acid
2		Nano-chitosan without TPP in 1% Acetic Acid (Conc. of chitosan oligomers: 3%)
3	Main Treatments	Nano-Chitosan & TPP in 1% Acetic Acid (Conc. chitosan olig.: 12% & TPP: 4.8%)
4		Nano-chitosan without TPP in 1% Acetic Acid (Conc. of chitosan oligomers: 12%)
5		Nano-Chitosan with TPP in 1% Acetic Acid + Laccase (1 mg/mL) + HQ (10 mmol) Before (Concentration of chitosan oligomers: 12% and TPP: 4.8%)
6		Nano-Chitosan without TPP in 1% Acetic Acid + Laccase (1 mg/mL) + HQ (10 mmol) Before (Concentration of chitosan oligomers: 12%)

Statistical Analysis

The experimental design was a completely randomized design, and the data for the fungal tests were analyzed using two-way ANOVA in a 2 x 17 factorial arrangement of the treatments to test for the main and interactive effects of leaching (leached and unleached) and the 17 nano-chitosan-TPP treatments. The statistical analysis was performed with SAS 9.4 (SAS Institute, Cary, NC) to generate the linear mixed models (PROC GLIMMIX) used for the fungi data analysis. The following model was used for analysis of the fungi data, as shown in Eq. 2,

$$Y_{ij} = \mu + L_i + N_j + (LN)_{ij} + E_{ij} \quad (2)$$

where μ is the population mean, L_i is the effect of the leached and unleached treatments ($i = 1$ to 2), N_j is the effect of the nano-chitosan-TPP treatments ($N = 1$ to 17), $(LN)_{ij}$ is the interaction of each of the leached and unleached treatments with the nano-chitosan-TPP treatments, and E_{ij} is residual error. Fungal resistance data were further tested *via* contrast analysis using the SAS 9.4 MIXED procedure. Effects of the quaternized nano-chitosan-TPP treatments *vs.* the non-quaternized nano-chitosan-TPP treatments, the control *vs.* the quaternized nano-chitosan-TPP treatments, and the control *vs.* the non-quaternized nano-chitosan treatments in both the leached and unleached samples were also tested. The fire test data were analyzed with a one-way ANOVA using the SAS 9.4 Proc GLM procedure. Differences were considered significant with a p-value less than or equal to 0.05.

RESULTS AND DISCUSSION

Fungal Resistance of the Nano-chitosan-TPP Treated Wood

There was significant interaction between the treatment and the leaching in terms of ML for both the brown rot samples (p-value was less than 0.0001), as shown in Fig. 1, and the white rot samples (p-value equaled 0.0009), as shown in Fig. 2.

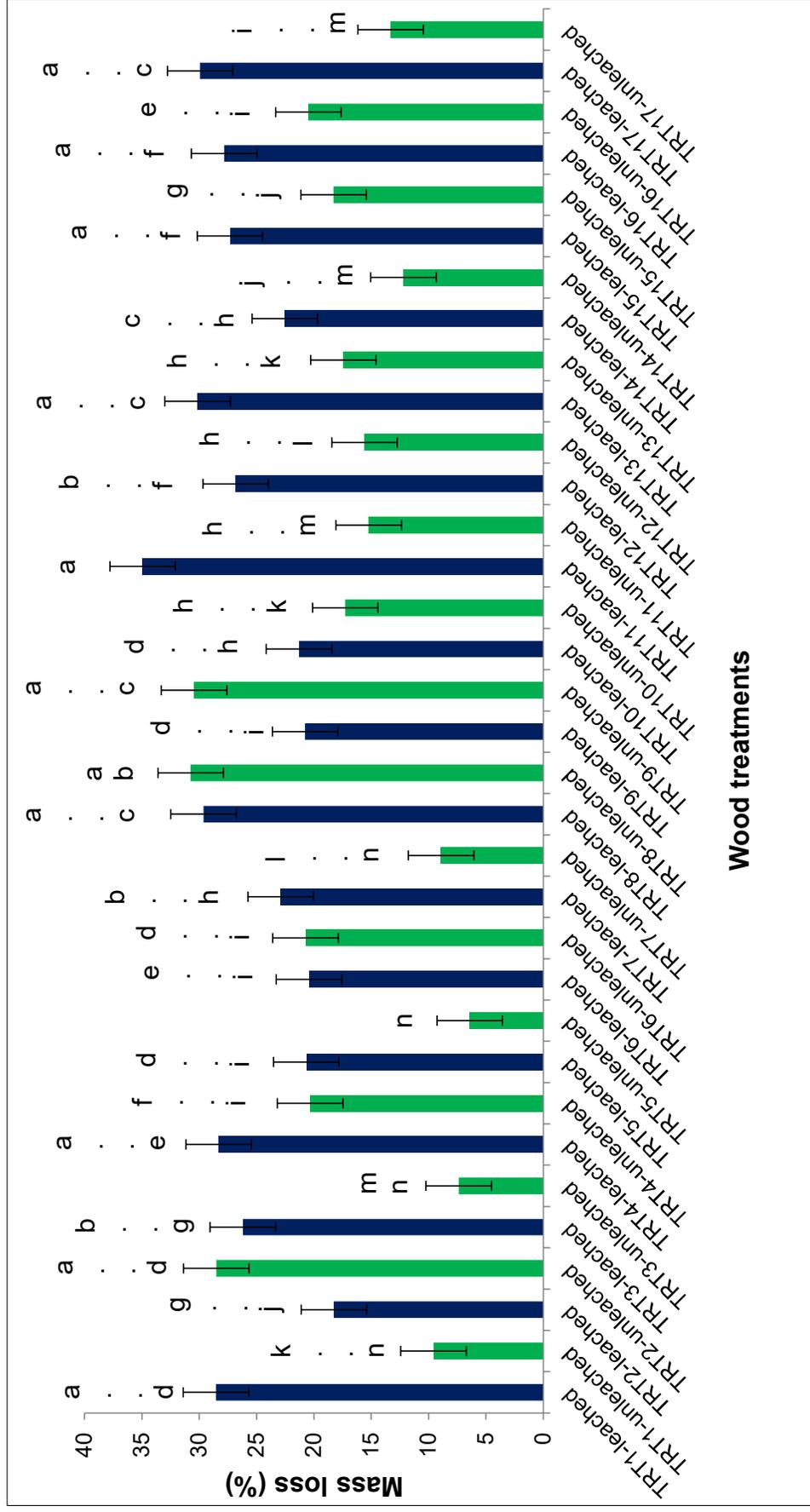


Fig. 1. The mass loss (ML) results of the 17 brown rot fungi (*Gloeophyllum trabeum*) wood treatments (leached and unleached samples). *All treatments from 1 to 17 (TRT1 to TRT17) were described in Table 1. In addition, treatments 1 to 17 were also tested in leached and unleached conditions which is illustrated in this figure. ^{a-n} Treatment means within the same column with no common superscripts are significantly different ($P < 0.05$).

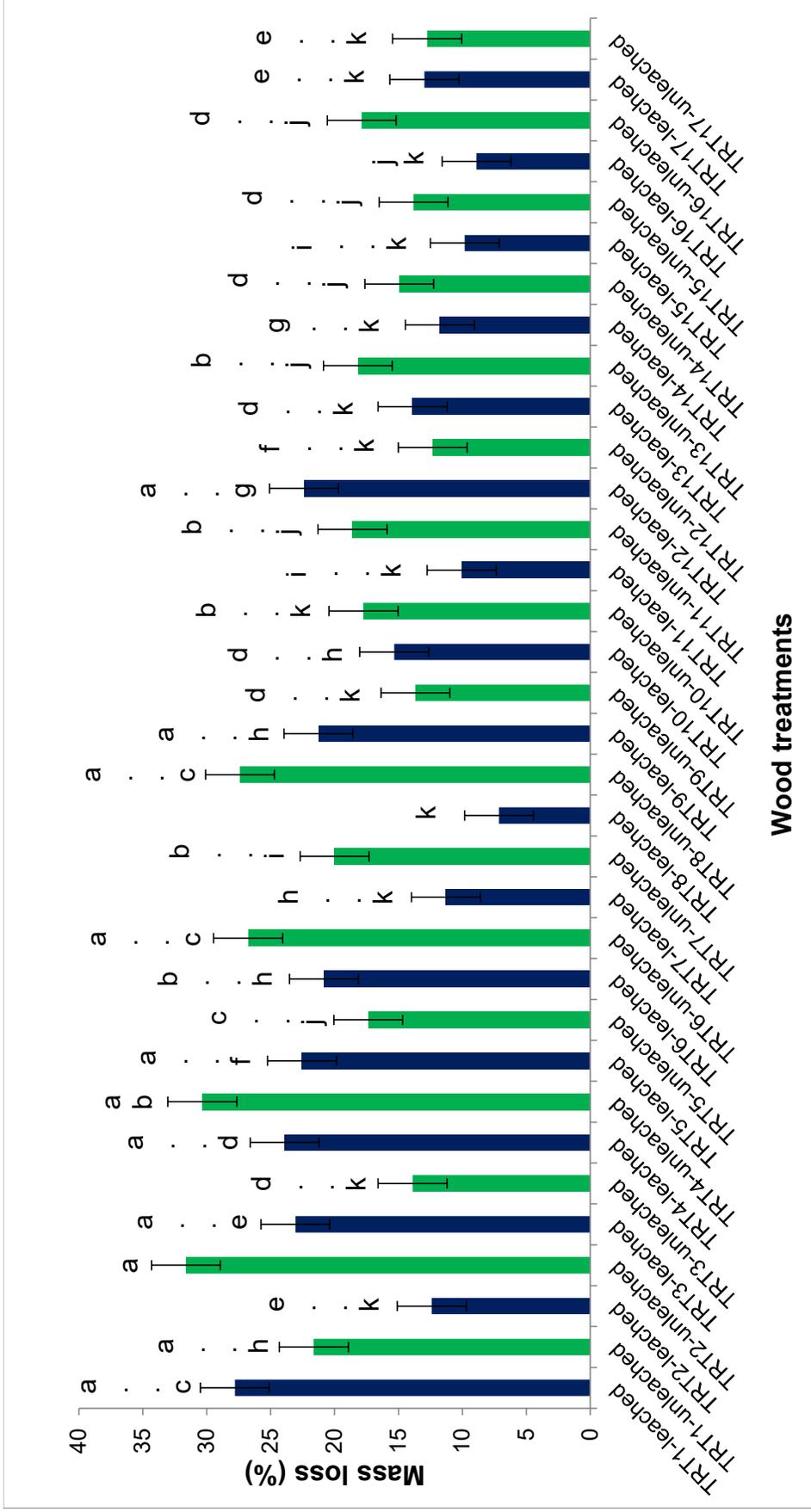


Fig. 2. The mass loss (ML) results of the 17 white rot fungi (*Trametes versicolor*) wood treatments (leached and unleached samples).

*All treatments from 1 to 17 (TRT1 to TRT 17) were described in Table 1. In addition, treatments 1 to 17 were also tested in leached and unleached conditions which is illustrated in this figure.

a-k Treatment means within the same column with no common superscripts are significantly different (P < 0.05).

For the unleached brown rot samples, the non-quaternized nano-chitosan-TPP in 1% acetic acid and LMS treatment (treatment 5) resulted in a lower ML when compared to the quaternized nano-chitosan treatments and the control. For the leached samples, the non-quaternized nano-chitosan without TPP in 1% acetic acid (treatment 2) had a lower ML in comparison to all the other treatments.

For the unleached white rot samples, the quaternized-nano-chitosan with TPP in 0.1 mol acetic acid and 0.2 mol NaCl (treatment 12) resulted in a lower ML when compared to the other treatments. However, the leached samples for treatment 12 had a higher ML in comparison to other samples. Furthermore, the leached samples with the non-quaternized nano-chitosan-TPP in 1% acetic acid and LMS treatment (treatment 8) resulted in a lower ML when compared to all the other treatments. However, the unleached samples with treatment 8 had a higher ML when compared to the quaternized nano-chitosan treatments as well as treatments 1 and 3.

The contrast analysis revealed that the non-quaternized nano-chitosan treatments had a lower (p-value equaled 0.05) ML for the leached samples when compared to the quaternized nano-chitosan treatment in the brown rot fungi. In addition, for the white rot samples, a lower ML was observed in the quaternized nano-chitosan treatment groups when compared to the non-quaternized nano-chitosan treatment groups, for both the leached samples (p-value equaled 0.01) and the unleached samples (p-value was less than 0.0001). For the brown rot samples, the ML in the unleached samples was decreased (p-value was less than 0.0001) for both the quaternized and non-quaternized nano-chitosan treatment groups in comparison to the control group (treatment 9), but there was no significant differences between the quaternized and non-quaternized nano-chitosan treatment groups and the control group in the leached samples.

The polycationic nature of chitosan has antifungal activity and is described by three mechanisms (Ing *et al.* 2012), including: (1) the positive charge of chitosan can react with the negative charge of fungi membranes, which are phospholipid components. This reaction causes the fungi membrane to be more permeable and consequently, due to the leakage of cell content, the fungi will die; (2) fungi need nutrients to grow. Chitosan makes these nutrients inaccessible for fungi by binding to the trace elements; and (3) chitosan can prohibit the production of necessary proteins and enzymes for fungi by penetrating the fungi cell walls and binding to its DNA. As such, the chitosan binding to DNA can prevent the synthesis of mRNA, as well as the synthesis of essential enzymes and proteins for the fungi. There are many factors that can influence the antifungal activity of chitosan and its derivatives, including the original source of chitosan, the molecular weight, the degree of deacetylation, the way that products are synthesized, its substituent sites, and the types of fungi and bacteria it interacts with (Xu *et al.* 2010; Ing *et al.* 2012). Chitosan has been previously shown to decrease the ML in Sugi wood exposed to *Tyromyces palustris* and *Trametes versicolor* in comparison to untreated Sugi wood (Kobayashi and Furukawa 1995). Additionally, similar results also reported the antifungal activity of chitosan in pine wood when they were exposed to *Cinifera puteana*, *Postia placenta*, and *Trametes versicolor* (Eikenes *et al.* 2005; Larnoy *et al.* 2006). Laccase contains copper phenol oxidase functioning in oxidize electron-rich substrates of phenolic and non-phenolic origin (Bourbonnais *et al.* 1997). Laccases contains four copper atoms that in the native form of laccase become fully oxidized (Cu²⁺) in order to decarboxylate, demethylate and demethoxylate of phenolic, and methoxy phenolic acids leading to the initial steps in lignin degradation (d'Acunzo *et al.* 2002). It is well documented that laccase has been used to degrade lignin through LMS (Christopher *et al.* 2014).

A mediator is a small chemical compound that plays role as a carrier of electrons between the laccase and the substrate (lignin, aromatic compounds, etc.) (Li *et al.* 1999). According to the Morozova *et al.* (2007), the ideal mediator was described as non-toxic, economic, and efficient compound with stable form. In current study, HQ was used that has been previously found to be the best mediator for LMS (Khademibami *et al.* 2020). In this study, brown rot fungi treatment 5, which was first treated with a laccase-mediator system (LMS) and then treated with nano-chitosan-TPP particles in a 1% solution of acetic acid, resulted in a lower ML in unleached wood samples. For brown rot fungi treatments 2, 4, 6, and 8 that excluded TPP had a negative impact on the ML in the non-quaternized unleached samples when compared to treatments 1, 3, 5, 7, which included TPP. The TPP had been previously reported as a commercial fire-retardant (Abraham 1972; Charuchinda *et al.* 2005). However, the anti-fungi activity of TPP had not been previously reported. Thus, this result indicated that the combination of nano-chitosan and TPP promoted the anti-fungal activity of chitosan when the pine wood sample was exposed to *Gloeophyllum trabeum* under unleached condition. For the white rot samples, quaternization lowered the ML under unleached conditions. Ing *et al.* (2012) investigated the antifungal activity of nanoparticles that were prepared from high, low, and trimethyl chitosan or TMC (quaternized chitosan) against three fungi, *Candida albicans*, *Fusarium solani*, and *Aspergillus niger*. Ing *et al.* (2012) reported that chitosan derivatives, *e.g.*, TMC, were highly soluble in water in comparison to chitosan itself and had weak antimicrobial activities. In contrast, the antibacterial activity of N,N,N-trimethyl chitosan against *Staphylococcus aureus* and *Escherichia coli* was investigated by Xu *et al.* (2010). The cited authors concluded that TMC was more active against *S. aureus* and *E. coli* in comparison to chitosan. Therefore, quaternization might have not been effective in lowering the ML of the samples for all pathogens and environmental conditions. In the current study, the quaternization of nano-chitosan was shown to reduce the ML in pine when exposed to *Trametes versicolor* under leached conditions. Additionally, without the quaternization of nano-chitosan, the ML in pine was reduced when exposed to *Gloeophyllum trabeum* under unleached conditions.

Fire Resistance of the Nano-chitosan-TPP Treated Wood

The heat release rate (HRR) and mass loss rate (MLR)

Fire-retardant treatments often reduce the flammability of wood by reducing the amount of heat released during the initial stages of fire. The spread of fires and volatiles released by the wood during fire exposure decreases with the application of a fire-retardant. The HRR is an essential parameter in fire testing. It is widely used to assess the flammability of a material and describe its behavior when subjected to fire. The HRR is based on the oxygen consumption during combustion and its relationship with the amount of heat released. This heat release rate is the total rate, as a function of time.

Table 3 illustrated the fact that the peak HRR of all specimens behaved similarly in the control groups and the treated group tests. There were no significant effects from the treatments on the peak HRR. There were no significant differences between the nano-chitosan-TPP treatments samples (both with TPP and without TPP) and the control samples in terms of the peak HRR. In addition, there were no significant differences between the nano-chitosan-TPP treatment samples (both with TPP and without TPP) and the control samples in terms of the peak MLR (as shown in Table 3).

While the peak HRR and peak MLR are important values, they are each only a single point during each test, and the overall response of the material to fire can be missed if other metrics are not considered. In addition to the peak values, it is common to look at the total heat release, the total mass loss, and the time to ignition. The total heat release is an integration over time of the HRR curve and the total mass loss is simply the mass change in the sample over the test time. Additionally, comparing the whole HRR or MLR curve among the samples can often show differences.

While there did not seem to be any significant difference between the control and treatment samples when comparing the peak HRR or peak MLR, as shown in Table 3, there appeared to be a difference in the total heat released, total mass loss, as well as a minor difference in the time to ignition. The total HRR was decreased in treatments 3, 4, and 5 when compared to the control treatment groups (1 and 2). Additionally, treatment 3 and 5 (which included TPP and a high concentration of nano-chitosan particles) resulted in a lower total MLR when compared to control group treatments excluding TPP. The treatment composed of only a low concentration of nano-chitosan particles (3%) had a higher ignition time value when compared to the treatment samples containing high concentrations of nano-chitosan particles, either alone or in combination with TPP. According to Dietenberger *et al.* (2012), fire-retardant treated wood reduced the initial HRR and MLR and increased the residual mass fraction, which led to lower average effective heat of combustion values and longer ignition times.

Phosphorus-based fire retardants (PFR) such as TPP are environmentally friendly products with low toxicity (Kandola *et al.* 1996). Also, it has been indicated that the PFR are highly effective fire retardants for cellulose and cellulose derivatives. These PFR compounds have been also shown to promote dehydration and char formation under combustion (Kandola *et al.* 1996). The presence of amino nitrogen in nano-chitosan particles provides a synergistic activity with the phosphoric acids by promoting the formation of intumescent chars (Kandola *et al.* 1996; Khaled *et al.* 2008). In addition to synergistic effect between chitosan and TPP, the durability of the fire retardancy to washing in the cotton fabric has been shown to increase when chitosan is combined with sodium polyphosphate in comparison to the untreated cotton fabric. The combination of chitosan with TPP has also been shown to result in the formation of film layer covering on the fabric surface. However, the exhibition of a film layer was not observed when chitosan alone was used (Charuchinda *et al.* 2005).

The fire-retardant activity has been shown to be restricted when higher concentration of chitosan combined with TPP. High concentration of chitosan (greater than 3%) decreased the activity of the flammability and performance properties of TPP (Khaled *et al.* 2008). It should be considered that there is potential to increase the fire-retardant activity by increasing the TPP concentration. However, as described by Huang *et al.* (2009), the best ratio for appropriate interaction between the nano-chitosan and TPP particles was 5 to 2, which was utilized for the current study. Thus, an increase in the ratio of nano-chitosan to TPP particles may exhibit a negative effect on the penetration of nanoparticles into the wood cell walls, due to the agglomeration of particles in high ratio. Therefore, the fire-retardant activity results revealed that 5:2 ratio of nano-chitosan to TPP particles, in general, improved the fire resistance characteristics for pine wood samples.

Table 3. Means of the Peak HRR, Peak MLR, Total HRR, Total MLR, and Ignition Time for Each Treatment

Treatment	Peak HRR	Total HRR	Peak MLR	Total MLR	Ignition Time
	kW/m ²	MJ/m ²	g/s	g	s
1	267	96.3 ^{ab}	0.167	66.7 ^{ab}	28.0 ^{ab}
2	286	98.8 ^a	0.180	69.1 ^a	31.7 ^a
3	266	79.6 ^c	0.231	62.7 ^c	25.0 ^b
4	298	84.3 ^c	0.370	64.9 ^b	27.0 ^b
5	352	80.2 ^c	0.271	63.3 ^c	24.3 ^b
6	297	93.7 ^b	0.208	69.1 ^a	25.0 ^b
SEM	41.2	2.27	0.1002	1.24	1.89
P-value	0.387	Less than .0001	0.412	0.001	0.0249

* a-c Treatment means within the same column with no common superscripts are significantly different ($P < 0.05$).

*All treatments were described in Table 2.

CONCLUSIONS

1. The impact of nano-chitosan-TPP particles on the resistance of pine wood to brown-rot fungus *Gloeophyllum trabeum*, white rot fungus *Trametes versicolor*, and fire was investigated. In addition, the efficacy of nano-chitosan-TPP particles as an environmentally friendly wood preservative agent, for both indoor and outdoor applications, was determined.
2. The investigation into the antifungal activities of nano-chitosan-TPP particles, after vacuum impregnation of the nanoparticle solution into southern yellow pine samples, indicated that treatment 5 (which was nano-chitosan-TPP in 1% acetic acid and LMS), showed promising results at lowering the ML when exposed to *Gloeophyllum trabeum* under unleached conditions. A lower ML was observed in quaternized treatments when exposed to *Trametes versicolor* under either leached or unleached conditions.
3. The results indicated that quaternization might have not been effective in lowering the ML for all pathogens and environmental conditions in comparison to non-quaternized treatments. Herein, the quaternization of nano-chitosan was shown to reduce the ML in pine wood when exposed to *Trametes versicolor* under leached conditions.
4. Nano-chitosan-TPP particles improved the fire-retardant activity of treated wood. The results of the current study demonstrated that TPP particles might be a suitable candidate as an effective fire-retardant. Therefore, further research is required to investigate the physical and chemical changes of the wood materials in response to the nano-chitosan-TPP particles.

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