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Original Article

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Resistance of TiO_2-treated *Eucalyptus botryoides* Wood to the Fungus *Ganoderma applanatum*

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ABSTRACT

The aim of the present study is to investigate the resistance of *Eucalyptus botryoides* treated with TiO_2 particles to attack by the fungus *Ganoderma applanatum*. The Bethel treatment method was applied to wood specimens (25 × 25 × 9 mm) and compared to CCB. The wood was subjected to the accelerated decay test and characterized through Rockwell Hardness and Fourier Transform Infrared Spectroscopy (FT-IR). The TiO_2-treated wood showed lower degradation and greater resistance to the penetration of a steel sphere (Rockwell Hardness) than the untreated wood. In addition, the results of the TiO_2 treatment were statistically equal to those of CCB. The FT-IR analysis showed that the fungus degraded lignin and hemicellulose in untreated samples. The present results showed the TiO_2 efficiency in forming a protective layer on the cell wall and in preventing the development of microorganisms, a fact that verifies its fungicidal action on wood.

Keywords: wood preservation, titanium dioxide, wood degradation.

1. INTRODUCTION

Wood is a natural material of versatile and renewable use, whose peculiar features stand out against other materials. The genus *Eucalyptus*, which encompasses many species, supplies raw material to the timber sector in Brazil. The species *Eucalyptus botryoides* presents potential for use in the timber industry, especially for sawn wood, since its basic specific mass varies from medium to high (Delucis et al., 2014).

The most critical economic concern in the construction industry lies in the selection of less environmentally-aggressive methods in association with the prevention of wood deterioration caused by xylophagous agents (Salem et al., 2016). Among the existing agents, fungi, mainly those belonging to the wood decaying group, cause more damage due to their highly aggressive attack (Fabbri et al., 1997; Mesquita et al., 2009). These fungi produce enzymes able to degrade chemical constituents (cellulose, hemicellulose and lignin) in the cell wall, as well as to turn into simple compounds, which are easily metabolized by hyphae (Oliveira et al., 2005).

Overall, the disruption caused by fungi in the chemical bonds of wood leads to changes in the material properties. After fungal attack, the wood presents mass reduction, reduced compressive strength parallel to the fibers, as well as reduced impact strength, hardness, static flexion, and esthetic pattern modifications, which compromises the material's useful life (Stangerlin et al., 2013; Bari et al., 2015; Witomski et al., 2016) and versatility.

Several biocides have been successfully used to mitigate wood susceptibility to fungi over the years. However, some formulations, such as the Chromated Copper Arsenate (CCA), have suffered certain restrictions because they pose risks to the environment and to human health (Shabir Mahr et al., 2013). These risks increasingly compromise the use of the material, mainly when it comes to the final destination of the treated-wood residues.

Accordingly, the wood technology field has undergone changes, which were based on studies aimed at analyzing products and processes with less damaging potential (Brand et al., 2006). Thus, the global trend is toward products that show minimized toxic element emission and that are able to replace, mainly, arsenic and chromium.

The use of inorganic materials impregnated in the wood surface and/or in its inner part to protect it against biotic agents is currently being investigated (De Filpo et al., 2013; Shabir Mahr et al., 2013; Harandi et al., 2016). Titanium dioxide (TiO_2) is one of the most promising substances used as a wood preservative or as dispersed phase in the production of wood/ceramic composites.

Semiconductivity is one of the most important TiO_2 features; it presents a band gap between 3.0 and 3.2 eV, which corresponds to UV-region wavelengths, a fact that makes it photoactive (presenting photocatalytic activity under UV light) (Wunderlich et al., 2004). This property assures fungicidal and bactericidal action on the material used as substrate (Huang et al., 2000; De Filpo et al., 2013). In addition, TiO_2 is a polymorph presented as rutile, anatase or brookite; the first two structures are tetragonal and the third is orthorhombic.

The aim of the present study was to assess the resistance of *Eucalyptus botryoides* treated with commercial TiO_2 particles to attack by the white-rot fungus *Ganoderma applanatum* in order to investigate the effect of TiO_2 on the wood of hardwood species.

2. MATERIAL AND METHODS

2.1. Material obtainment and source

Eucalyptus botryoides specimens were obtained from four 60-year-old trees (approximate age) derived from a homogeneous stand located in Charqueadas region, Rio Grande do Sul State. The specimen dimensions were 25 × 25 × 9 mm - the smallest dimension followed the axial direction. They were stored in a controlled air-conditioned room (20 °C and 65% relative humidity) until reaching 12% equilibrium humidity. The overall mean of the basic specific mass (0.735 g/cm^3) of the woods used here was calculated according to the methodology by ASTM (1995).

The wood resistance to the accelerated decay resulting from *G. applanatum* fungal attack was assessed. The isolates used in the current study were provided by the Wood Biodegradation and Preservation Sector of the Forestry Products Laboratory (LPF - Laboratório de Produtos Florestais) of the Brazilian Forestry Service (SFB - Serviço Florestal Brasileiro), located in Brasília – DF. These isolates were stored in a Biological Oxygen Demand (BOD) incubator at 25 ± 3 °C, without relative humidity control and under 12-hour photoperiod.

2.2. Wood treatment

Different treatments were carried out using two preservative products - TiO_2 particles and Chromated Copper Borate (CCB). These products were individually applied in the absence and presence of light, totaling six treatments, namely: TiO_2 + light, TiO_2 + dark, CCB + light, CCB + dark, control + light, and control + dark.

The commercial TiO_2 from Sigma Aldrich was prepared at a concentration of 25 µg/ml (99% purity) in distilled water and its particles were used to impregnate the wood surface and inner part. X-Ray Diffraction analysis (D8 Advance from Bruker) was carried out at 0.02° step and 25-70° scan to check the impregnated material phase and crystallinity in order to compare them to the results obtained here.

The specimens subjected to the CCB treatment were treated with a homogeneous solution composed of distilled water containing 3% active ingredients such as sulfate copper (26%), sodium dichromate (63.5%) and boric acid (10.5%), according to the ABNT (1986).

All treatments were performed in a 30cm-long and 10cm-diameter laboratory autoclave. The specimens were introduced in the cylinder and treated through the Bethel process at maximum pressure 8 $kgf.cm^{-2}$, for 1.5 hours.

2.3. Accelerated decay test

The accelerated decay test using the *G. applanatum* fungus was conducted according to ASTM (2005). The fungal isolate was subcultured in potato-dextrose-agar (PDA) medium at the following ratio: 200g potato, 17g agar and 20g dextrose to 1000 mL distilled water. The properly sterilized culture medium was transferred to Petri dishes placed in a laminar flow chamber, wherein they received a disc containing fungal mycelium and were kept in BOD at 25 ± 3 °C, under 12-hour photoperiod, until the fungal colony developed over the dishes.

The experiment used 500 mL glass vials filled with 100 g soil (pH 5 and 40% retention capacity). This data was used to adjust soil moisture through the addition of distilled water until the soil reached 130% retention capacity.

After preparation, each vial received a support plate (dimensions 3 × 29 × 35 mm) made of sapwood belonging to species *Pinus elliottii*. The support plate was used as a substrate for the initial establishment of the fungal colony. The assembled vials were autoclaved for two 40-minute periods at 120 °C and 1 atm pressure at an interval of 24-hour. A disc (approximately 7 mm) containing the fungal colony from the PDA medium was aseptically transferred to the vials containing the soil and the support plates. Next, they were kept in an air-conditioned room under controlled conditions for 15 days, which was the time the fungal mycelium took to fully cover the support plate.

The treated and untreated specimens were oven dried at 50 °C until reaching constant weight in order to find the initial mass, before being exposed to the wood-decay fungus. After the initial mass was determined, the specimens were autoclaved for 1 hour at 120 °C and aseptically placed in contact with the fully-colonized support plate. The specimens were maintained under these conditions for 16 weeks. During this period, the reference wood (low density) showed mass loss greater than 50%. According to the ASTM (2005), the specimens should be kept in a dark environment during the aforementioned period. However, since TiO_2 presents photoactivity under light conditions, 6 samples were randomly subjected to the absence of light and 6 were subjected to a 12-hour photoperiod in each treatment, for comparison purposes.

After the fungal attack period was over, the specimens were withdrawn from the vials to remove their mycelia. Next, they were oven dried again at 50 °C until reaching constant weight in order to record their final mass.

The wood resistance to fungal attack was set according to mass loss, which was calculated through the means of the initial and final masses of the specimens at 0% humidity. The resistance rates were classified according to mean mass loss intervals, in compliance with the ASTM (2005), as shown in Table 1.

Table 1. Wood decay-resistance classes according to ASTM (2005).

Resistance class	Mass loss (%)	Residual mass (%)
Highly resistant	0-10	90-100
Resistant	11-24	76-89
Moderately resistant	24-44	56-75
Non-resistant	≥ 45	≤ 55

2.4. Rockwell hardness and chemical modification

In addition to the mass loss assessment, treated and untreated specimens were subjected to the Rockwell hardness mechanical test after the accelerated decay test. Based on the study by Stangerlin et al. (2013), a Digemess Durometer with 1/4-inch spherical penetrator was used at 100 Kgf (load) by following the technical specifications set for polymeric materials in the equipment table. Six (6) readings were taken at different points in the cross section of each specimen and the resistance rate was read on the analogue display.

The specimens were characterized through the Fourier-Transform Infrared Spectroscopy (FT-IR) technique to allow visualizing the chemical modifications taking place in the wood structure before and after the fungal attack. In order to do so, three specimens from each treatment were ground in a Willey-type mill and the material used for analysis was retained in a 60-mesh sieve. The equipment used in the analysis was configured to perform 32 absorbance scans at 4 cm^{-1} resolution and readings between 4000 and 600 cm^{-1}.

2.5. Statistical analysis

The experiment followed a completely randomized design, with six repetitions for each treatment. It was bifactorial – preservative products × luminosity (3 × 2) – and comprised 6 treatments, therefore with 36 specimens in total. The mass loss and Rockwell hardness rates were subjected to analysis of variance using the Statgraphics Centurion software in order to check significant differences through the F test. The Tukey test at 5% significance was used to test the means.

3. RESULTS AND DISCUSSION

Figure 1 shows the X-Ray Diffraction of the TiO_2 product, which was compared to the standards available in specific databases from the Joint Committee on Powder Diffraction Standards (JCPDS). The peaks identified at angles such as 27°, 37°, 39°, 42°, 44°, 54°, 56°, 63°, 64° and 69° corresponded to the standard crystallographic peaks of the indexed PDF 21-1276 datasheet. Results showed that the commercial TiO_2 impregnated in the wood was purely rutile, which is more stable and less photoactive than anatase.

The bifactorial interaction found here did not present statistically significant differences, i.e., the luminosity variation did not influence the mass loss in the accelerated decay test regardless of the treatment. Therefore, the means of mass loss were compared between preservative products, only. The two treatments applied in the current study increased wood resistance to fungal attack; in other words, both treatments made the wood resistant to the fungus used here (Table 2).

With respect to the natural durability of the species in question, there were no reports in the literature about how resistant it is to attack by white-rot fungi, for comparison purposes. However, other species belonging the same genus, such as *E. grandis* and *E. cloeziana*, showed approximately 58 and 28% mass loss, respectively, after they were attacked by white-rot fungi (Vivian et al., 2014). Thus, it is possible to affirm that *E. botryoides* showed resistance levels higher than those of the aforementioned species subjected

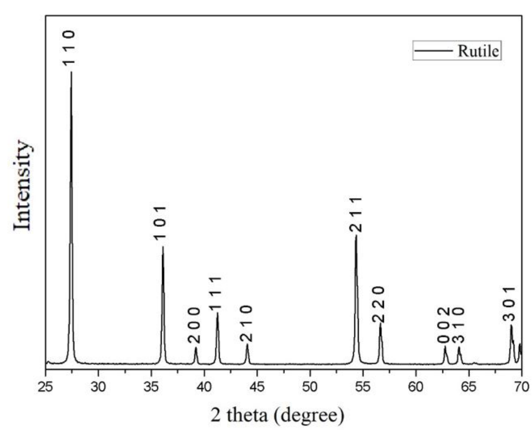

Figure 1. X-Ray Diffractogram of the commercial TiO_2 used to impregnate the wood.

Table 2. Mean weight loss values (%) of *E. botryoides* wood subjected to white-rot fungi.

Treatment	Mass loss (%)	Classification
Control	14.95 (± 3.15) a	Resistant
CCB	0.75 (± 0.37) b	Highly resistant
TiO_2 particles	0.84 (± 0.21) b*	Highly resistant

*Means followed by the same letter in the column do not differ from each other, according to the Tukey test, at 5% significance. Values in parentheses refer to the standard deviation of each treatment.

to white-rot fungi, although the lack of a preservation process caused wood deterioration 94.7% higher than that found in the treated wood.

The TiO_2 particles reduced the development of wood-decay fungi in *E. botryoides*, regardless of the light conditions applied for fungal development, and showed results statistically equal to those recorded for the commercial product (CCB). This occurred due to two TiO_2 features that effectively influence its fungicidal action: the TiO_2 kept in environments without luminosity may attribute a hydrophobic character to the wood surface or work as photocatalyst in environments subjected to luminosity. Both conditions were efficient in preventing fungal growth.

Studies using the two materials (TiO_2-wood) proved that the fungicidal action of TiO_2 is related to photocatalytic activity, to pore size reduction in the cell wall of the wood through impregnation, and/or to decreased moisture absorption capacity (Weigenand et al., 2008; Chen et al., 2009; De Filpo et al., 2013). The TiO_2 absorbs energy from the ultraviolet (UV) region and forms reactive oxygen species such as the OH^- radicals, which cause inactivation by oxidation or deterioration in the cell wall of microorganisms, thus inhibiting cell breathing and, consequently, leading to mortality (Markowska-Szczupak et al., 2011; Foster et al., 2011).

The photoactivity of the particles was sufficiently high to prevent and avoid fungal development, even under simple radiation conditions (fluorescent light - 5% UV radiation in the electromagnetic spectrum). De Filpo et al. (2013) investigated commercial TiO_2 properties (P25 - 80% anatase and 20% rutile) and found that TiO_2 application prevented the growth of white-rot (*Hypocrea lixii*) and brown-rot (*Mucor circinelloides*) fungal colonization in several forest species subjected to the same light conditions proposed in the methodology applied here.

The TiO_2 was able to make the wood surface hydrophobic in the absence of light. Thus, the fungus presented slow development, since it requires moisture higher than 20% to do so. Furthermore, these results may be attributed to the obstacle provided by the TiO_2 between the cell wall of the wood and the fungus, since the digestive enzymes released for the cleavage of organic chemical compounds are not able to degrade inorganic materials. Shabir Mahr et al. (2013) conducted a biological test using brown-rot fungi (*Coniophora puteana* and *Poria placenta*) in the absence of light, by impregnating the wood of *Pinus silvestres* plants with solutions containing titanium oxide. Their results corroborate those observed in the present study.

The Rockwell hardness test associated mass loss resulting from fungal deterioration with sphere-penetration resistance. The untreated wood showed penetration resistance lower than that of woods treated with TiO_2 and CCB, which showed similar behavior (Table 3). The formation of a protective layer resulting from the TiO_2-particle treatment prevented the enzymes from accessing the wood polymers, and kept the structure intact after deterioration. On the other hand, the integrity of the cell wall polymers in the untreated wood was affected.

Accordingly, the decreased hardness in the untreated wood after deterioration caused by fungal attacks was mainly related to hemicellulose loss, which affected the integrity of the cell wall polymers and, consequently, decreased the strength against mechanical loads (Bari et al., 2015).

The FT-IR of the *E. botryoides* control wood decayed by *G. applanatum* showed spectral changes (Figure 2),

Table 3. Mean Rockwell Hardness (DRM) values of *E. botryoides* subjected to white-rot fungi.

Treatment	Hardness (DRM)
Control	73.8 (± 5.16) b
CCB	88.6 (± 2.65) a
TiO_2 particles	87.7 (± 2.59) a*

*Means followed by the same letter in the column do not differ from each other, according to the Tukey test, at 5% significance. Values in parentheses refer to the standard deviation of each treatment.

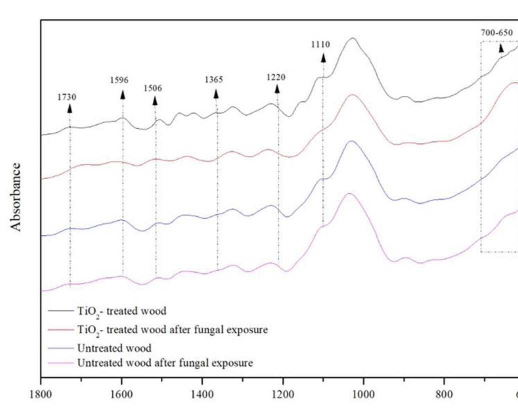

Figure 2. FT-IR spectra of untreated and TiO_2-treated woods before and after the accelerated decay test.

mainly in the peaks of the spectral signature region referring to the wood (600 to 1800 cm^{-1}). The fungal attack led to the emergence of peaks, as well as to the reduction and displacement of others, which, according to Bari et al. (2015), results from enzyme action.

The bands found in the FT-IR analysis applied to the treated woods overall showed similar behavior. Although these woods did not show significant mass loss after the accelerated decay test, it was possible to see that the attenuated fungal growth caused slight displacements in characteristic peaks, as well as the intensification and emergence of others.

Spectrum changes happened mainly in bands corresponding to lignin-hemicellulose bonds due to *G. applanatum* preference for these components. Most peak displacements and emergences in the TiO_2-treated wood resulted from the titanium dioxide found in the wood, which showed characteristic vibrations in the band identified in the FT-IR spectrum.

The region close to 1730 cm^{-1}, which refers to stretching vibrations of the C=O group of xylans, acetyl or carboxylic acid (Cademartori et al., 2012), diminished in the untreated decayed wood due to modifications in the lignin structure and/or the weakening of the hemicellulose aromatic skeletal bonds, which resulted from the enzyme attack. The phenolic lignin units - 1506 cm^{-1} and 1596 cm^{-1} bands (Santos et al., 2016) - diminished in the untreated wood due to fungal attack, whereas the TiO_2-treated specimens did not show significant changes.

There was a small change at the 1365 cm^{-1} peak, which was attributed to C-H cellulose deformation. Similarly, Bari et al. (2015) attributed the 1223 cm^{-1} peak emergence to the lignin syringyl ring, which refers to the C-O stretching between lignin and xylose. The 1220 and 1110 cm^{-1} bands featured vibrational stretching of many lignin and carbohydrate groups (Poletto et al., 2012), which showed that lignin-hemicellulose bonds were cleaved during biological deterioration.

The shortest wavelengths, between 700 and 650 cm^{-1} (Chen et al., 2007), corresponded to characteristic TiO_2 bands found in crystal-like shape in the region. The hypothesis for the increased peak in this region, in the TiO_2-treated wood, after fungal action may be due to the high TiO_2 rate on the cellulose surface, since the fungus used here was able to simultaneously degrade hemicellulose and lignin to a similar extent.

Given this, the efficiency of the purely-rutile TiO_2 particles in forming a protective layer on the material cell wall and in preventing the access and development of white-rot fungi became evident, a fact that demonstrated TiO_2 fungicidal action and wood preservation potential.

4. CONCLUSION

E. botryoides wood durability increased after TiO_2-particle impregnation, in comparison to untreated specimens, after wood exposure to the fungus *G. applanatum*. The efficiency of the TiO_2-particle treatment in the rutile phase did not depend on the light conditions for fungal development, similar to what happened in CCB-treated woods. The TiO_2 particles were able to form a protective layer on the cell wall surface, as well as to prevent fungal attack. They hindered the deterioration of constituent polymers and helped to preserve their sphere-penetration resistance.

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