Evaluation of the Antifungal Activity of Treated *Acacia saligna* Wood with Paraloid B-72/TiO_2 Nanocomposites Against the Growth of *Alternaria tenuissima*, *Trichoderma harzianum*, and *Fusarium culmorum*

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Acacia saligna wood was impregnated with 5% and 10% concentrations of Paraloid B-72/TiO_2 nanocomposites using a soaking technique and evaluated for their antifungal activity against the growth of three molds *in vitro*, namely, *Alternaria tenuissima*, *Trichoderma harzianum*, and *Fusarium culmorum*. The Titanium (Ti) element peak of 0.14% and 0.23%, was found in the *A. saligna* wood treated with Paraloid B-72/TiO_2 nanocomposites at 5% and 10%, respectively. Consolidant polymer Paraloid B-72 mixed with TiO_2 nanocomposites at 5% and 10% showed antifungal activity against the three studied molds, while the linear growth of the studied molds reached the maximum in the control and Paraloid B-72 treatments. The results concluded that using synthesized Paraloid B-72/TiO_2 nanocomposite could be considered as a new agent in the wood preservation field by prevention of mold fungal growth over the wood surfaces.

Keywords: Durability improvement; Molds; Paraloid B-72/TiO_2 nanocomposites; Surface characterization

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INTRODUCTION

Wood is a natural material that can be attacked in service by several biological pathogens such as wood-destroying fungi and molds. Molds including *Fusarium* and *Alternaria* have been found in such places where humid conditions are available (Fogel and Lloyd 2002; Xu *et al.* 2013). Branches and leaves of fallen trees into water are decomposed by *Fusarium* sp. (Wylloughby and Archer 1973; Révay and Gönczöl 1990), where *F. culmorum* has been isolated from water of the Andarax riverbed in the provinces of Granada and Almeria in southeastern Spain (Palmero *et al.* 2009) and an aquatic system (Smither-Kopperl *et al.* 1998). *A. tenuissima* has been isolated from different wood species (Sivanesan 1991) and has been shown to cause discoloration for wood and wood-based products (Andersen *et al.* 2002, 2011; Yang 2005; Lee *et al.* 2014).

Molds such as *Alternaria* and *Fusarium* could be isolated even if preservatives have previously treated the wood (Bridžiuvienė and Raudonienė 2013). In addition, Sohail *et al.* (2011) found that the *Alternaria* species produces an enzyme that hydrolyzes cellulose into glucose. *Trichoderma* species have been found to colonize *Pinus radiata* sapwood of freshly cut timber (Butcher 1968; Dowding 1970), whereas the poles of Scots pine heartwood have little colonization (Bruce and King 1986).

Titanium dioxide (TiO_2) gel deposited in a high amount in the cell lumens of wood did help enhance the hygroscopicity of the wood; thereupon, thermal stability and stiffness of the wood cell walls were improved (Wang *et al.* 2012).

Nanoparticles have a broad-spectrum of uses in different areas, for example, a significant reduction in the mycelium biomass (28% to 35%) of *A. niger* and *P. chrysogenum* was found when using silver nanoparticles (AgNPs) in the growing medium (Pietrzak and Gutarowska 2015). In addition, a reduction in bacterial numbers (92.9% to 94.6%) was obtained after treating the sewage with AgNPs (Patil 2014). The resistance of coatings containing ZnO nanoparticles was improved against the microbial attack by *Trichoderma reesei* and *Aspergillus niger* (El-Feky *et al.* 2014). Significant antifungal activity was found against *Aspergillus flavus*, *A. niger*, and *Candida albicans* when using ZnO nanoparticles (Jayaseelan *et al.* 2012).

The treated wood with consolidants (synthetic and natural chemicals) resulted in higher strength than untreated wood, but can be attacked by fungi, molds, and insects (Clausi *et al.* 2011). Paraloid B-72 is an acrylic resin that has been used as a wood surface consolidant (Yang *et al.* 2007; Vaz *et al.* 2008). However, this polymer at 2% or 10% has a weak resistance against the growth of fungi (Tiralová and Reinprecht 2004; Pohleven *et al.* 2013).

The authors' previous studies reported *F. culmorum* was grown over an *Acacia saligna* wood surface that was treated by Paraloid B-72 (5% and 10%). Also, the enhancement of wood resistance was found with a combination of Paraloid B-72 and some natural extracts (Mansour *et al.* 2015; Mansour and Salem 2015). For example, the combination of Paraloid B-72 with *Cupressus sempervirens* wood (methanol extract) could be useful against *T. harzianum* (Mansour and Salem 2015), whereas borate base and voriconazole synergy effects were found useful in the inhibition of *A. niger, P. chrysogenum*, and *Trichoderma viride* (Clausen and Yang 2007). Titanium dioxide and Ag-doped titanium dioxide have been tested with consolidants and have been applied to several surface materials against biological colonization (La Russa *et al.* 2012; Ruffolo *et al.* 2013). Furthermore, the treatments of nano-compounds (CuO, ZnO, B_2O_3, TiO_2, and CeO_2) combined with Paraloid B-72 increased the biological performance of treated Scots pine wood against decay fungi tested but no improvements were obtained in mold resistance tests (Muhcu *et al.* 2017).

Therefore, in this present work the authors continued to increase the wood resistance by using a combination of Paraloid B-72 and TiO_2 in the form of nanocomposites to treat the wood. The antifungal activity of the treated *A. saligna* wood with Paraloid B-72 polymer and Paraloid B-72/TiO_2 nanocomposites is evaluated at concentrations of 5% and 10% against the growth of three molds (*Alternaria tenuissima*, *Trichoderma harzianum*, and *Fusarium culmorum*) *in vitro*.

EXPERIMENTAL

Materials

Synthesis of paraloid B-72/nano TiO_2 by in situ emulsion polymerization

A co-polymer emulsion lattice with a 50/50 composition ratio of methyl methacrylate/ethyl acrylate (MMA/EA) monomers (Aldrich, Darmstadt, Germany) was used to produce poly(MMA-Co-EA). It was prepared by an emulsion polymerization technique with different solid contents (5% and 10%) in the presence of 3% of TiO_2 nanoparticles. The polymerization was carried out according to the following procedure: in a 250-mL three-nicked flask, 1 g of emulsifier sodium dodecyl sulfate (SDS) (El Gomhouria Company, Cairo, Egypt) was dissolved in a desired amount of distilled water.

The desired amount of the monomers with the selected composition ratio (50/50 MMA/EA), was added and well emulsified for 30 min at room temperature using a mechanical stirrer (500 rpm) in the presence of 0.03 g of TiO_2 nanoparticles (a mixture of rutile and anatase nanopowder, < 100 nm particle size (BET), 99.5% trace metals basis obtained from Sigma-Aldrich, Schnelldorf, Germany). Then, the mixture was heated to 80 ºC (Shafei *et al.* 2008). Next, the redox initiation system composed of potassium persulphate (PPS) (0.27 g) and sodium bisulphite (SBS) (0.416 g) (Sigma-Aldrich, Schnelldorf, Germany) dissolved in 50 mL of distilled water was added dropwise to the reaction mixture under continuous stirring for 3 h. To obtain the solution of Paraloid-B72/TiO_2 nanocomposites, 100 mL of anhydrous xylene was added to the desired amount of Paraloid-B72/TiO_2 nanocomposites in the presence of N_2 and heated to 80 °C. The properties of the used concentrations are shown in Table 1.

Table 1. Concentrations of Used Coating Materials to Produce Polymer-based Inorganic Nanoparticle Composite

	Solids content	Total volume	Water volume	3 % TiO_2 weight %	Monomer Volume	
					MMA	EA
Poly (MMA/EA)/TiO_2	5%	100 mL	95 mL	0.15 gm	2.5 mL	2.5 mL
	10%	100 mL	90 mL	0.30 gm	5 mL	5 mL

MMA: methyl methacrylate
EA: ethyl acrylate

Morphological analysis of the prepared nanocomposites

The morphological analyses of the prepared paraloid B-72/TiO_2 nanocomposites were investigated by transmission electronic microscopy (TEM), where the TEM images were obtained by a JEM-1230 electron microscope operated at 60 KV (JEOL Ltd., Tokyo, Japan). Before taking a TEM image, the sample was diluted at least 10 times by water. A drop of well-dispersed diluted sample was placed onto a copper grid (200-mesh and covered with a carbon membrane) and dried at ambient temperature.

The prepared Paraloid B-72/TiO_2 nanocomposite was investigated using TEM and from the figure, it was noticed that, a homogenous nanocomposite between Paraloid B-72 and TiO_2 nanoparticles was prepared and the obtained particle size reached 38 nm in the case of Paraloid B-72 (5%) in presence of 3% TiO_2 (Fig. 1) and reached 54 nm in the case of Paraloid B-72 (10%) and in presence of 3% TiO_2 (Fig. 2).

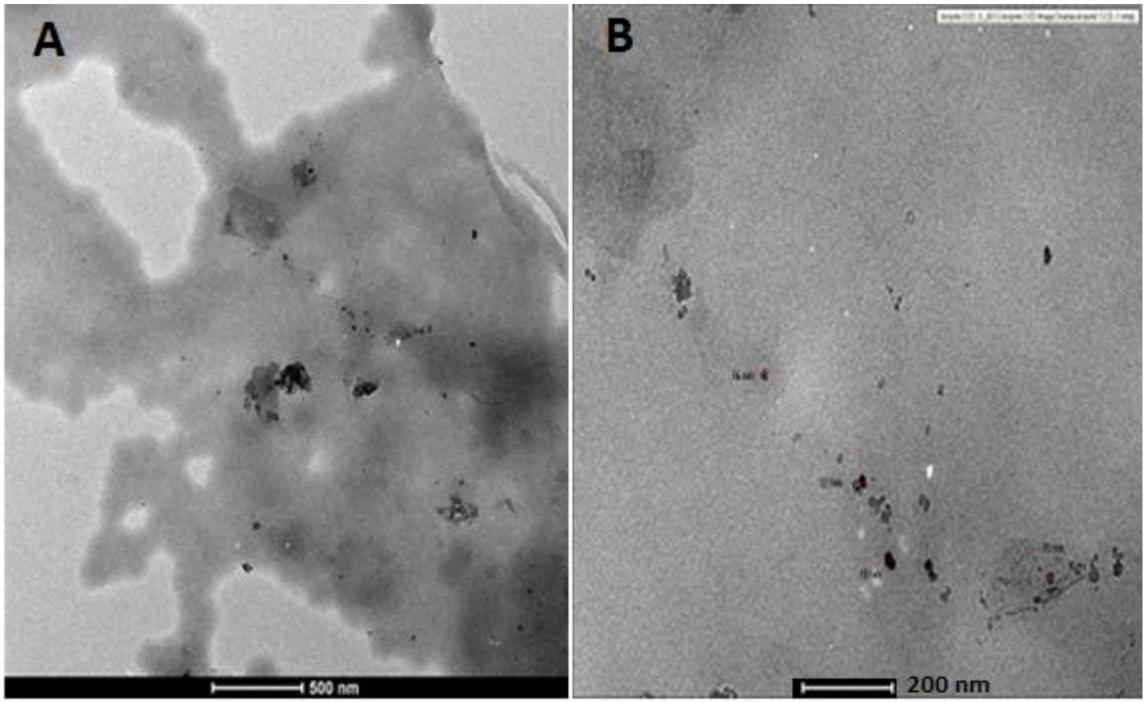


Fig. 1. TEM images of nanocomposite for Paraloid B-72 (5%) in presence of 3% TiO_2; A) Photo at 500 nm of magnification and B) at 200 nm of magnification

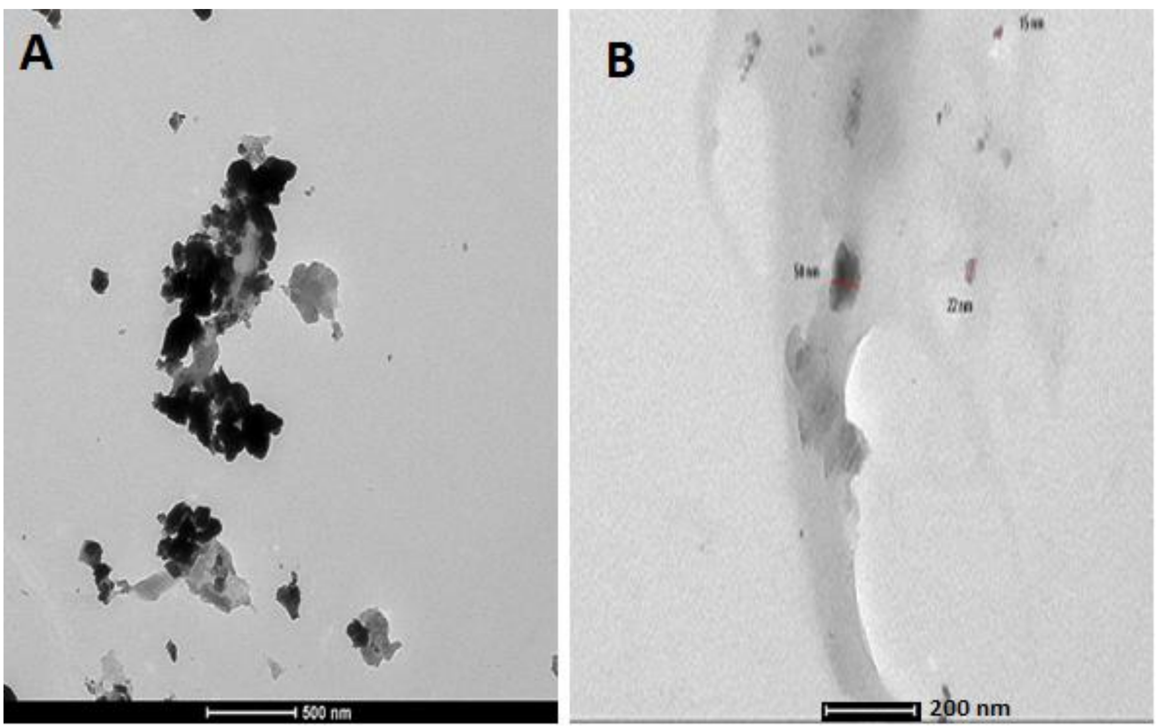


Fig. 2. TEM images of nanocomposite for Paraloid B-72 (10%) in presence of 3% TiO_2; A) Photo at 500 nm of magnification and B) at 200 nm of magnification

Preparation and treating wood samples

Wood samples of *A. saligna* were prepared at the Department of Forestry and Wood Technology, Alexandria University (Alexandria, Egypt) during January 2017 with the dimensions of 0.5 cm × 1 cm × 2 cm. The wood samples were soaked for complete saturation in a solution of Paraloid B-72/TiO_2 nanocomposites (5% and 10%) and Paraloid B-72 polymer (5% and 10%) in Petri dishes for three sequential days and left to dry at room temperature for 15 days (Mansour and Salem 2015). The surfaces of the treated wood with 5% and 10% of Paraloid B-72/TiO_2 nanocomposites were examined for their elemental composition by dispersive X-ray spectroscopy (EDX) (FEI Company, Eindhoven, Netherlands) (Danilatos and Robinson 1979).

Retention value of Paraloid B-72 and Paraloid B-72/TiO_2 nanocomposite in A. saligna wood samples

The retention value was calculated based on BS EN 113 standard test method (BS EN 1997) and the recommendation of Mańkowski *et al.* (2015) with some modification. *A. saligna* wood samples were firstly weighed based on oven-dry weight, and then saturated by soaking method with the concentrated Paraloid B-72/TiO_2 nanocomposites (5% and 10%) and Paraloid B-72 polymer (5% and 10%). After all the treatments, the wood samples were air-dried in the laboratory for 48 h, then conditioned at 20 ± 2 ºC and 65 ± 5 % RH until constant weight, and the final weight after treatment was recorded, then the retention value was calculated as kg/m^3.

Antifungal activity

The three common molds, namely *Alternaria tenuissima*, *Trichoderma harzianum*, and *Fusarium culmorum*, were used in the present study and supplied from the Regional Center for Mycology and Biotechnology, Al-Azhar University (Cairo, Egypt). Plates of potato dextrose agar (PDA) medium were prepared and inoculated with a 5-mm disc of 7-day-old culture from each of the tested molds. The treated wood samples with two levels of the concentrated Paraloid B-72/TiO_2 nanocomposites and the Paraloid B-72 polymer were put over the inoculated plates and incubated for 2 weeks at 25 °C. The fungal liner growth (mm) of the three mold fungi was measured after 14 days according to the methods described in the following sources (Satish *et al.* 2007; Essa and Khallaf 2014; Mansour and Salem 2015; Mansour *et al.* 2015; Salem *et al.* 2016 a, b) with some modification, where the linear growth was measured by a ruler from the fungal growth to the marge of the clear inhibition zones (no growth of fungus) formed around the treated wood once the control treatment (wood without any treatments) reached 9 cm-diameter in the growth. The wood samples were used for each treatment. Furthermore, after 6 months of inoculation at room temperature, the fungal colonization was visually evaluated (Mansour *et al.* 2015; Mansour and Salem 2015).

Methods

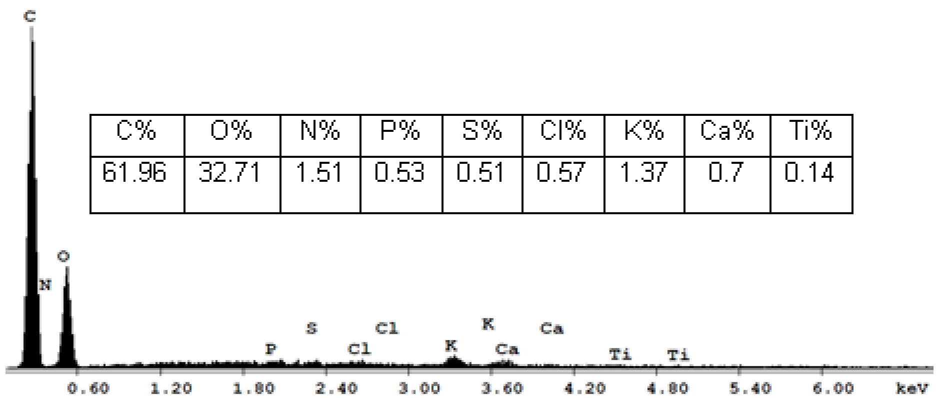
Statistical analysis

The effects of five different treatments [control (wood without any treatment), Paraloid B-72 polymer (5 and 10%), and Paraloid B-72/TiO_2 nanocomposites (5 and 10%)] on the linear growth values of *A. tenuissima*, *T. harzianum*, and *F. culmorum*, were statistically analyzed with one-way ANOVA using SAS version 8.2 software (2001) (Cary, NC, USA) in a completely randomized design. Least significant difference (LSD) at the $\alpha = 0.05$ level was used to test the differences among the treatments.

RESULTS AND DISCUSSION

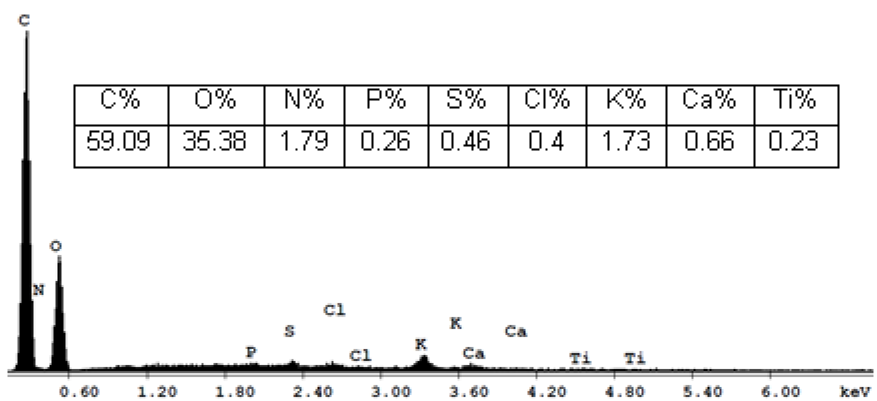
EDX Measurements of Treated Wood with Paraloid B-72/TiO_2 Nanocomposites

The elemental chemical compositions of the treated wood with Paraloid B-72/TiO_2 nanocomposites at 5% and 10% are shown in Figs. 3 and 4, respectively. The C element was 61.96% and 59.09% in wood treated with Paraloid B-72/TiO_2 nanoparticles at 5% and 10%, respectively. The Titanium (Ti) element peak was found in percentages of 0.14% and 0.23%, on the *A. saligna* wood treated with Paraloid B-72/TiO_2 nanocomposites at 5% and 10%, respectively.



C%	O%	N%	P%	S%	Cl%	K%	Ca%	Ti%
61.96	32.71	1.51	0.53	0.51	0.57	1.37	0.7	0.14

Fig. 3. EDX analysis of treated wood with Paraloid72/TiO_2 nanocomposite at 5%



C%	O%	N%	P%	S%	Cl%	K%	Ca%	Ti%
59.09	35.38	1.79	0.26	0.46	0.4	1.73	0.66	0.23

Fig. 4. EDX analysis of treated wood with Paraloid B-72/TiO_2 nanocomposite at 10%

Retention of Paraloid B-72 and Paraloid B-72/TiO_2 Nanocomposite in Wood

Table 2 presents the retention values in the Paraloid B-72/TiO_2-treated wood specimens as well as Paraloid B-72. Statistically significant higher mean of retention value was recorded after double concentration for the treated samples. These results are in agreements with Muhcu *et al.* (2017) and Mańkowski *et al.* (2015, 2016).

Table 2. Retention Levels of Paraloid B-72 and Paraloid B-72/TiO_2 Nanocomposite in Wood Specimens after Treatments

Treatment	Retention value (kg/m^3)
Control	-
Paraloid B-72 (5%)	114.90 (3.38)
Paraloid B-72 (10%)	164.09 (4.08)
Paraloid B-72/TiO_2 nanocomposite (5%)	120.44 (3.52)
Paraloid B-72/TiO_2 nanocomposite (10%)	175.09 (4.11)

Retention levels in treated wood specimens by soaking method.
Values in parentheses are standard deviations.

Fungal Linear Growth

The linear growth of the studied three molds (Table 3) reached its highest (90 mm) in the control treatment, while it decreased significantly ($p < 0.0001$) to 38.3 mm, 38.3 mm, and 23.3 mm with *T. harzianum*, *A. tenuissima*, and *F culmorum*, respectively, as *A. saligna* wood was treated with Paraloid B-72/TiO_2 nanocomposites at a concentration of 5%. Furthermore, the linear growth was significantly ($p < 0.0001$) reduced with values of 13.3 mm, 13.3 mm, and 11.3 mm, for *T. harzianum*, *A. tenuissima*, and *F culmorum*, respectively, using the wood treated with Paraloid B-72/TiO_2 nanocomposites at a concentration of 10%.

Table 3. Means ± Standard Deviation (Standard Error of Linear Growth of *T. harzianum*, *A. tenuissima,* and *F. culmorum* as Affected by Paraloid B-72/TiO_2 Nanocomposites (5% and 10%) and Paraloid B-72

Treatment	Linear Growth of Fungal Mycelia (mm)		
	T. harzianum	*A. tenuissima*	*F. culmorum*
Control	90.00 ± 0.00a (0.00)	90.00 ± 0.00a (0.00)	90.00 ± 0.00a (0.00)
Paraloid B-72 (5%)	85.33 ± 5.03a (2.90)	85.33 ± 5.03a (2.91)	84.66 ± 4.51a (2.60)
Paraloid B-72 (10%)	88.33 ± 2.88a (1.66)	88.33 ± 2.88a (1.66)	87.66 ± 2.51a (1.45)
Paraloid B-72/TiO_2 nanocomposites (5%)	38.33 ± 2.88b (1.66)	38.33 ± 2.88b (1.66)	23.33 ± 5.77b (3.33)
Paraloid B-72/TiO_2 nanocomposites (10%)	13.33 ± 2.88c (1.66)	13.33 ± 2.88c (1.66)	11.33 ± 1.15c (0.66)
$LSD_{0.05}$	5.77	5.77	6.37
R^2	0.99	0.99	0.99
CV %	5.03	5.03	5.89

Means with the same letter within the same column are not significantly difference according to LSD at a 0.05 level of probability

From Table 3, the linear growth of the three molds incubated with the treated wood samples with Paraloid B-72 reached the maximum, which was not significantly different from the control treatments. These results are in agreement with previous works (Tiralová and Reinprecht 2004; Yang *et al.* 2007; Vaz *et al.* 2008; Pohleven *et al.* 2013; Mansour *et al.* 2015; Mansour and Salem 2015; Reinprecht and Vidholdová 2017). Also, Paraloid B-72-only treated wood specimens were observed to have the highest weight losses in decay tests (Reinprecht *et al.* 2015; Muhcu *et al.* 2017) and practically the polyacrylate Paraloid B-72 had no effects against molds (Reinprecht and Vidholdová 2017).

Several studies reported that TiO_2 applied as a coating has high photoactivity and non-toxicity, as well as a strong self-cleaning property (Meng and Lu 2010; Zawadzka *et al.* 2016). Suitable antifungal properties of the photocatalyst TiO_2 nanoparticles have been observed against *Candida albicans* biofilms (Akiba *et al.* 2005; Shibata *et al.* 2007; Haghighi *et al.* 2013). Cotton fabric impregnated with TiO_2 nanoparticles showed a maximum zone of inhibition against *Aspergillus niger* and *Trichoderma reesei* and was demonstrated to be an effective antimicrobial agent (Durairaj *et al.* 2015). The poly(lactic acid)/TiO_2 nanocomposites with 8 wt.% were effective (99.99%) against *Aspergillus fumigatus* under the ultraviolet A (UVA) irradiation (Fonseca *et al.* 2015).

Nano-particle as fillers in a polymer structure combined with consolidant materials has the ability to neutralize acid, has high durability, and is a better coating (Christensen *et al.* 2012). The nano-materials (ZnO, TiO_2, SiO_2, CuO, Fe_2O_3) mixed consolidation products increased the biological, UV radiation scratch, abrasion and water resistance of wood (Blee and Matisons 2008; Kartal *et al.* 2009; Tuduce-Trãistaru *et al.* 2010; Clausi *et al.* 2011).

Unger *et al.* (2001) reported that Paraloid B-72 was provided some antifungal activity to the consolidated wood, but other studies have shown that Paraloid B-72 cannot protect wood itself against wood-destroying fungi and molds, which needs the additions of fungicides (Nakhla 1986), chemical preservative (Olstag and Kucerova 2009), natural products (Mansour *et al.* 2015; Mansour and Salem 2015), or nanoparticles (Muhcu *et al.* 2017). Furthermore, the choosing of ideal consolidant is very important to be compatible with wood-protecting biocides (Unger *et al.* 2001; Mansour *et al.* 2015; Mansour and Salem 2015). Therefore, the combination of Paraloid B-72 with TiO_2 nanoparticle at 5% and 10% showed strong antifungal activity against the growth of *T. harzianum*, *A. tenuissima*, and *F. culmorum*.

Visual Observation after 14 Days

After 14 days from the inoculation, wood samples treated with Paraloid B-72 were completely colonized by the three molds (Mansour *et al.* 2015; Mansour and Salem 2015). In contrast, the treated wood samples with either Paraloid B-72/TiO_2 nanocomposites at 5% or 10% showed significant inhibition to the linear growth of the three tested molds after 14 days from the inoculation. By contrast, the consolidant polymer Paraloid B-72 mixed with TiO_2 nanoparticles was shown to be a strong antifungal agent against the three studied molds.

Previous studies about using nanoparticles for preventing mold infestation showed that a 90 ppm concentration of AgNPs (10 nm to 100 nm particle size) was effective in removing the molds present on the surface of objects from six different museums and archives in Poland (Gutarowska *et al.* 2012). Consolidation polymers (acrylic and silicon polymers) functionalized with AgNPs were found used as a potent biocide and consolidation material for historic monuments and artifacts (Essa and Khallaf 2014). Consolidants and water repellents mixed with copper nanoparticles were effective in the protection of stones from biodeterioration (Pinna *et al.* 2012).

Visual Observation of the Incubated Wood Samples after 3 Months

The visual observations of the treated wood samples with Paraloid B-72/TiO_2 nanocomposites at (5% and 10%) and inoculated with *T. harzianum*, *A. tenuissima*, and *F. culmorum* are evaluated after Petri dishes were stored at room temperature for 3 months. It was observed that no visual growths of *A. tenuissima* were found on the surface of *A. saligna* wood samples treated with Paraloid B-72/TiO_2 nanocomposites at 10%, but some growth was observed for the treated wood with Paraloid B-72/TiO_2 nanocomposites at 5%. Some growth of *T. harzianum* was visually observed for both treatments (Paraloid B-72/TiO_2 nanocomposites at 5% and 10%). Growths of *F. culmorum* were apparent all over the surface of the treated wood samples, regardless of treatment.

CONCLUSIONS

1. Increasing saturation wood with the concentrated Paraloid B-72 solution or Paraloid B-72/TiO_2 nanocomposites significantly increased the retention values.
2. The linear growth of *T. harzianum*, *A. tenuissima*, and *F. culmorum* reached the maximum in the Paraloid B-72 and control treatment (wood without any treatments), while it decreased as *A. saligna* wood was treated with Paraloid B-72/TiO_2 nanocomposites at the concentration of 5% and 10%.
3. After 3 months from the incubation at room temperature, no visual growth of *A. tenuissima* was found for treated wood (Paraloid B-72/TiO_2 nanocomposites 10%), *T. harzianum* growth was visually observed (Paraloid B-72/TiO_2 nanocomposites at 5% and 10%) and growth of *F. culmorum* was observed regardless of treatment.
4. From the present results, these combinations of nanocomposites (Paraloid B-72/TiO_2) could be useful as a potential treatment against molds and staining fungi that colonize wood.

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