Durability of Wood Treated with Propolis

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Propolis is an important antifungal agent found naturally in beehives and used as a food supplement for many purposes. This study aimed to use methanolic propolis extract (MPE) as a treatment material as an antifungal agent for wood preservation. Scots pine and paulownia woods were exposed to *Trametes versicolor* and *Neolentinus lepideus* fungi for 12 weeks, and untreated woods were used as the controls. Compared with the control, paulownia wood exposed to *N. lepideus* had a 47.2% mean mass loss, while the treated wood with 7% MPE had an 11.6% mean mass loss. In addition, a 27.2% mass loss occurred with the control for Scots pine when exposed to *N. lepideus*, and a 2.5% mass loss occurred with the 7% propolis-treated specimens. Total phenolic content and the phenolic profile of the raw propolis samples were also analyzed. Scanning electron microscopy images showed that the propolis extracts still remained in the wood cells without being degraded after the fungal destruction and the propolis-treated specimens were more durable against fungal decay compared to the untreated control specimens. The results from this study indicated that propolis could be used as an environmentally compatible and natural wood preservative to protect wood against fungal attack.

Keywords: Propolis; Wood; Decay fungi; Impregnation

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INTRODUCTION

Wood is widely used all over the world and is an abundant renewable material that is composed of lignin, cellulose, and hemicelluloses. However, wood is susceptible to decay and biodegradation by fungi (Susi *et al*. 2011). When wood material is used in an outdoor environment and left unprotected, it can deteriorate, resulting in great economic and resource losses (Hassan *et al*. 2016). Therefore, wood has to be treated with wood protection agents. Many wood protection agents and methods have been tried to protect wood against biological factors, such as fungi, insects, termites, and molds. Chromated copper arsenate (CCA) formulations have been used to extend the service life of wood for many decades due to their biological toxicity (Barraj *et al*. 2009). After 2003, with increasing environmental pressures and legal regulations, CCA was prohibited in residential applications due to its health risks (Clausen 2004). Researchers developed new wood protection agents that are less harmful to humans and the environment (Hsu *et al*. 2009; Bakar *et al*. 2013). Extracts, tannins, and resins obtained from various parts of plants have great promise as wood preservation agents because they contain phenolic compounds (Tascioglu *et al*. 2013; Valette *et al*. 2017).

Propolis or bee glue is a natural product collected from plant buds and shoots by honeybees; it is responsible for the protection of bees and their hives. Bees use propolis to cover holes and to fix inner walls of hives. Due to its waxy structure and mechanical properties, honeybees cover the sleeve cracks with propolis to maintain constant temperature and humidity in their hives (Crane 1990; Zabaiou *et al*. 2017). The color of the propolis may range from light yellow to dark brown depending on the source of the resin. It is soft, flexible, and sticky at temperatures between 25 °C and 45 °C. It can be easily dissolved in methanol, ethanol, chloroform, and acetone, but it is insoluble in water (Wagh 2013). Propolis shows appreciable bioactive features (*e.g.*, antioxidant, anti-inflammatory, antimicrobial, antiviral, antifungal, and antiparasitic activities). Since propolis has bioactive features, it is used as food supplements in apitherapy (Bankova *et al*. 2006; Salas *et al*. 2016). In the literature, propolis is considered a food preservative that can be used to protect foods. Propolis extracts are added to foods or are superficially applied by immersing food directly in propolis extracts. Foods are coated with specifically developed coatings based on polymers containing propolis extracts. Both methods reduce / eliminate pahhogens or saprophytic microbiota in foods (Pobiega *et al.* 2017, 2019). Propolis is also used as an antimicrobial and antifungal agent against a wide spectrum of microorganisms, such as *Schizophyllum commune*, *Penicillium italicum*, *Lenzites elegans*, and *Ganoderma applanatum* (Quiroga *et al*. 2006; Yang *et al*. 2011). Over 500 compounds have been identified in propolis, as noted in the scientific literature as of 2012 (Huang *et al*. 2014). Though its composition is dependent upon many environmental conditions, raw propolis is composed of resin (50%), wax (30%), essential compounds (5 to 10%), pollen (5%), and numerous other chemicals, such as polyphenols, vitamins, and sugars (Li *et al*. 2008). The total amount of propolis production in the world is estimated to be between 100 to 200 tons/year. China produces more propolis than other countries; some South American countries, like Brazil, have notable propolis production levels (Crane 2009). As the demand for bee products has increased over the years, it is expected that propolis production will continue to increase more in the future.

Paulownia is a fast-growing tree species that is especially found in China and Japan. Paulownia needs to be considered carefully because it is a fast-growing tree species that has a light-colored wood with higher resistance properties when compared to similar tree species with the same density (Gonçalves *et al*. 2008). In China and other Asian countries, its wood is used in various applications, such as pulp and paper, furniture, musical instruments, building materials, particleboard, plywood, and packaging (Kalaycioglu *et al*. 2005). However, this wood has been known to resist decay in ancient times (Hua *et al*. 1986); contemporary studies have shown that paulownia wood is non-durable, particularly to brown-rot fungal attack (Kirker *et al*. 2013).

The aim of this study is to investigate the antifungal properties of Turkish propolis against wood-decaying fungi. Impregnated Scots pine and paulownia wood specimens were used to investigate the antifungal effects of propolis. Fourier transform infrared spectroscopy (FTIR) was used to determine the chemical changes in wood structure, whereas scanning electron microscopy (SEM) was used to observe the distribution of the propolis extracts in the wood cells. When the wood materials are treated with the impregnation chemicals and exposed to outdoor conditions, it will be under the influence of degrading factors and leaching. An efficient wood preservative should resist leaching. Therefore, leaching experiments were also conducted in this study.

EXPERIMENTAL

Materials

Randomly selected first grade Scots pine (*Pinus sylvestris* L.) (0.433 g/cm^3) and *Paulownia elongata* (0.210 g/cm^3) wood specimens were obtained from the Kastamonu province in Turkey. Wood samples were cut from the sapwood with the dimensions of 20 mm × 20 mm × 10 mm (longitudinal × radial × tangential direction). The ring widths were 5 mm for both wood species. A total of 168 specimens were prepared, with 84 samples from each wood species. Wood samples were fully dried at 103 °C for 24 h and weighed.

Samples and preparation of propolis extracts

In this study, Turkish propolis samples were collected in 2018 with traps placed into beehives located in the provinces of Kırklareli and Demirköy (Turkey). After grinding the frozen raw propolis, 100 g of the ground powder was dissolved in 250 mL of 98% methanol in a glass flask (500 mL). The mixture was stirred using a Promax 2020 shaker (Heidolph; Schwabach, Germany) at room temperature for 72 h; afterwards, the particles were removed by filtration, the solution was evaporated with a rotary evaporator, and the dry-extract residual was recovered and stored at room temperature. The residue was re-dissolved in methanol to prepare a 7% propolis solution in methanol.

Treatment

Treatment solutions were prepared at 1%, 3%, 5%, and 7% concentrations by dissolving the propolis in methanol. Wood samples were placed into glass containers according to intended treatment concentration levels. To determine whether the factor providing the activity in the fungus tests was caused by the solvent or propolis, 12 wood samples from each wood species were also treated with only methanol and exposed to the fungi. An 80 Pa vacuum was applied to a glass desiccator, which contained the wood samples, for 20 min. After treatment, the treated wood samples were immediately removed and weighed. Retentions of wood samples (kg/m^3) were calculated as,

$$R\ (\mathrm{kg/m^3}) = \frac{(Ma - Mb) \times C}{V} \times 10 \quad (1)$$

where *Mb* is the wood mass before treatment (g), *Ma* is the wood mass after treatment (g), *C* is the concentration of the propolis treatment solution (%), and *V* is the volume of the wood (m^3).

Methods

Decay tests

The decay resistance of treated Scots pine and paulownia specimens against white rot (*Trametes versicolor*) and brown rot (*Nelolentinus lepideus*) was conducted according to the JIS K 1571 (2004) standard. Malt extract agar (MEA) medium (3.7%) was used to grow the fungi cultures. The media and all wood samples were sterilized at 121 °C at 1.1 atm pressure for 20 min. Then, the media were transferred to Petri dishes. After inoculation, the dishes were kept at 26 °C and 70% relative humidity. When the media surfaces were completely colonized by the test fungi, the treated and untreated wood samples were placed into the Petri dishes. The wood samples were exposed to decay fungi in an incubator at 26 °C and 70% relative humidity for 12 weeks. At the end of the exposure time, the exposed wood samples were cleaned of the fungi's mycelium and weighed again. Then, the percent

mass loss caused by the fungi was calculated from mass differences before and after the decay. Decay resistance tests were conducted at the Forest Biology and Wood Preservation Laboratory at Duzce University (Duzce, Turkey).

Total phenolic contents

Measurement of polyphenols and antifungal activity of propolis extracts is an important tool to understand the value of propolis. Because the active ingredients of propolis are mostly polyphenols which have antifungal properties, the polyphenol composition of the propolis used was examined. Total phenolic compounds were determined using the Folin-Ciocalteu assay. A total of 680 µL distilled water, 20 µL diluted propolis extract, 400 µL of 0.5 N Folin-Ciocalteu reagent (Sigma Aldrich, Darmstadt, Germany), and 400 µL Na_2CO_3 (10%) were added in a test tube (Singleton *et al*. 1999). After 2 h of incubation at room temperature, the solution's absorbance was measured at 760 nm wavelength. The result was expressed as mg of gallic acid equivalents (GAE) per 100 g propolis. All experiments were performed in triplicate.

Phenolic profile analyses of propolis

Phenolic profile analyses of the samples were performed by high-performance liquid chromatography coupled with an ultraviolet detector (HPLC-UV). The residue was dissolved in 15 mL acidified distilled water (pH 2), and liquid-liquid extraction was completed with 5 × 3 mL diethyl ether and 5 × 3 mL ethyl acetate, consecutively. Both diethyl ether and ethyl acetate phases were combined and the solvents were evaporated using a rotary evaporator (IKA-Werke, Staufen, Germany) at 40 °C. The pellet was re-suspended in 2 mL ethanol, and the resulting solution was filtered using syringe filters (RC-membrane, 0.45 µm) prior to injecting the solution into the HPLC. The results are expressed as mg/100 mL extracts

The HPLC-UV analyses of the ethanolic extracts were performed on an Elite LaChrom HPLC (Elite LaChrom Hitachi Ltd., Tokyo, Japan) using an ultraviolet (UV)-visible detector (Elite LaChrom Hitachi, Tokyo, Japan). An analytical Fortis C18 column (150 mm × 4.6 mm, 5 µm silica particle size (Fortis Technologies Ltd., Neston, UK) was used. Gradient elution was applied; the mobile phase consisted of (A) 2% acetic acid in water and (B) acetonitrile-water (70:30). The sample injection volume was 25 µL. The column temperature was set at 30 °C with a flow rate of 0.75 mL/ min. The programmed solvent profile used began with a linear gradient of A held at 95% for 3 min, and then decreased A to 80% at 10 min, to 60% at 20 min, to 20% at 30 min, and finally to 5% at 50 min.

Leaching test

The leaching procedure used was performed according to the AWPA E11 test method (AWPA E11 2000). Scots pine samples, which were treated with 5% propolis solution, were leached by immersion in distilled water at 10× the specimen's volume in a glass container. The water was stirred with a magnetic stirrer at 500 rpm for 1, 2, 4, 8, 16, and 48 h. The water was replaced with new distilled water at the end of each leaching period. Untreated control samples were leached for only 48 h. Leachate water was collected and kept in a plastic box at 4 °C to determine the total phenolic compounds that were leached. The amount of propolis from the samples suspended in methanol to the solvent was determined in terms of total polyphenols.

FTIR characterization and SEM analysis

The FTIR analysis was one of the methods used to examine the effect of rot fungi on wood. Both FTIR and SEM analyses were performed at Duzce University Scientific and Technological Researches Application and Research Center (Duzce, Turkey). The FTIR analyses were performed using an IR Prestige-21 instrument (Shimadzu Benelux B.V., Hertogenbosch, Netherlands) with ATR attachment, and the spectra were recorded in the wavenumber range from 400 to 4000 cm^{-1}. SEM images were recorded using a Quanta FEG 250 instrument (FEI Europe B.V., Eindhoven, Netherlands).

SEM and FTIR analyzes were performed for both wood species, all concentrations, un-treated controls, and following treatments with both fungal species used in the study. FTIR analysis were performed for samples with the highest weight loss among the untreated control specimens exposure to decay and the specimens having the lowest weight loss were selected for the samples treated with propolis at concentrations of 1-3-5-7%. Selected wood samples were dried in an oven at 60 °C for 48 h and used for FTIR and SEM.

For SEM analyses, the samples were mounted onto aluminum stubs with double-sided carbon tape, and mounted specimens were coated with 10 nm gold film using a sputter coater (Desk V- Standard, Denton Vacuum, LLC, New Jersey, USA) before analyses. Surface morphologies of the samples were investigated with an SEM Quanta FEG 250, which used an electron acceleration voltage of 10 keV. The measurements were taken in radial direction of the woods.

Statistical analyses

Statistical analyses of the data were performed using SPSS 19 software (IBM Corp., Armonk, NY, USA). The mean mass losses caused by the decay fungi species and the gross solution uptake (retentions) for wood species were evaluated using a one-way analysis of variance (ANOVA). A mean separation test (*i.e.*, Duncan's multiple range test) was applied using an $\alpha = 0.05$ level to determine the significant differences among the paired comparisons.

RESULTS AND DISCUSSION

Phenolic Profile Analyses of Propolis

Total phenolic content was measured either with the raw propolis sample or the methanolic propolis extracts. The results were 135.04 ± 2.33 mg GAE/g raw propolis and 122 ± 2.14 mg GAE/mL, respectively.

Phenolic propolis of the sample was analyzed also both of the propolis samples fractions, raw and used extract. The results are given in Table 1. Caffeic acid, epicatechin, ferulic acid, rutin, luteolin, and *t*-cinnamic acid were the major phenolic compounds found in the samples.

Protocatechuic acid, catechin, *p*-benzoic acid, and daidzein were not detected in the samples. The total phenolic content and the phenolic composition of propolis depended on the floral sources that the bees used to produce the propolis (Li *et al*. 2008; Keskin *et al*. 2019).

Table 1. Characteristic Properties of the Propolis Extract

Phenolic Profiles	Raw Propolis (µg/g)	Used Methanolic Extracts (µg/100 mL)
Gallic acid	3.28	0.10
Protocatechuic acid	Not detected	Not detected
p-Benzoic acid	Not detected	Not detected
Catechin	Not detected.	Not detected
Vanillic acid	101.05	17.56
Caffeic acid	2883.59	156.40
Syringic acid	11.64	2.14
Epicatechin	4176.70	645.07
p-Coumaric acid	852.38	42.50
Ferulic acid	3010.43	408.12
Rutin	1719.44	189.07
Daidzein	Not detected	Not detected
t-Cinnamic acid	210.08	34.15
Luteolin	737.80	87.42

Retentions

Mean retentions of the wood samples calculated from solution uptake and propolis concentrations are given in Table 2. As expected, the retention values increased as solution concentration increased from 1% to 7%. The greatest mean retention value, 42.1 kg/m^3, was observed for the Scots pine at a concentration level of 7%. The mean retention amounts for Scots pine were higher than the values for paulownia for all concentration levels examined. The low retention values for paulownia when compared to Scots pine is attributed to the presence of tylose formation on the vessels contained in the paulownia. Tylose formation prevents the penetration of the impregnation solution from the cross-sections into the cells (Hua *et al*. 1986; Ates *et al*. 2008).

Table 2. Mean Retentions (kg /m^3) of Species Treated with Propolis

Propolis Concentration (%)	Paulownia	Scots Pine
1	3.9 (0.3) *a*	6.0 (1.0) *a*
3	13.8 (0.8) *b*	17.7 (0.8) *b*
5	23.6 (1.3) *c*	30.5 (1.6) *c*
7	30.0 (2.0) *d*	42.1 (1.8) *d*
Means within each column followed by the same letter are not significantly different from one another ($p < 0.50$); numbers in parenthesis are standard deviations of mean retention values		

Decay Tests

Table 3 shows the effects of different concentration levels of propolis on the mass loss of the wood species caused by white- or brown-rot fungi. When the effect of the methanol solvent on fungi resistance was taken into account, the results showed that the mass loss values were similar to those of the control samples; hence, the propolis was not an effective factor with fungi resistance. In the samples impregnated with methanol solvent, the mass loss was higher and was only significant to paulownia exposed to *T. versicolor versus* the control samples.

Table 3. Mean Mass Loss of Wood Species

Concentration (%)	Mass Losses (%)			
	Paulownia		Scots pine	
	T. versicolor	*N. lepideus*	*T. versicolor*	*N. lepideus*
Control	39.2 (9.54) a	47.2 (4.32) a	28.4 (3.71) a	29.7 (3.17) a
Solvent	46.7 (5.16) b	43.6 (7.43) ab	30.8 (4.84) a	31.6 (3.43) a
1	37.5 (4.55) a	38.0 (5.53) b	28.3 (3.90) a	28.3 (2.69) a
3	26.8 (5.21) c	26.4 (3.86) c	19.5 (3.06) b	20.2 (4.42) b
5	17.8 (3.47) d	17.9 (6.00) d	13.4 (3.22) c	14.4 (2.83) c
7	12.3 (3.60) d	11.6 (5.31) d	4.2 (2.28) d	2.5 (1.82) d

The values in parentheses are standard deviations of mean mass losses. Means within each column followed by the same letter are not significantly different from one another ($p < 0.50$).

The highest mass loss (47.2%) was observed with the paulownia wood samples exposed to *N. lepideus* fungi in the control samples. In the Scots pine, control samples exposed to *T. versicolor* fungus had a 28.4% mass loss while a 29.7% mass loss occurred with the samples exposed to *N. lepideus* fungi. According to the experimental data, it was determined that the natural resistance of Scots pine was higher than that of paulownia wood when the control samples of the wood species were compared. As the concentration level increased, the mean mass loss decreased for both wood species, and the differences between the control and treated samples were statistically significant ($p < 0.05$). In the paulownia samples treated with 1% propolis and exposed to *N. lepideus* fungi, 43.6% mass loss was found while the mass loss decreased to 11.6% when the concentration of propolis level was increased to 7%. Similarly, Scots pine samples treated with 1% propolis and exposed to *N. lepideus* were observed to have a 28.3% mass loss, while it dropped to 2.5% when the concentration level increased to 7%. It was found that Scots pine treated with 7% propolis prevented mass losses against *T. versicolor* by 85.2% and against *N. lepideus* by 91.5% when compared to the control samples. Mass losses with paulownia treated with propolis were higher than that of Scots pine for all concentration treatments.

The higher mass loss in paulownia samples impregnated with methanol was attributed to the solvent removing the extractives from the wood during the impregnation process. Similar results were also reported by Kirker *et al*. (2013).

There are many studies published on the antifungal efficacy of propolis. Most of the studies investigated the antifungal properties of propolis on *Candida sp.* (Ota *et al*. 2001; Dota *et al*. 2011). Quiroga *et al*. (2006) reported on the anti-fungal effect of propolis against several wood-decaying fungi (*Ganoderma applanatum, Lenzites elegans*, *Pycnoporus sanguineus*, and *Schizophyllum commune*), as well as phytopathogenic fungi (*Aspergillus niger*, *Fusarium sp*., *Macrophomina sp*., *Penicillium notatum*, *Phomopsis sp*., and *Trichoderma sp*.). The authors showed that even a low amount of propolis (1.16 mg/ mL) inhibited the fungi hypha growth. Ratajczak *et al*. (2018) studied the biological durability of wood treated with a mixture of 30% ethanol extract from propolis, caffeine, and organosilanes against *Coniophora puteana*. These authors concluded that propolis-treated wood was seven times more durable than untreated wood. Budija *et al*. (2008) also examined the efficacy of propolis against *Antrodia vaillantii*, *Gloeophyllum trabeum*, and *T. versicolor*. Jones *et al*. (2011) examined the efficacy of propolis in an aqueous soda solution against *Coniophora puteana* and *Poria placenta* as wood-decaying fungi (Ratajczak *et al*. 2018). Many scientific publications have reported that propolis exhibits

good antifungal properties and that these properties are mostly attributed to polyphenolic compounds, such as phenolic acids and flavonoids, that are found in the propolis (Baltas *et al*. 2016; Oliveira *et al*. 2016; Sardi *et al*. 2016; Khatkar *et al*. 2017). The lowest mass loss (2.5%) was observed for Scots pine treated with 7% propolis when the wood was exposed to *N. lepideus* fungi in this study. This result indicated that a 7% concentration of propolis has sufficient efficacy against wood-decaying fungi.

Leaching Tests

Table 4 shows the results from the leaching test to determine whether propolis was affixed to the wood after treatment. In the leaching tests, phenolic compounds were not detected in the leachate after 1, 2, 4, 8, and 16 h of leaching, whereas only 0.014 mg/100 mL of phenolic compounds were found in the leachates after 48 h of leaching. Total phenolic content was determined to be 0.010 mg/100 mL in control samples after a 48-h leaching period. The total amount of phenolics detected by the 48 h leaching for the control samples and the propolis-treated samples were almost identical. According to these findings, propolis was not leached from Scots pine during the leaching period examined.

Table 4. Total Phenolic Content After Leaching Test

Time (h)	Total Phenolic Content (mg/100 mL)
1	Not detected
2	Not detected
4	Not detected
8	Not detected
16	Not detected
48	0.014
48 (untreated control)	0.010

An effective wood preservative should resist leaching when it comes in contact with water in outdoor conditions. Because propolis consists of a high amount of insoluble resin, wax, and oils by water (Wagh 2013), phenolic compounds in the leachate waters were not detected after 16-h leaching tests, and after 48 h they were present in a low amount. Hence, wood materials treated with propolis could have long service times in outdoor environments.

FTIR characterization

The FTIR analyses of brown and white-rot tests of Scots pine and paulownia samples are shown in Fig. 1. When Fig. 1a and 1c were examined, it was observed that the lengths of peaks at 1740, 1380, 1327, 1165, and 903 cm^{-1} representing carbohydrate components of the untreated wood samples (Bari *et al*. 2015) exposed to *N. lepideus* fungi (A) were decreased compared to the control sample (B). This decrease in the absorptions was less prominent in these spectra than in the spectra from the other wood samples impregnated with propolis. The reason for the partial decrease of the density of the band at 1165 cm^{-1} indicated that the average polymerization degree of polycarbonates decreased. The decrease in the absorption intensity of the band at 903 cm^{-1} indicated changes to the cell wall carbohydrates. Hence, the observed changes at 1165 and 903 cm^{-1} were important to determine the resistance of the wood samples against fungal decay (Wozniak *et al*. 2015). In this case, as the propolis concentration increased, the ability of *N. lepideus* fungi

to degrade the carbohydrates decreased. There was a slight increase in the absorption peaks at 1510, 1465, 1425, and 1268 cm^{-1}, which are attributed to lignin components (Silverstein *et al*. 2005). When the Scots pine and paulownia samples were exposed to the *N. lepideus* fungi, most of the degradation of the cell wall components occurred with the carbohydrates (*i.e.*, hemicelluloses and cellulose), whereas the lignin degradation was limited.

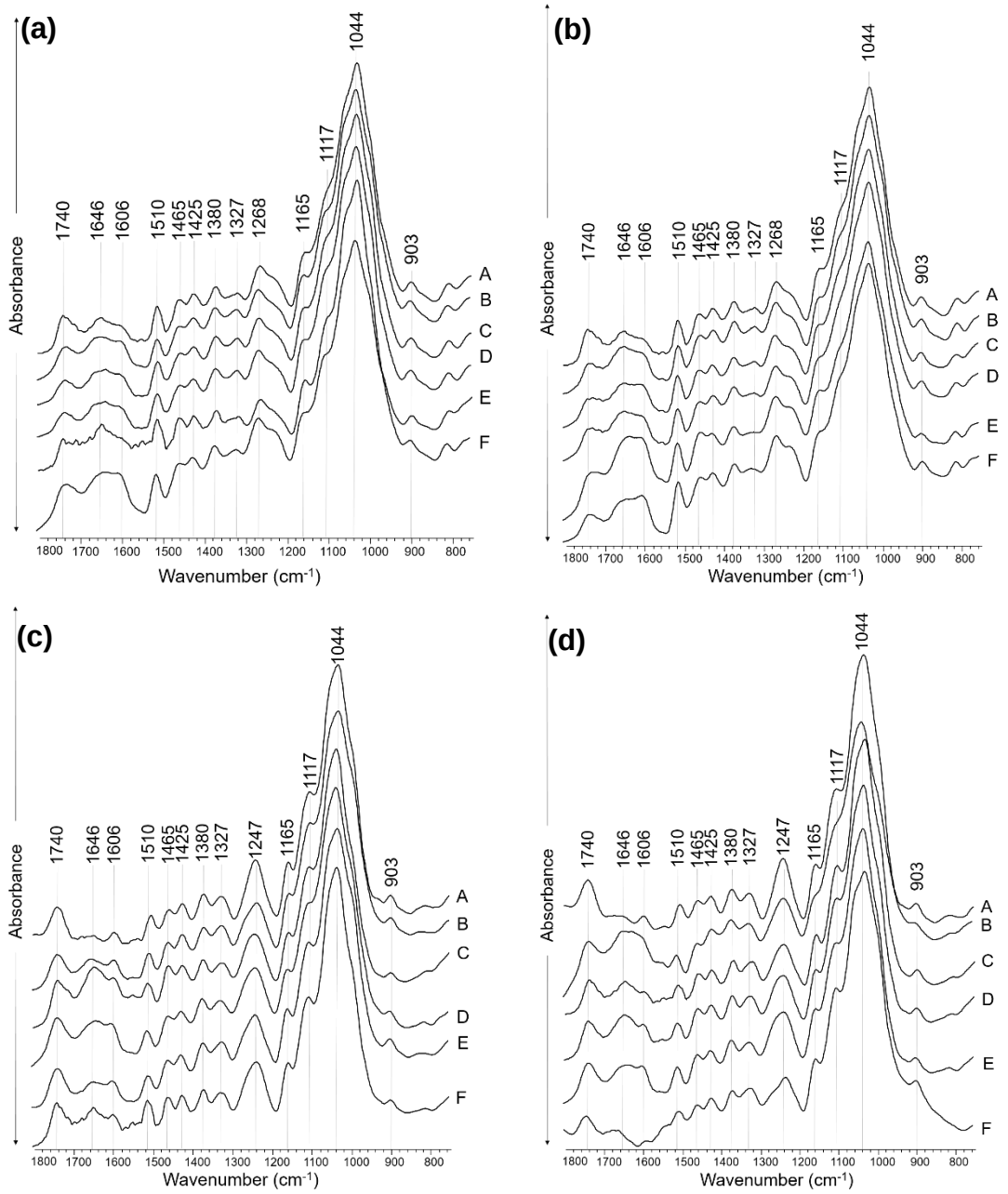

Fig. 1. FTIR analysis of Scots pine ((a) and (b)) and paulownia ((c) and (d)) samples at the end of brown- ((a) and (c)) and white- ((b) and (d)) rot fungal tests (A: untreated and not decayed, B: untreated and decayed, C: treated with 1% propolis and decayed, D: treated with 3% propolis and decayed, E: treated with 5% propolis and decayed, F: treated with 7% propolis and decayed)

The FTIR spectra of Scots pine and paulownia samples exposed to *T. versicolor* for 12 weeks are shown in Fig. 1b and 1d, respectively. With the effect of white-rot, the strain peak in holocellulose at 1740 cm^{-1} did not changed significantly for both wood species due to the C=O double bond. The C=C aromatic stress absorption at 1510 cm^{-1} and the absorption from the C–O group of lignin at 1606 cm^{-1} were weaker in the B spectrum when compared to the A spectrum. These absorption peaks of C, D, E, and F in the FTIR spectra of wood samples treated with propolis were not appreciably different when compared to FTIR spectrum of A. It was deduced that *T. versicolor* consumed lignin from the cell wall of the wood; however, this ability was inhibited when the treatment concentration of propolis increased.

SEM analysis

The SEM analyses of the Scots pine and paulownia wood after the fungal tests are shown in Fig. 2.

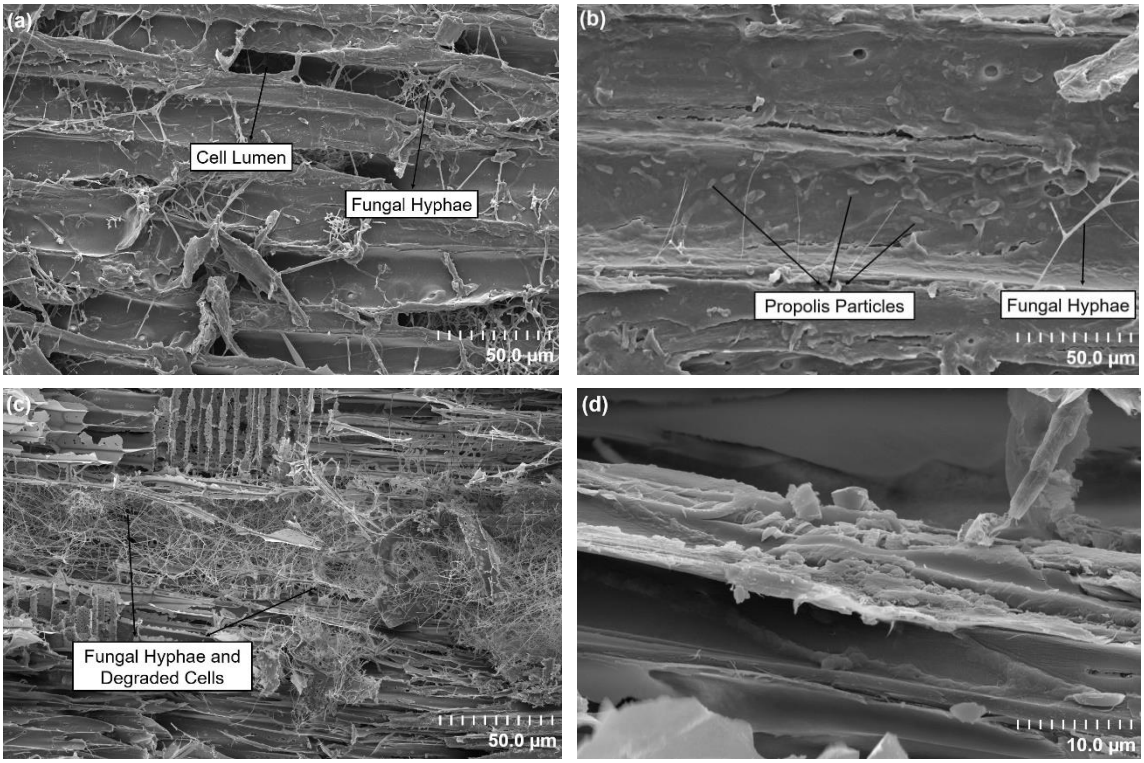

Fig. 2. Scots pine and paulownia specimens exposed to *N. lepideus* and *T. versicolor* (a: untreated Scots pine and exposed to *N. lepideus*, b: 7%-treated Scots pine and exposed to *N. lepideus*, c: untreated paulownia and exposed to *T. versicolor*, and d: 7%-treated paulownia and exposed to *T. versicolor*)

Figure 2 shows dense fungal hypha/mycelium in vessel and tracheid cells in the control samples that were not impregnated with propolis in both tree and fungal species. However, it is shown that there were much less fungal hyphae in the samples impregnated with 7% propolis *versus* the control samples. Propolis extracts were located as sediments in the tracheids and vessels. The SEM analyses clearly showed that both wood species samples impregnated with propolis extracts were much more resistant to fungal attack than the untreated control samples.

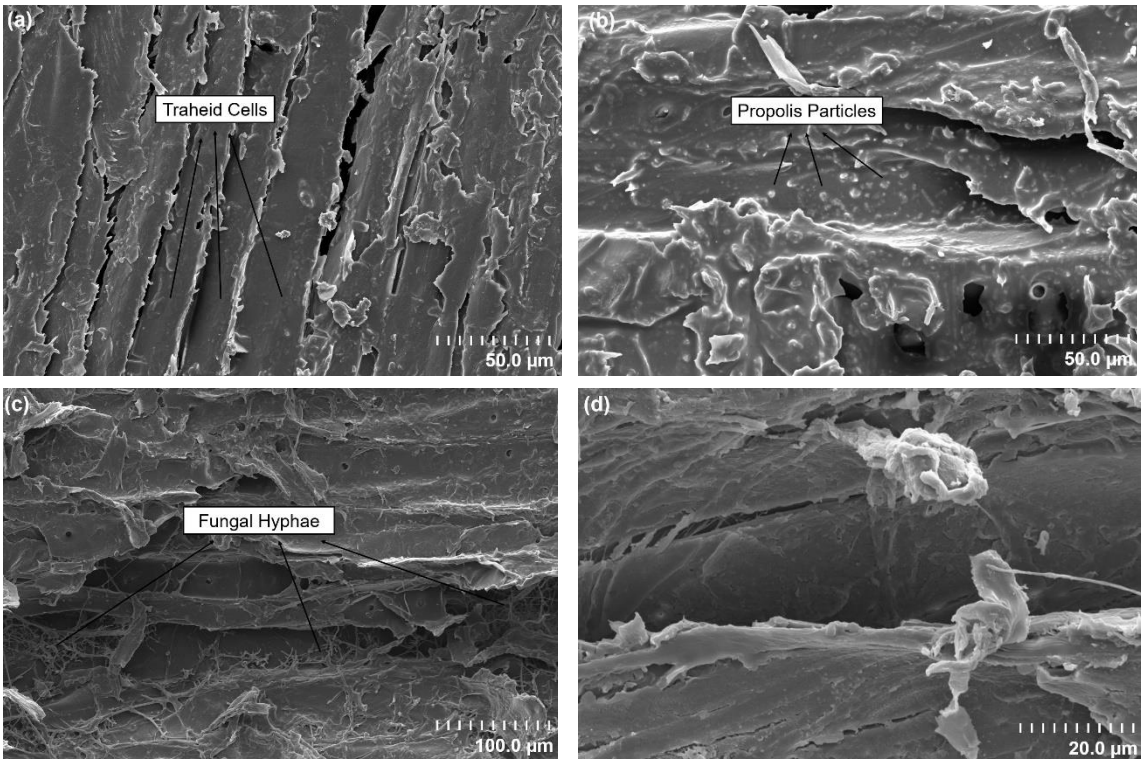

Fig. 3. Scots pine specimens (a: untreated and not exposed to decay, b: 7%-treated and not exposed to decay, c: untreated and exposed to *T. versicolor*, and d: 7%-treated and exposed to *T. versicolor*)

Fungal hyphae penetrated into the cell lumens of the wood *via* the pits and began to change the structure of the wood by spreading through the lumens (Goodell *et al*. 2003). Subsequently, the cell wall components of wood were decomposed by the fungal enzymes, and/or low molecular agents, which changed the carbohydrate decomposition products (Durmaz *et al*. 2016; Can *et al*. 2018). Figures 2a, 2c, 3a, 3c, 4a, and 4c express that the fungal hyphae played an important role in the degradation of Scots pine and paulownia woods.

Dense fungal hyphae were mostly located in the untreated paulownia wood cells where the walls were almost completely degraded. It was thought that the white-rot fungi (*T. versicolor*) caused the destruction of all wood components (cellulose, hemicelluloses, and lignin) in the observed micrographs (Dashtban *et al*. 2010). In addition, the white-rot fungi preferred hardwoods, while the brown-rot fungi preferred softwoods (Rudakiya and Gupte 2017). The enzyme systems of the fungi played an important role in this degradation (Tuor *et al*. 1995). When the wood samples were impregnated with propolis extracts, fungal hyphae were not observed in the wood cell lumens in the SEM images. In addition, dense mycelium covered on the untreated Scots pine and paulownia specimens while micelle density decreased sharply on the impregnated specimens when the concentration level of propolis reached 7% (Fig. 5).

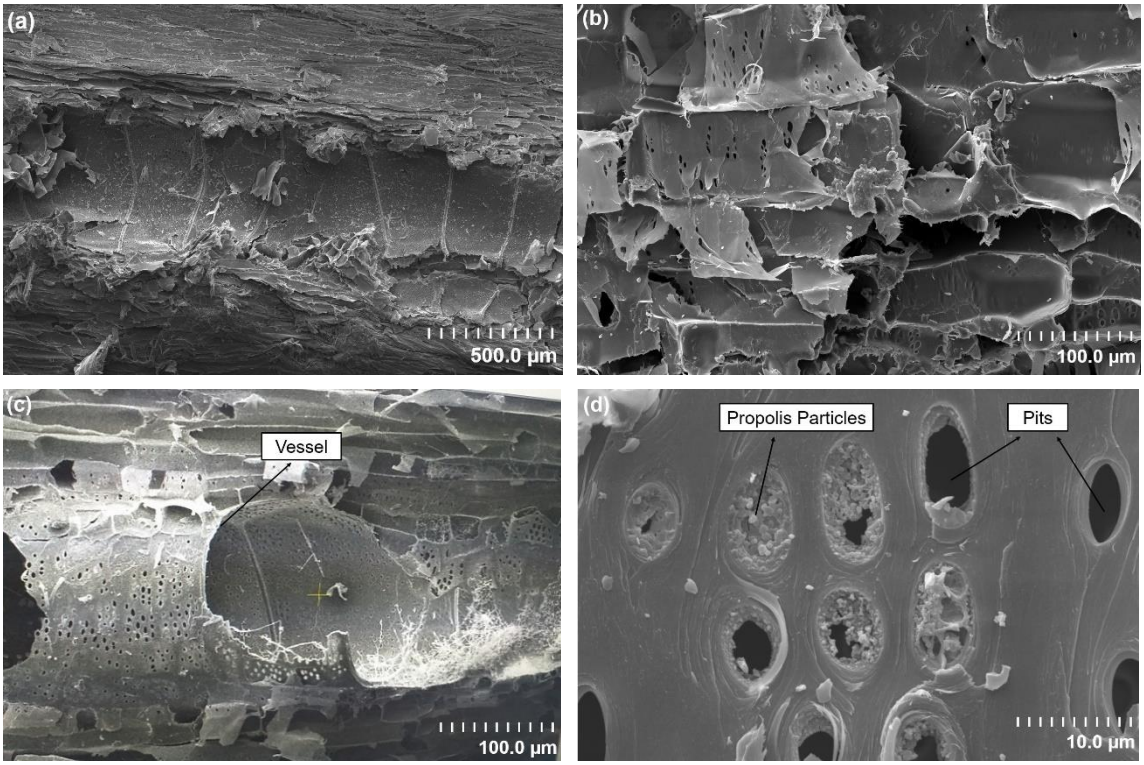

Fig. 4. Paulownia specimens exposed to *N. lepideus* (a: untreated and not exposed to decay fungi, b: 7%-treated and not exposed to decay, c: untreated and exposed to *N. lepideus*, and d: 7%-treated and exposed to *N. lepideus*)

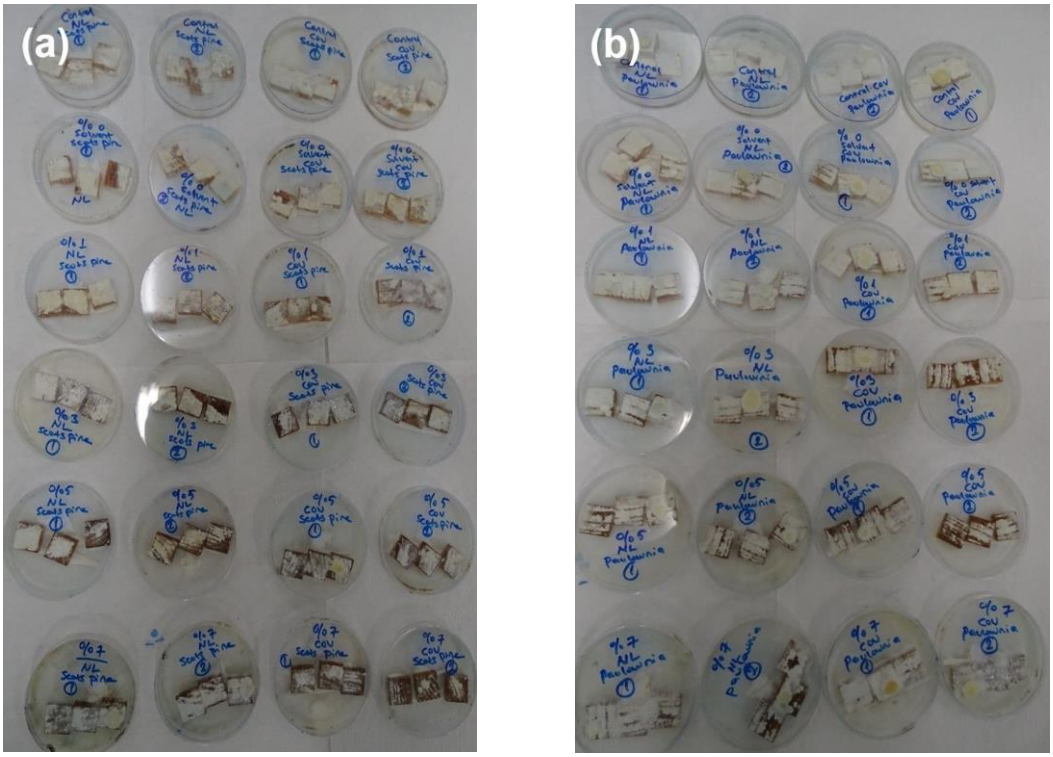

Fig. 5. Images of fungal test for Scots pine (a) and paulownia (b) wood samples exposed *to N. lepideus* and *T. versicolor*

CONCLUSIONS

1. In this study, a 91.5% protection rate against wood-rot fungi was observed with the Scots pine wood treated with propolis extracts *versus* the control.
2. Scots pine treated with propolis was more durable against decay than paulownia when the wood species were considered.
3. Absorption peaks at 1510, 1465, 1425, and 1268 cm^{-1} in samples exposed to *N. lepideus* generally showed a slight increase. The main reason for this increase is that brown rot fungus consumes cell wall carbohydrates and proportionally increases the lignin ratio in the cell wall. In order to see this increase in FTIR spectra more clearly and better determine the ability of propolis to protect wood against fungi, it is necessary to increase the duration of decay test and propolis concentration.
4. SEM images showed that the cell walls of the untreated control specimens were intensely degraded by the fungus when compared to the samples impregnated with propolis.
5. No phenolic compounds were detected in the leachates until an exposure of 16 h during the leaching tests, while a very low amont of phenolic content had been leached at 48 h, which was almost identical compared with that of untrated control samples.

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Article submitted: October 9, 2019; Peer review completed: December 1, 2019; Revised version received and accepted: December 4, 2019; Published: January 14, 2020.
DOI: 10.15376/biores.15.1.1547-1562