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THE EFFICIENCY OF *Pistacia atlantica* GUM FOR INCREASING RESISTANCE OF RAPESEED OIL-HEAT TREATED WOOD TO FUNGAL ATTACKS

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ABSTRACT

In this research, we used *Pistacia atlantica* gum during cooling phase of oil-heat treatment of poplar wood (*Populus deltoids*) to improve its resistance to the white-rot fungus *Trametes versicolor* and growth of the mold fungus *Penicillium expansum*. Thermal modification was carried out using rapeseed oil at 180 °C, 200 °C and 220 °C for 2 hours and 4 hours. The modified wood specimens were then directly cooled in the oil containing 0 %, 5 % and 10 % (w/w) of the gum at 25 °C for 30 minutes. The chemical constituents of the essential oil extracted with a Clevenger type apparatus were determined by chromatography–mass spectrometry (GC-MS). The amounts of α-pinene, β-pinene and α-terpinolene of the essential oil were 60,2 %, 8,7 % and 3,9 %, respectively. The mold resistance was greatly improved, while the improvement against the decay fungus was only observed for the specimens modified at 180 °C. Our results confirmed that the enhanced fungal resistance was not only due to the presence of monoterpenes in the essential oil, but also to a further reduction in the hygroscopicity of the treated wood.

Keywords: Fungal resistance, oil-heat treated wood, *Penicillium expansum, Pistacia atlantica, Populus deltoids, Trametes versicolor.*

INTRODUCTION

Natural compounds of plants, such as essential oils, and extractives from very durable wood species, can be used as alternatives to harmful chemical preservatives for biological protection of wood (Pánek *et al.* 2014, Xie *et al.* 2017, Fernández-Costas *et al.* 2017, Zhang *et al.* 2016, Bahmani and Schmidt 2018). Essential oils are mostly composed of terpenes (e.g. mono-, di- and sesqui-terpenes), which have antimicrobial activity (Dhifi *et al.* 2016). So far, the potential uses of several essential oils from different parts of plants like *Syzygium aromaticum*, *Betula pendula*, *Lavandula angustifolia*, *Origanum vulgare*, *Acorus calamus*, *Satureja hortensis*, *Salvia officinalis*, *Melaleuca alternifolia*, *Thymus vulgaris* (Pánek *et al.* 2014), *Cymbopogon citratus*, *Pelargonium graveolens*, *Cinnamomum zeylanicum*, *Eugenia caryophyllata* (Xie *et al.* 2017), *Eucalyptus camaldulensis, Pinus rigida* (Salem *et al.* 2016), *Cedrus atlantica* (Fidah *et al.* 2016), *Artemisia monosperma, Cupressus sempervirens*, *Citrus limon*, *Thuja occidentalis* and *Schinus molle, Pelargonium graveolens (*Mohareb *et al.* 2013), *Cymbopogon winterianus*, *Eucalyptus globolus*, *Foeniculum vulgare*, *Ilium verum*, *Juniperus mexicana*, *Matricaria chamomilla*, *Melaleuca alternifolia*, *Melea arachdirachta*, *Mentha arvensis*, *Mentha piperita*, *Oenothera biennis*, *Trachyspermum copticum* (Bahmani and Schmidt 2018) have been investigated for protection of wood against mold and decay fungi.

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Pistacia atlantica is a deciduous tree species which grows from Iranian plateau to North Africa. The exudate gum from the tree trunk which is rich in monoterpene hydrocarbons is used in pharmaceutical and food industries (Barrero *et al.* 2005, Benhammou *et al.* 2008). It was historically used by some philosophers such as Abu Ali Sina as a medicine for abdominal pain and stomach ulcers. The gum exudate is obtained by injuring the trunk using a sharp adze at the end of spring. The production process of the gum was described in detail by Ahmed (2017). Antifungal and antibacterial properties of the essential oil extracted from different parts of *P. atlantica* tree (leaves, fruits and gum) have been reported in numerous previous works (Benhammou *et al.* 2008, Talibi *et al.* 2012, Habibi Najafi *et al.* 2014, Rezaie *et al.* 2015, Hamelian *et al.* 2018), but the question is whether the essential oil can improve the resistance to wood-decay fungi. Unlike molds, which generally feed on starch, simple sugars and proteins stored in the ray and axial parenchyma cells of sapwood, the decay fungi consume the cell wall components (i.e. cellulose, hemicellulose and lignin). Sadeghi *et al.* (2016) found that the gum, fruit and leaves essential oils of *Pistacia atlantica* subsp. Kurdica had insecticide activities against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) beetle. The insecticidal properties of the essential oils of this plant against *Callosobruchus maculatus* (Fabricius) (Coloeptera: Bruchidae) was also reported by Pourya *et al.* (2018).

Oil-heat treatment (OHT) is one of the most environmentally friendly methods of wood modification, which involves the heating of wood in oil at relatively high temperatures (usually 180 °C to 260 °C). Thermal modification is well known to be effective in improving the dimensional stability and decay resistance of wood (Lee *et al.* 2018). Various vegetable oils with high thermal stability (e.g. linseed, canola, palm, soy and coconut oil) can be used for the modification. The oil provides a fast and uniform heat transfer in wood and prevents its oxidation by formation of a barrier between the wood and oxygen. The characteristics of oil-heat treated wood depend on several factors, such as oil temperature, wood species, period of heating and weight percent gain (Lee *et al.* 2018). Although oil heat treatment improves the resistance of wood against biodeterioration agents, the modified wood remains susceptible to decay fungi and molds. Therefore, some researchers have recently used several additives with oil to improve the performance of oil-heat treated wood (Lyona *et al.* 2007, Mohebby *et al.* 2014). It was found that impregnation of wood with a 1,0 % w/w boric acid solution prior to oil treatment reduced the leaching of the preservative, and improved the decay and termite resistance of the wood (Lyona *et al.* 2007). Mohebby *et al.* (2014) also improved the physico-mechanical properties of olethermal modified fir wood by using soybean oil combined with maleic anhydride.

This study aimed to improve the resistance of oil-heat treated poplar wood to growth of the mold *Penicillium expansum* and the white-rot fungus *Trametes versicolor* by using *P. atlantica* gum during the cooling stage of the thermal modification process. Poplar is a fast-growing species that is widely used for the manufacture of a broad range of forest products. However, modification or preservative treatment is generally required to extend the service life of this non-durable wood.

MATERIALS AND METHODS

Materials

A poplar tree species (*Populus deltoids* L.), growing in an experimental forest (Nowshahr, Mazandaran Province, Iran), belonging to University of Tehran was felled. Then, the sapwood specimens with dimensions required for each test were cut. *P. atlantica* gum with density of 1100 kg/m^3 and pH of 5 was prepared from Zagros forest located in Kurdistan province of Iran. Rapeseed oil with density of 920 kg/m^3 and dynamic viscosity of 0,078 Pa s at 20 °C was used for thermal modification. The oil was purchased from Zeyton Talaei Co. in Qazvin, Iran.

Extraction of essential oil

In order to determine the type and amount of components in the essential oil of *P. atlantica*, the oil was initially extracted from the gum by hydrodistillation method using a Clevenger type apparatus. For this purpose, 50 g of gum was heated with distillated water at the boiling temperature of about 100 °C for 2 hours. The extracted essential oil was subsequently dried over anhydrous sodium sulfate and stored in a dark glass inside a refrigerator at 4 °C until tested.

Gas chromatography/mass spectrometry analysis

Gas chromatography-mass spectrometry (5975C Series GC/MSD system) with column length of 30 m and inside diameter (id) of 0,25 mm was used to identify the essential oil components. Helium gas was used as the carrier gas at a flow rate of 1 ml/min. The column temperature ranged from 45 °C to 250 °C at 3 °C/min.

Attenuated total reflectance/Fourier transform infrared (ATR-FTIR) spectroscopy

The chemical structure of the gum was also determined by Equinox 55 ATR-FTIR spectrometer (Bruker Optics GmbH, Ettlingen, Germany). The spectroscopy was carried out at the wavenumber of 400 cm^{-1} to 4000 cm^{-1} using 64 scans at a resolution of 4 cm^{-1}. The obtained spectra were baseline corrected by the concave rubber band method and max-min normalized.

Thermal modification

Prior to thermal modification, the wood samples were conditioned at 20 °C and relative humidity (RH) of 65 % to 12 % moisture content (MC). Then, the specimens were immersed in a cylinder containing rapeseed oil. Thermal modification was carried out at 180 °C, 200 °C and 220 °C for 2 hours and 4 hours. The cooling stage of the process was done in the rapeseed oil containing *P. atlantica* gum at concentrations of 5 % and 10 % (w/w) at 25 °C for 30 minutes. After cooling, the temperature of the solution varied from 50 °C to 60 °C. The modified samples were dried at 103 °C ± 2 °C for 24 hours and weighed to determine the weight percent gain (WPG).

X-ray scanning

The vertical density profile of the modified wood specimens was measured using a commercial X-ray scanner (Siempelkamp's Sicoscan, Germany) to determine the uniformity of the modification process. The specimens were prepared with dimensions of 50 mm × 50 mm × 50 mm according to the instruction manual of the device. The measurements were performed by scanning across the thickness in the middle of the specimens.

Moisture exclusion efficiency

The oven-dried samples were placed in a conditioning room at 20 °C and 65 % RH for 3 weeks to determine the moisture exclusion efficiency (MEE) of the modified woods. The efficiency was determined by Equation 1:

$$MEE = \left(\frac{E_u - E_m}{E_u} \right) x\ 100 \qquad (1)$$

Where E_u and E_m are the equilibrium moisture content (EMC) of the control and modified woods.

Decay test

The resistance of the wood samples to the white-rot fungus *Trametes versicolor* (strain: CTB 863A) was evaluated. The fungal strain was obtained from Wood Preservation Laboratory, Research Unit, BioWooEB, CIRAD, Montpellier, France. Wood blocks with dimensions of 15 mm × 25 mm × 50 mm (L × R × T) were prepared according to European standard test method CEN EN113 (1996). A uniform culture medium (malt extract agar, MEA) was prepared by adding 45 g of malt agar to 1000 ml of distilled water, followed by heating the solution for 15 minutes. Then, the medium was sterilized inside a steam autoclave at 120 °C for 20 minutes. Cubic glass containers with metal lids were used for fungal cultivation. Each container was filled with 70 ml of 4,8 % (w/v) malt agar solution. A 20-mm diameter hole was made on the glass lid and compressed cotton was placed inside the hole for the air exchange. One modified sample and one unmodified sample were placed inside each container. A plastic mesh was used to prevent the direct contact of the wood samples with the medium. Five replicates were used for each treatment. The specimens were incubated at 22 °C and 75 % RH for 16 weeks. After this period, they were removed from incubator, cleaned from the surface fungal mycelium and

dried for 24 hours in an oven at 103 ± 2 °C for 24 hours and the weight loss were finally calculated.

Mold resistance test

Wood blocks with dimensions of 7 mm × 20 mm × 70 mm (T × R ×L) were cut according to the ASTM D4445-91 (1996). Control samples were also prepared from freshly-cut boards with moisture content of about 60%. The strains of *Penicillium expansum* was provided from Department of Plant Protection at University of Tehran. The wood samples were sterilized in an autoclave at 120 °C for 20 minutes. Mold spore suspension was prepared by adding 10 ml of distillated water to petri dishes, containing mold spores. Eight filter papers sprayed with the distilled water were placed into each petri dish. Glass tubes with diameter of 3 mm were put under the wood specimens to prevent the direct contact of the specimens with the wet filter papers. The specimens were sprayed with 1ml of mold spore suspension and incubated at 25 °C and 70 % RH for 4 weeks. After this period, the mold coverage was visually determined on a scale of 0-5 and reported as 0 (free of mold growth), 1 (1 % to 5 %), 2 (6 % to 25 %), 3 (26 % to 50 %), 4 (51 % to 75 %) and 5 with heavy mold growth (mold coverage of 76 % to 100 %). Data analysis was done using SPSS software and the mean of data was compared using Duncan test at 5 % level.

RESULTS AND DISCUSSION

Chemical structure of *P. atlantica* essential oil

The chemical components of *P. atlantica* essential oil are shown in Table 1 α-pinene, β-pinene and α-terpinolene were the most constituents of the oil with amount of 60,15 %, 8,68 % and 3,93 %, respectively. The type and amount of chemical compounds of *P. atlantica* essential oil determined in this study were slightly different with those reported in some previous researches (Alma *et al.* 2004, Barrero *et al.* 2005, Salimi *et al.* 2011). Barrero *et al.* (2005) identified the α-pinene and β-pinene as the main components of the essential oil with amount of 42,9 % and 13,2 %, respectively. These differences may be due to variation in tree species, sampling time, and growth conditions (Alma *et al.* 2004).

Table 1: Chemical composition of *P. atlantica* gum.

Chemical name	Molecular weight	Formula	CAS number	Percentage
α -pinene	136,13	$C_{10}H_{16}$	8-56-000080	60,15
β -pinene	136,13	$C_{10}H_{17}$	3-91-000127	8,68
α -terpinolene	136,13	$C_{10}H_{16}$	9-62-000586	3,94
Trans-verbenol	152,12	$C_{10}H_{16}O$	3-09-001820	3,03
Del- limonene	163,13	$C_{10}H_{16}$	3-86-000138	2,67
P-mentha-1,5-dien-8-ol	152,12	$C_{10}H_{16}O$	0-20-001686	2,57
Pinocarveol, trans	152,12	$C_{10}H_{16}O$	5-61-000547	2,49
α -terpineol	154,14	$C_{10}H_{18}O$	5-55-000098	2,29
Camphene	136,13	$C_{10}H_{16}$	5-92-000079	1,99
β -myrcene	136,12	$C_{10}H_{16}$	3-35-000123	1,70
α -bornyl acetate	196,15	$C_{12}H_{20}O_2$	8-61-005655	1,65
1,8-cineole	154,14	$C_{10}H_{18}O$	6-82-000470	1,57
Camphor aldehyde	152,12	$C_{10}H_{16}O$	1-03-026882	1,32
Delta-3-carene	136,13	$C_{10}H_{16}$	9-78-013466	1,31
Hexane	86,11	C_6H_{14}	3-54-000110	1,24
Pi- mirsen	134,11	$C_{10}H_{14}$	6-87-000099	1,04
Mirtenol	152,12	$C_{10}H_{16}O$	4-00-000515	0,75
Trans-(+)carveol	152,12	$C_{10}H_{16}O$	5-07-001197	0,66
2-pinen-4-one	150,10	$C_{10}H_{14}O$	9-57-000080	0,55
Exo-2-hydroxycineole	212,14	$C_{10}H_{20}O_3$	2-95-057709	0,41

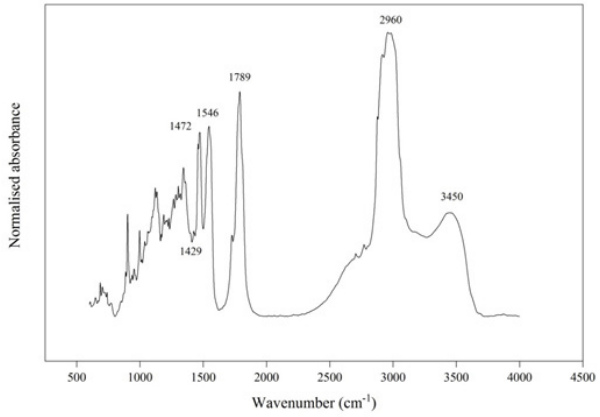

Figure 1: ATR-FTIR spectrum of *P. atlantica* gum.

The FTIR spectrum of the gum is given in Figure 1. The peaks at wavenumbers of 3450 cm^{-1}, 2960 cm^{-1} and 1789 cm^{-1} are related to the stretching vibration of O-H, C-H, and C=O, respectively. The absorption bands at wavenumbers of 1429 cm^{-1} and 1472 cm^{-1} are due to vibration of C-H group. The peak occurred in the wavenumber of 1546 cm^{-1} is also caused by vibration of N-H and C=N.

Weight percent gain and moisture exclusion efficiency

The oil-heat treatment yielded a WPG in the range of 60,7 % to 77,6 % (Figure 2). The WPG after thermal modification in oil was previously reported to be in the range of 50 % to 90 %, depending on the process variables (time and temperature) and wood species (Sailer *et al.* 2000, Lee *et al.* 2018). The weight of wood is normally reduced due to thermal degradation of the cell wall polymers; however, the amount of oil uptake during thermal modification with oil is much more than the weight loss caused by the thermal degradation, resulting in the weight gain. The WPG increased by using *P. atlantica* gum, which was directly proportional to its concentration. In agreement with previous works (Lee *et al.* 2018), the WPG decreased by increasing the modification temperature from 180 °C to 220 °C when the cooling stage was carried out in the oil without *P. atlantica* gum. This can be due to further destruction of the wood cell wall compounds at higher temperatures. In contrast, the WPG was greater at higher temperatures when the specimens were cooled in the oil containing 5 % *P. atlantica* gum. It is believed that the oil is significantly absorbed during the cooling stage of the oil-heat treatment process due to the pressure gradient (Lee *et al.* 2018).

A uniform pattern of density profile for the modified wood specimens (Figure 3) indicates a roughly homogeneous thermal modification and oil uptake through the specimen thickness. As expected, thermal modification reduced the EMC of wood samples (Figure 4). The moisture exclusion efficiency is usually attributed to degradation of the cell wall polymers, reduction in the hydroxyl (OH) accessibility, polycondensation reactions in the lignin, and formation of thermal degradation products which reduce the microporosity of the cell walls (Esteves and Pereira 2009). The efficiency was also improved by using *P. atlantica* gum.

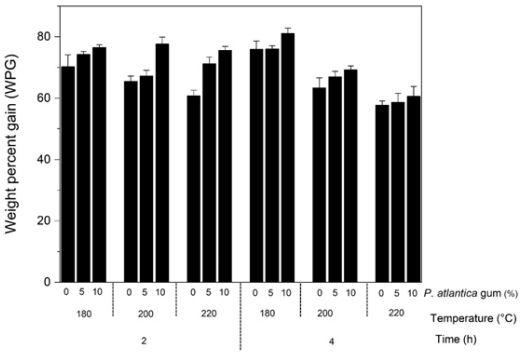

Figure 2: Weight percent gain (WPG) of the poplar wood samples after heat treatment with oil under different treatment conditions.

Means with similar letters are not statistically significantly different (α=5 %) based on Duncan's multiple range test.

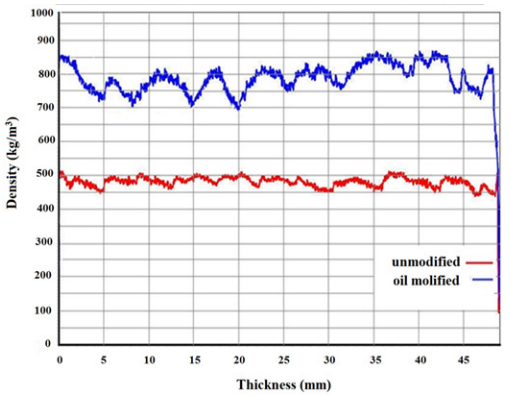

Figure 3: Density profile across the thickness of the control (unmodified) and oil-heat treated poplar wood (treatment condition: 200 °C, 2 h).

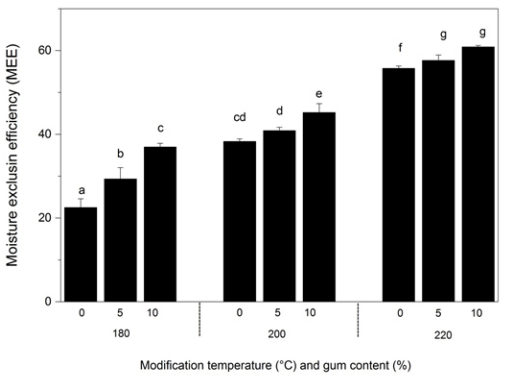

Figure 4: Moisture exclusion efficiency of the poplar wood specimens modified at different temperatures and *P. atlantica* gum contents for 2 hours.

Means with similar letters are not statistically significantly different (α=5 %) based on Duncan's multiple range test.

Fungal resistance

Results showed that resistance of the wood samples to *Trametes versicolor* was improved by oil heat treatment (Figure 5). The improvement in the fungal resistance of wood by thermal modification can be explained by the bulking effects, reduction in the accessible hydroxyl (OH) groups and moisture content (Esteves and Pereira 2009, Hill 2006, Thybring 2013). Various toxic extractives, such as phenolic compounds are formed due to thermal modification, which can reduce the fungal growth until they remain in the modified wood. Diffusion of low molecular weight degradative agents within the cell walls is the most likely mechanism responsible for wood decay during initial biodegradation (Schmidt 2006). The presence of water in the cell walls is essential for such a diffusion process. A reduction in the cell wall moisture content caused by thermal modification limits the diffusion rate (Esteves and Pereira 2009, Thybring 2013). Changes in the crystallinity of wood due to thermal modification can be another factor in controlling the natural durability of the modified wood (Esteves and Pereira 2009). In agreement with previous findings (Esteves and Pereira 2009, Calonego *et al*. 2010, Lee *et al.* 2018), our results showed that the decay resistance was improved by increasing the temperature of thermal modification from 180 °C to 220 °C. Hakkou *et al.* (2006) also reported a strong correlation between the temperature of thermal modification and fungal durability of heat-treated beech wood to *T. versicolor*. We found no improvement in the decay resistance by increasing the modification time. The decay resistance of wood specimens modified at 180 °C was slightly improved by using *P. atlantica* gum. The improving effect can be due to monoterpenes of the gum that have antifungal activity (Mohareb *et al.* 2013). However, at higher modification temperatures, i.e. 200 °C and 220 °C, the use of *P. atlantica* gum was not effective in improving the decay resistance. Bahmani and Schmidt (2018) showed the inhibition of surface colonization of *Fagus orientalis* wood samples by the oils from *Cybopogon winterianus*, *Lavandula angustifolia*, *Thymus vulgaris* and *Trachsperum copticum*.

Mold resistance of the specimens was also improved after oil heat treatment. The improvement was more pronounced at higher temperatures (Figure 6). This is mainly due to reduction in the hygroscopicity of wood after heat treatment (Ahmed *et al.* 2017). On the other hand, similar to what was observed with the decay resistance, the growth of mold was not significantly affected by increasing the heat treatment duration from 2 hours to 4 hours. According to the classification system of mold attacks (Waals *et al.* 2003), all oil heat-treated specimens were in the same class of the mold growth. The control specimens with 76 % to 100 % mold coverage were in class 5, while the modified specimens with 51 % to 75 % mold coverage were in class 4.

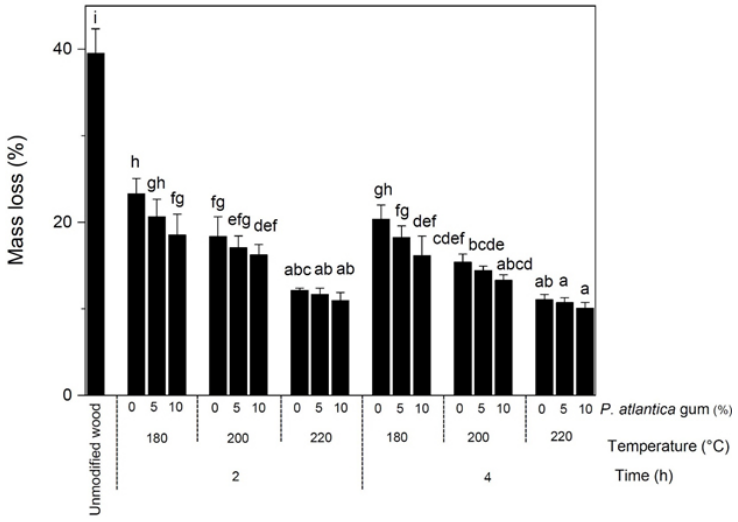

Figure 5: Mass loss of the control and oil heat-treated poplar wood specimens due to degradation by *Trametes versicolor*.

Means with similar letters are not statistically significantly different (α=5 %) based on Duncan's multiple range test.

Although the oil heat treatment improved the mold resistance, the modified woods were not completely safe from the mold attack. Unlike decay fungi that feed on the cell wall compounds, molds feed on the stored starches, sugars and proteins, and thus the cell wall alteration caused by thermal modification has little influence on the mold control. Thus, it is recommended to use mold-resistant coatings for protection of ther-

mally modified wood against molds. Boonstra *et al.* (2007) also found that the heat-treated radiata pine and Norway spruce were susceptible to mold growth due to formation of some thermal degradation products like surges. The addition of *P. atlantica* gum to the rapeseed oil significantly reduced the mold coverage. All wood specimens treated by using *P. atlantica* gum with mold coverage of 26 % to 50 % were in class 3. However, the gum could not completely prevent the mold growth. The enhanced mold resistance is due to the antimicrobial properties of the gum, along with the improved moisture exclusion efficiency of the modified wood. Antifungal efficiency of *P. atlantica* essential oil against *Penicillium italicum* (Talibi *et al.* 2012), *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* (Shialy *et al.* 2015), *Rhizopus stolonifer*, *Trichoderma sp* and *Fusarium sp* (Benhammou *et al.* 2008) was also previously reported. Bahmani and Schmidt (2018) showed complete growth inhibition of *A. niger* and *P. commune* on *Pinus taeda* wood samples by the oils from *Cymbopogon winterianus*, *Lavandula angustifolia* and *Thymus vulgaris* and additionally by *Trachsperum copticum* oil on *Fagus orientalis* samples.

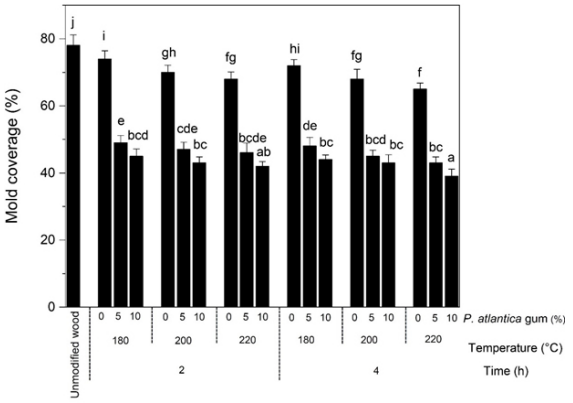

Figure 6: Mold resistance of the control and oil heat-treated poplar wood.
Means with similar letters are not statistically significantly different (α=5 %) based on Duncan's multiple range test.

CONCLUSIONS

We found that the *P. atlantica* gum had more pronounced effect on the mold growth of the oil-heat treated wood than the decay resistance. The improvement can be due to the presence of monoterpenes such as α-pinene, β-pinene and α-terpinolene in the *P. atlantica* essential oil as well as a further increase in the moisture exclusion efficiency of the treated wood. The results of this study also showed that increasing the heat-treatment temperature was more successful than increasing the heat-treatment time to improve the resistance of the oil-heat treated wood to the fungal attacks. Considering the high amount of α-pinene and β-pinene in the *P. atlantica* essential oil, study on its efficiency for preservation of wood against wood-destroying insects is recommended.

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