# Investigation of Fungal Decay of Poplar Wood Treated with Pistachio Resin

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The density, swelling, and fungal decay of poplar (*Populus deltoides*) wood treated with pistachio resin (PR) obtained from *Pistacia atlantica* were investigated. The white-rot fungus *Trametes versicolor* and the brown-rot fungus *Coniophora puteana* were used. Methanolic solutions of PR with different concentrations of 1%, 6%, 12%, and 15% were used as the preservative solution. Wood samples were saturated by two different vacuum/pressure (V/P) and dipping methods. The density, volumetric swelling of treated wood, and their mass loss (ML) caused by fungal decay were determined. The density of treated species increased to 15.4% and 5.8% for V/P and dipping methods, respectively, at 15% PR concentration. The volumetric swelling of the treated samples was reduced to 24.5% and 16.8% for V/P and dipping procedure, respectively, at 15% PR concentration. The mass loss of treated samples after exposure to *T. versicolor* was less than the untreated one (17.4% for V/P and 22.6% for dipping methods at 15% PR concentration). The results showed the better performance of V/P treatment in promotion of wood durability against fungal decay than the dipping method.

*Keywords: Pistachio resin; Populus deltoides; Fungal decay; White-rot and brown-rot fungi*

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## INTRODUCTION

Microorganisms such as fungi and bacteria, as well as insects, usually attack wood and wood products under unfavorable environmental conditions, including high humidity and moisture (Yang and Clausen 2007). Fungi are some of the most important degrading agents. Decay fungi destroy wood by consuming the woody substrate, changing the chemical and physical properties of wood and reducing its mechanical performance. Wood is protected against these biological damages by wood drying and chemical treatment. Synthetic fungicides such as chromium(VI)-copper-arsenic (CCA), phenol–formaldehyde, and urea–formaldehyde resin (Yue *et al.* 2018) have been used to prevent the growth of damaging fungi. Despite the inhibitory effects of these substances against microorganisms, they can be harmful to humans and the environment (Singh *et al.* 2010). However, these preservatives contain arsenic and formaldehyde, which may pose serious health risks even after their service time ends and the treated wood becomes waste. Environmental performance and sensitivity play an increasing role in the development and use of antifungal chemicals that are not toxic to humans and the surrounding environment.

Wood extractives are natural compounds that are rich in bioactive substances such as tannins, polyphenols, and lignans, which are toxic to wood-degrading organisms (Syofuna *et al.* 2012). Various natural plants extracts from *Nerium oleander* L. (Goktas *et al.* 2007), pine resin (Nunes *et al.* 2004), and cinnamon leaves (Wang *et al.* 2005; Cheng

*et al.* 2006; Lin *et al.* 2007; Maoz *et al.* 2007), and essential oils from lemon grass, rosemary, tea tree, and thyme (Yang and Clausen 2007; Bahmani and Schmidt 2018) are effective against wood decay fungi at certain concentration levels.

The wild pistachio tree belongs to *Pistacia*, a genus of many species in the Anacardiaceae family, distributed in the Mediterranean area to center and west Asia (Cheng *et al.* 2006; Humar and Lesar 2008). Five species are more popular including *P. vera* L., *P. atlantica* Desf., *P. terebinthus* L., *P. khinjuk* Stocks, and *P. lentiscus* L. Three *Pistacia* species naturally occur in Iran: *P. vera* L., *P. khinjuk* Stocks, and *P. atlantica* Desf. *P. atlantica* has three subspecies or varieties, which have been described as *cabulica*, *kurdica*, and *mutica* (Hanafi *et al.* 2012).

A kind of resin is exuded from trunks of wild pistachio trees in two natural and synthetic ways. In the natural way, resin is exuded from the grooves that are naturally created in bark and branches of tree, and extracted resin is collected in clay pots. In the synthetic way, the trunks of trees are cut, and resin flows down. The most important feature of this resin is its antibacterial properties (Hanafi *et al.* 2012). The methanol extract of this resin is rich in phenolic and flavonoid compounds (Amri *et al.* 2015), and it inhibits the growth of *Penicillium* molds (Ostowar *et al.* 2014). The ethanol extract from fruit, bark, and core of wild pistachio is characterized by antimicrobial properties against *Escherichia coli* and *Staphylococcus aureus* bacteria (Mortazavi *et al.* 2014).

This study investigated antifungal effects of pistachio resin as an environmentally friendly material to augment the decay resistance of poplar wood against the growth of *T. versicolor* and *C. puteana*.

## EXPERIMENTAL

### Materials

The natural pistachio resin (PR) of wild pistachio (*Pistacia atlantica*) was obtained from trees growing in Kurdistan province in the western area of Iran. Methanol of 99% purity was purchased from the Shiraz Company (Marvdasht, Iran). The sapwood of poplar (*Populus deltoides*) was obtained from the northern forests of Iran. The chosen specimens were free from cracks, decay, insect damage, and other defects.

### Methods

*Gas chromatography- mass spectrometry (GC-MS) of the pistachio resin*

The pistachio resin was analyzed by an Agilent 7890A gas chromatograph (Tokyo, Japan) to determine its components. The PR was dissolved in ethyl ether before injection into the GC device. The gas chromatograph was equipped with an Rtx 5MS capillary column (30 m × 0.25 mm; 0.25 μm film thickness) with He as the carrier gas (1.3 mL/min). The oven temperature was programmed from 40 to 200 °C at 5 °C/min. The temperatures of the injector and quadrupole and ion sources were 250 °C, 150 °C, and 230 °C, respectively.

*Preparation of wood samples*

The dimensions of samples were 50 mm × 25 mm × 15 mm (longitudinal, radial, and tangential) according to the EN 113 (1996) and EN 84 (1995) standards for mass loss and leaching tests, respectively. The sample dimensions of 20 mm × 20 mm × 20 mm (longitudinal, radial, and tangential) were used for volumetric swelling and density tests

according to the ISO 13061 (2014) standard. All specimens were conditioned at 20 ± 2 °C and 65 ± 3% relative humidity for 4 weeks before the subsequent treatments.

*Preparation of treatment solution*

The resin extracted from pistachio tree was used to prepare treatment solution without any purification. The desired amount of resin was dissolved in methanol (w/w) to prepare treatment solution with 1%, 6%, 12%, and 15% concentration.

*Treatment processes*

The test wood samples were dried in the oven at 103 ± 2 °C until reaching a constant weight ($m_1$). The wood samples were impregnated with 1%, 6%, 12%, and 15% methanolic PR solutions by two vacuum/pressure (V/P) and dipping methods. In the V/P method, the anhydrous specimens were first vacuumed at -800 Pa for 30 min. After PR solutions were added, impregnation was continued at $4 \times 10^5$ Pa and 20 °C for 2 h. In the dipping method, the specimens were immersed in methanolic PR solution for 2 h at 50 °C. After impregnation, the samples were kept to an acclimatized room at 20 °C and 65% RH for 2 weeks and then oven dried at 103 ± 2 °C. The weight ($m_2$) of treated oven dried samples was measured. The PR retention (R in $kg.m^{-3}$) in each cubic meter of woods were calculated using Eq. 1,

$$R = \frac{m_2 - m_1}{V} \quad (1)$$

where $m_1$ is weight of oven dried sample before impregnation (kg), $m_2$ is weight of oven dried sample after impregnation (kg), and $V$ is initial volume of sample ($m^3$).

## Density

The density of treated specimens was calculated by Eq. 2,

$$D_{od} = \frac{m_{od}}{V_{od}} \quad (2)$$

where $D_{od}$ is density of oven dried samples ($kg.m^{-3}$), $m_{od}$ is oven-dried weight of samples (kg), and $V_{od}$ is volume of oven dried samples ($m^3$). Ten replications were conducted for each treatment.

## Volumetric Swelling (VS)

The treated specimens were placed in beakers filled with distilled water and were kept at 23 °C for 8 days. The water was replaced by fresh distilled water at 2 h, 4 h, 6 h, 8 h, and 24 h and was repeated daily. The volume of samples was measured, and the volumetric swelling (VS) was calculated according to Eq. 3,

$$VS = \frac{V_2 - V_1}{V_1} \times 100 \quad (3)$$

where $V_2$ is the volume of the sample after immersion in water, and $V_1$ is the volume of the sample before immersion in water.

## Leaching Test

Half of the treated samples were leached before the decay testing. The leaching test was conducted according to European standard test method EN 84 (1997). Deionized water (5 volumes of water for 1 volume of wood) was added to specimens and then 4 kPa vacuum

was applied for 20 min. After 2 h, 24 h, and thereafter at 24 h intervals, leachates were replaced by fresh water. The process was continued for 14 days, and then the samples were conditioned in an acclimatized room at 20 °C and 65% RH for 2 weeks in order to reach moisture equilibrium.

### Wood Decay

The treated samples were exposed to both white-rot (*Trametes versicolor*; CTB 863 A) and brown-rot fungi (*Coniophora puteana*; BAM Ebw. 15) according to European standard EN 113 (1996). The samples were sterilized at 121 °C for 15 min. Petri dishes with malt extract agar (50 mL per plate) prepared from malt (40 g) and agar (20 g) in one liter distilled water, were inoculated with a piece of mycelium of a freshly grown fungus. When the fungal mycelium reached the border of the plate, one untreated and one treated wood samples were added on separate metal grids to prevent moisture uptake by the wood sample. The specimens were incubated for 16 weeks at 22 ± 2 °C and 70 ± 5% relative humidity. The decay test was repeated six times for each set of samples. After incubation, the mycelia were removed from the samples surfaces, and the specimens were dried at 105 °C for 24 h to obtain the dry weight ($m_3$). The mass loss ($ML$%) values of samples were calculated from the difference between oven dried weights of specimens before ($m_2$) and after the decay test ($m_3$) by Eq. 4.

$$ML\% = \frac{m_2 - m_3}{m_2} \times 100 \quad (4)$$

### Statistical Analysis

The two-way analysis of variance (ANOVA) was carried out using SPSS v.20 (IBM CO., New York, USA) to determine the differences between treatment conditions. P values of less than 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

### Pistachio Resin Analysis

More than 20 components were detected by GC analysis of pistachio resin. α-pinene was the main component (85.61%), and α-terpinene (1.94%), β-pinene (1.72%), α-terpinolene (1.32%), camphene (0.70%), and limonene (0.69%) were the other identified components. Mahjoub *et al.* (2018) reported 82.64% for α-pinene and 3.04% for β-pinene for *Pistacia atlantica* of Kordestan province in Iran. Sharifi *et al.* (2011) also obtained 92.7% for α-pinene, 1.26% for β-pinene, 0.41% for camphene, and 0.06% for limonene in *Pistacia atlantica* Kurdica. The current results confirm previous research showing that the monoterpenes are the main fraction of *Pistacia atlantica* and α-pinene is the major component.

### Pistachio Resin Retention

The mean PR retention of treated wood specimens, applied with two different treatment procedures, is provided in Table 1. The PR retentions of the vacuum pressure (V/P) samples were higher than those undergoing the dipping procedure. The highest retention was obtained in the V/P treated sample (71.8 $kg.m^{-3}$) at 15% PR concentration, which was approximately 3.36 times higher than the dipping procedure (21.3 $kg.m^{-3}$). Žlahtič *et al*. (2017) investigated the tung oil impregnation in European larch, sweet

chestnut, and Norway spruce by vacuum-pressure method and reported 39 kg.m$^{-3}$, 186.6 kg.m$^{-3}$, and 119.3 kg.m$^{-3}$ retention, respectively.

**Table 1.** Retention Amounts of Treated Wood Samples

| PR Concentration (%) | Retentions (kg.m$^{-3}$) (V/P method) | Retentions (kg.m$^{-3}$) (dipping method) |
|---|---|---|
| Control | - | - |
| 1 | 5.35 (0.32) | 2.84 (0.62) |
| 6 | 25.78 (1.91) | 7.58 (2.20) |
| 12 | 56.02 (3.28) | 17.08 (6.02) |
| 15 | 71.78 (7.19) | 21.34 (8.48) |

Note: The data are mean of 12 replicates, and the numbers in parenthesis are standard deviations.

## Density

The oven dry densities of treated samples by V/P and dipping methods are given in Fig. 1. The density of PR treated wood increased to 555.9 kg.m$^{-3}$ and 505.7 kg.m$^{-3}$ at 15% PR concentration for V/P and dipping methods, respectively, corresponding to an increase of 15.4% and 5.8% compared with untreated wood. The higher density in V/P treated samples compared with the dipping method can be related to greater PR retention in treated wood (Table 1).

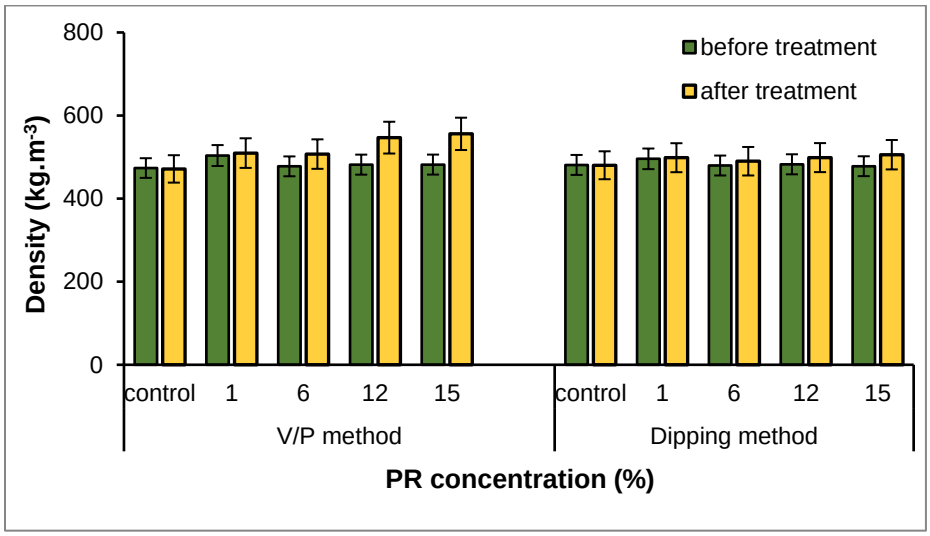


**Fig. 1.** Density of treated samples in different pistachio resin concentrations by vacuum pressure (V/P) and dipping methods

## Volumetric swelling

The volumetric swelling of treated samples by V/P and dipping methods is shown in Fig. 2. The volumetric swelling of untreated wood specimens reached 13.3% within 168 h from their storage in water, while the swelling of treated ones reached 10.1% and 11.1% in V/P and dipping methods, respectively, for 15% PR concentration during the same period. The volumetric swelling of samples was reduced by increasing PR concentrations in both procedures. The volumetric swellings in V/P procedure were less than dipping method in all PR concentrations. These differences could be attributed to greater PR retention in V/P method (Table 1). The volumetric swellings of treated wood were

decreased 24.5% and 16.8% compared with untreated wood for V/P and dipping procedures, respectively in 15% PR and 168 h from immersing in water.

The reduced swelling and improved the dimensional stability of the treated wood could be attributed to the high water repellency of pistachio resin. In addition, the resin molecules are too large to penetrate in the cell wall and mostly remain in the cell lumens, blocking water-absorbent sites.

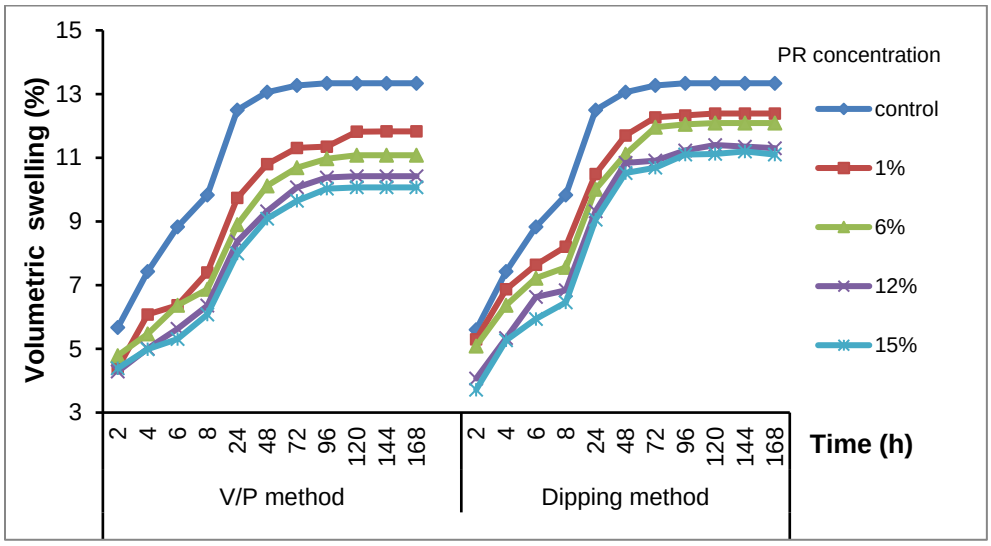


**Fig. 2.** Volumetric swelling of wood samples treated with different concentrations of pistachio resin (PR) by vacuum pressure (V/P) and dipping methods

## Decay Tests

The mass losses results of the treated samples are shown in Fig. 3. The sample mass losses were decreased from 33.7% to 17.4% for V/P and from 33.7% to 22.6% for the dipping method. The treated samples by highest PR concentration (15%) had the greatest resistance to fungal decay in both methods. The differences between mass losses in leached and non-leached samples were not significant. The mass loss of samples exposed to the white rot fungus revealed that the V/P method demonstrated better performance than the dipping procedure, which could be related to the high retention of resin in V/P method (Table 1).

Similarly, Talibi *et al.* (2013) demonstrated that the extract of *Pistacia atlantica* prevents the growth of the yeast-like mold fungus *Geotrichum candidum*. Amri *et al.* (2015) also reported that the antifungal activity of *Pistacia atlantica* extracts exhibit a strong antifungal activity against the mold fungus *Penicillium digitatum* at a concentration of 1.25 $mg.mL^{-1}$ by approximately 50% reduction in fungus growth. Indeed, *Pistacia atlantica* resin has a high content of polyphenols (more than 40%) that are capable of inhibiting mycelium growth (Ostowar *et al.* 2014; Santos Junior *et al.* 2014; Amri *et al.* 2015). Cavaleiro and Salgueiro (2015) studied antifungal activity of *Angelica major* plant essential oil and showed that the α-pinene displayed broadest fungicidal activity against yeast and dermatophyte strains.

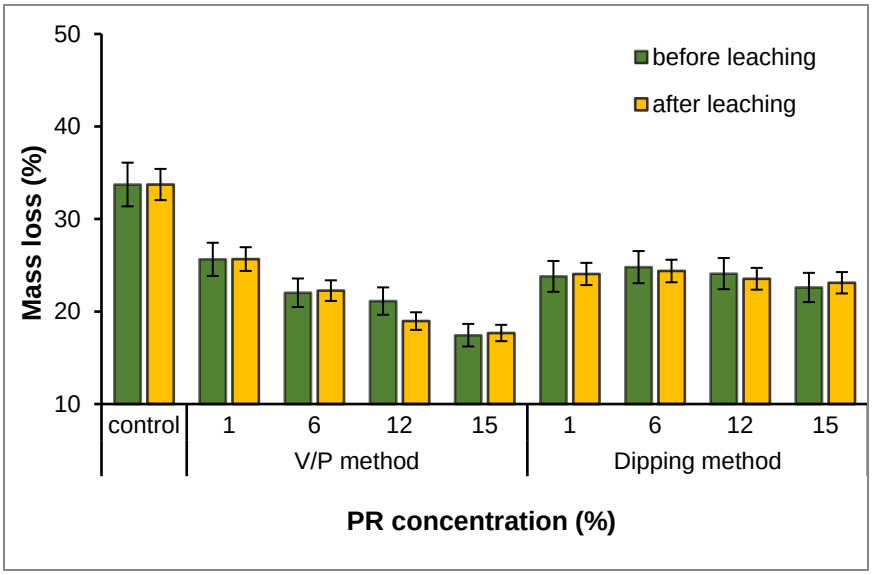


**Fig. 3.** Mass loss of treated samples by vacuum pressure (V/P) and dipping methods before and after leaching against *T. versicolor*

The mass loss caused by C. *puteana* on treated samples by the V/P method with different PR concentrations is shown in Fig. 4. Unleached and leached samples showed mean mass losses from 39.7% to 46.3%. After exposure to the fungus, the 15% PR treated samples showed mass losses of 39.7%, which were improved compared with the control samples. The losses in mass decreased by increasing PR concentration for both leached and unleached wood samples. The mass losses caused by *C. puteana* were greater than those found with the *T. versicolor* (Fig. 3).

The results showed that PR as a preservative substance decreased wood decay against *C. puteana* and *T. versicolor* fungi, but none of the treatments had a mass loss of less than 3%, which is sufficient for complete protection of wood according to EN 113 (1996). Although these treatments did not completely inhibit decay of wood at the PR concentrations tested, but this study provides a new perspective on using natural pistachio resin for extending the service life of wood products. Future studies will be need for investigation PR combination with other preservatives in order to evaluation its efficacy against fungi.

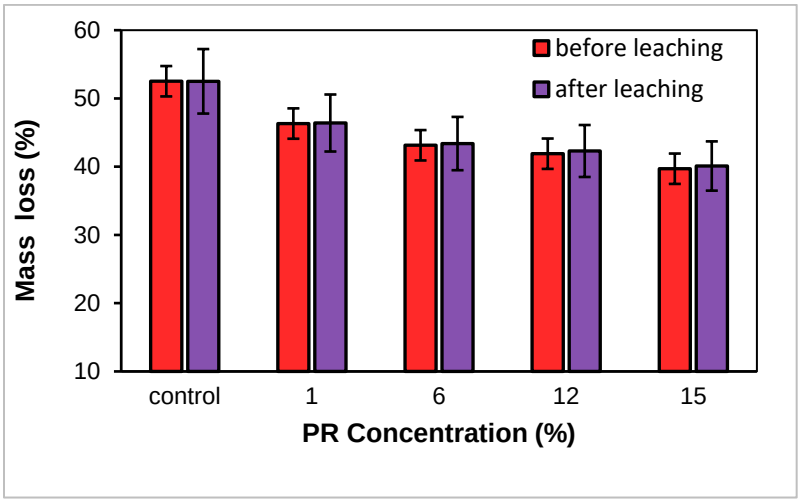


**Fig. 4.** Mass loss of treated samples by vacuum pressure (V/P) method against *C. puteana.*

## CONCLUSIONS

1. The maximum retentions of resin in poplar wood were 71.8 $kg.m^{-3}$ for the vacuum/pressure (V/P) method and 21.3 $kg.m^{-3}$ for the dipping method.
2. The volumetric swelling of treated specimens was decreased by 24.5% and 16.8% in V/P and dipping methods, respectively, in comparison to untreated samples.
3. Pistachio resin (PR) treatments effectively improved the wood decay resistance against *T. versicolor*, and the treatment of 15% PR showed a mass loss of 17.4% and 26.7% for V/P and dipping methods, respectively.

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Article submitted: November 1, 2020; Peer review completed: December 1, 2020; Revised version received and accepted: December 3, 2020; Published: December 8, 2020.
DOI: 10.15376/biores.16.1.779-788