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ASSESSMENT OF PRELIMINARY RESULTS FOR BIOFILM FORMATION ON WOOD

Ümit Cafer YILDIZ
Karadeniz Technical University Faculty of Forestry, Dept. of Forest Industry Engineering
61080 Trabzon, TURKEY
Tel: +90 462 377 1502, Fax: +90 462 325 7499, E-mail: yildiz@ktu.edu.tr

Sibel YILDIZ
Karadeniz Technical University Faculty of Forestry, Dept. of Forest Industry Engineering
61080 Trabzon, TURKEY
E-mail: sibelyildiz@ktu.edu.tr

Özge Nur ERDEYER
Karadeniz Technical University Faculty of Forestry, Dept. of Forest Industry Engineering
61080 Trabzon, TURKEY
E-mail: nurerdeyer@ktu.edu.tr

Mustafa Selçuk GİRGİNER
Karadeniz Technical University Faculty of Forestry, Dept. of Forest Industry Engineering
61080 Trabzon, TURKEY
E-mail: sibelyildiz@ktu.edu.tr

Kazım SEZEN
Karadeniz Technical University Faculty of Science, Dept. of Biology
61080 Trabzon, TURKEY
E-mail: sibelyildiz@ktu.edu.tr

Ebru GÜNEY AYDIN
Karadeniz Technical University Faculty of Science, Dept. of Biology
61080 Trabzon, TURKEY
E-mail: sibelyildiz@ktu.edu.tr

Abstract:

Protective biofilm formation on wood is an emerging, environmentally friendly and sustainable wood preservation technique. In this study, biofilm formation was investigated on Scots pine (Pinus sylvestris) and Eastern spruce (Picea orientalis) wood samples impregnated with various vegetable oils. The fungal strain Aureobasidium melanogenum was used as the biofilm-producing fungus. Wood samples impregnated with oils and treated with the fungal strain on their surface were exposed to outdoor weather conditions for a 3-month period (June-July-August-2025). Following the outdoor exposure test, the physical properties (equilibrium moisture content and compression strength) and the surface characterization properties (color changes, glossiness, surface roughness) of wood specimen was determined. The preliminary results obtained can be considered promising.

INTRODUCTION

The “ecological approach” is becoming increasingly important in wood preservation and surface treatments. In the last twenty-five years, several important review articles on the subject have been published. The field of biological control has essentially been developing rapidly for the past sixty years (Schoeman 1999, Mai *et al.* 2004, González-Laredo *et al.* 2015, Teaca *et al.* 2019). The use of more environmentally friendly wood preservatives against nature, people, and animals has gained importance recently. Therefore, it has become almost essential to develop natural, organic, and plant-based preservatives for commercial markets. Vegetable oils that do not contain any toxic chemicals in their compositions are considered as an alternative impregnation material for wood preservation, as they create a hydrophobic layer in the wood cells and reduce water uptake (Tomak ve Yıldız 2012, Teaca *et al.* 2019).

Protective biofilm formation on wood is an emerging, environmentally friendly and sustainable wood preservation technique. The biofilm wood protection surface treatment is one of the latest innovations in wood coating field. Biofilms are communities of microorganisms structured within a protective extracellular polymeric substance (EPS) matrix, attached to living or non-living surfaces (Donlan and Costerton 2002, Gün and Ekinci 2009). Although bacteria are more prominent in terms of biofilm formation, it is known that some fungal species also form biofilm layers similar to bacteria. It has been reported that fungal species such as *Candida*,

Aspergillus, *Trichosporon*, and *Aureobasidium* in particular have the potential to form biofilms (Sheppard and Howell 2016, Uzundağ *et al.* 2020).

The initial findings related to biofilm and wood coating surface treatment were obtained in 1996 in Germany (Hamburg). To extend the lifespan of wood using a more environmentally friendly method, a homogeneous black color developed over time on wood samples impregnated with natural oils. Further research determined that this was caused by a common endophytic fungus called *Aureobasidium pullulans*, one of the biofilm-forming fungi. Thus, one of the environmentally friendly wood preservation approaches emerged in this way. If the wood is covered with a hydrophobic coating such as oil, liquid water cannot penetrate deep into the wood, and the necessary moisture for the growth of *A. pullulans* on the surface is provided. On the other hand, as the temperature increases, water can evaporate. Although this creates an excessively dry surface that constitutes negative conditions for other microorganisms, it has been reported that *A. pullulans* can somehow survive even under these conditions, even if the temperature rises to 70°C (Castenmiller 2004, Sailer *et al.* 2010, Xyhlo Biofinish, 2023). The biofilm, developed based on an innovative preservation principle, is designed entirely with natural substances. It is a self-healing and sustainable system.

Biofilm-forming fungi (*Aureobasidium* spp.) frequently appear all over the world, even under variable climatic conditions. Because it is of biological origin, the wood-biofilm combination is completely recyclable and environmentally friendly, and is not harmful to humans. Because the fungal cells that form the biofilm are relatively inactive, they do not require much nutrition. That is, they do not harm the material they inhabit. Therefore, the surface they are applied to is durable, and the system's service life is long. Furthermore, compared to a conventional surface treatment substance, the biofilm has a somewhat softer texture but is more water-repellent. This increases the dimensional stability of the wood and reduces crack formation on the surface. In examinations carried out after biofilm applications, it was observed that the samples remained intact even after being exposed to the open air for a period of more than ten years (Sailer 2009). However, the protection provided by classical surface coating materials is much more superficial. For example, varnish layers can crack within a few years. Over time, water, fungal spores, and insect larvae can enter the wood material through these cracks, and the material can be damaged. Therefore, they require frequent maintenance and renovation, which becomes a very expensive process (Var 2001, Poohphajai *et al.* 2021). According to research, a biofilm of this kind formed on wood surfaces possesses the following characteristics (Sailer *et al.* 2010, Sailer 2023, Xhylo Biofinish 2023):

1) It acts as a protective layer that is resistant to UV rays and does not damage the wood it inhabits in any way.
2) It adheres through fungal growth within the wood cells.
3) It attaches to the wood surface by forming adhesion polymers.
4) It must be applied with a water-repellent oil to ensure the wood remains dry.
5) Thanks to lower moisture, it provides less degradation and increased protection.
6) It protects the wood against other harmful microorganisms and insects.
7) Liquid oil contributes to the interaction between wood and fungus and to the self-healing properties.

OBJECTIVE

The goal of the study is to determine the surface characterization features of the biofilm formation that occurs on the surfaces of pine and spruce wood impregnated with various oils after a 3-month outdoor test, as well as some physical properties of the wood.

MATERIAL AND METHOD

Fermentation Stage

Fermentation method is one of the most critical stages of the project. This is because the adhesion and aesthetic appearance properties of the biofilm depend on a series of factors such as fermentation formation and biofilm formulation. For the fermentation method, the fungal strain *Aureobasidium melanogenum* (CBS 140241) was procured from the Westerdijk Fungal Biodiversity Institute in a freeze-dried form. Fungal suspensions were obtained according to the method described below, after various fermentation trials.

Revival Process: The powdered sample was suspended with 1 mL of sterile dH_2O, shaken gently, and spread in a 0.5 mL volume onto malt extract agar (Merck) medium, and finally incubated at 25ºC.

A. melanogenum was first grown on dichloran 18% glycerol agar for 10 days. A number of cells were taken from the edges of the grown *A. melanogenum* colonies and transferred to a flask. For 1L, the following

were added and suspended in sterile water: 0.6 g sodium nitrate, 0.1 g potassium dihydrogen phosphate, 0.5 g potassium chloride, 0.2 g magnesium sulfate,100 g glucose (D+) anhydrous. The culture medium was prepared and transferred to the fermenter. The liquid medium inside the fermenter was sterilized using an autoclave at 121ºC for 20 minutes under 1.1 atm pressure. *A. melanogenum* cells were transferred into the prepared medium in the fermenter as a 1% inoculation culture (1% culture in 98-99% medium). The total cell concentration in the suspension was initially adjusted to be between 5 x 10^5 and 10 x 10^5 cells/mL. Subsequently, the fermenter was set to various pH levels via a computer program, at 25ºC and 200 rpm, and incubated for 24 hours. After incubation, it was washed twice with sterile dH_2O. It was then resuspended in a medium containing 2% glucose. After a second incubation at 25ºC for 24 hours at 200 rpm, *A. melanogenum* was washed again and suspended in the medium to make it ready for use. The appearance of *A. melanogenum* cells on 'Malt Extract Agar' after the fermentation processes is given in Fig. 1.

Fig. 1.
The image of A. melanogenum cells on malt extract agar.

Studying the Effect of pH on Suspension Formation

The fermentation method described above was repeated separately for pH 3, 5, and 7. After 24 hours, the density of the grown cells was measured both with a Thoma lam and a spectrophotometer. For the Thoma lam: First, dilutions up to 10^{-5} were prepared from the grown cells. The dilutions were prepared in microcentrifuge tubes by mixing 900 µL of sterile water with 100 µL of cell suspension. A portion of these prepared dilutions was transferred to the Thoma lam, and cell counting was performed under a light microscope at 100x magnification. The results were calculated using the formula given below (1):

$$A \times SF \times 10{,}000 \quad (1)$$

where:

A: Number of cells counted;
SF: Dilution factor;
10,000: Constant used to convert the count to the number of cells per mL.

For spectrophotometric measurement: cuvettes were taken and 1 mL of sterile water was added to one of them. This cuvette was used as the blank. Then, for each sample, 900 µL of sterile water and 100 µL of cell suspension were placed into separate cuvettes, measurements were taken at 600 nm in the spectrophotometer, and the results were recorded.

The effect of pH level on cell count is presented in Table 1. Accordingly, it was observed that as the pH level increased, the cell count increased according to both the Thoma lam and spectrophotometer results.

Table 1

Effect of pH on cell counting

pH	Thoma Lam	Spectrophotometer
3	196 x10^8	202x10^8
5	328 x10^8	325x10^8
7	440 x10^8	409x10^8

According to various literature studies, it has been reported that as the pH of the fungal culture medium decreases, pullulan production by *A. pullulans* is stimulated. Several studies have investigated the effect of culture medium pH on fungal polysaccharide synthesis. From these studies, it was concluded that the optimum initial pH of the culture medium for fungal pullulan synthesis is 6.0–6.5, and that the optimum pH of the medium also depends on the carbon source added to the medium (Catley 1971, Catley 1980, Lacroix *et al.* 1985, West and Reed-Hamer 1993). In a different study, the optimal initial pH for *Aureobasidium pullulans* was determined to be 6.48 (Müjdeci *et al*., 2024). It can be stated that the findings obtained in the study work are consistent with the literature and that applying an initial pH level between 5 and 7 is appropriate.

Impregnation with Oils

In the study, biofilm formation was investigated on Scots pine (*Pinus sylvestris*) and Eastern spruce (*Picea orientalis*) wood samples impregnated with various vegetable oils such as sunflower oil, waste sunflower oil, raw linseed oil and soybean oil. Wood samples were impregnated with the oils in two stages; first keeping in a hot oil bath at 160°C for 30 minutes; and then in a cold oil bath at 20°C for 30 minutes. Wood samples obtained from Scots pine and spruce timbers were dimensioned to 150 x 75 x 20 mm (length x width x thickness) and were conditioned to equilibrium moisture content at 20°C and 65% relative humidity before and after the oil impregnation process. Five replicates were used for each variation. During the hot oil treatment, to prevent cracks that could occur in the cross-sections, the samples were placed in oil at room temperature instead of being directly immersed in hot oil, and the oil temperature was gradually increased (at a rate of 5°C per minute). The immersion process began once the target temperature was reached. The vacuum created by the temperature difference facilitated the penetration of the oil into the wood.

Weight percent gain (WPG) values were calculated using the initial oven-dry weight (M_0) and the oven-dry weight after the oil impregnation process (M) according to the following equation (2):

$$WPG\ (\%) = [(M - M_0) / M_0] \times 100 \quad (2)$$

Oil retention values were calculated based on the initial oven-dry volume (V_0) of the samples according to the following equation (3):

$$Oil\ Retention\ (kg/m^3) = [(M - M_0) / V_0] \quad (3)$$

Physical Tests

In order to determine whether the samples exposed to the outdoor environment had suffered any loss of resistance, the compressive strength parallel to the grain was determined according to TS ISO 13061-17. For the compressive strength test, the samples were dimensioned to 2 x 2 x 3 cm (tangential x radial x longitudinal direction), and the equilibrium moisture content was also determined on the same samples.

Surface Characterization Tests

A Linshangs-LS173 colorimeter was employed to evaluate the color changes of the studied wood samples. Color measurements were determined using the CIELab color system. Color measurements were taken from three different points on the longitidunal surface of the wood samples. The total color change (ΔE) in the samples was determined by comparison with the initial color measurement.

Gloss measurements of the test and control samples were performed at 85° angle using a gloss meter (PCE-GM 100). The measurements were obtained as the arithmetic mean of 10 measurements for each surface, taken both parallel and perpendicular to the wood fibers. Since the 85° angle gives more sensitive results on matte surfaces, it became the most suitable measurement angle to reveal the surface differences of the wood samples used in the experiment.

In the study, Ra (arithmetic mean) values, which is one of the measurement parameters for surface roughness, were obtained using the Mitutoyo Surftest SJ-301 as the surface roughness tester and in accordance with the DIN 4768 (1990) standard. For the surface roughness measurements, the cut-off length was 2.5 mm, the evaluation length was 12.5 mm, the measuring range was 0.01 µm – 100 µm, the resolution was 350 µm, and the stylus tip radius was 5 µm. Twenty-five separate measurements were taken for each test group created in the study.

RESULTS AND DISSUCCIONS

Isolation of *A. melanogenum* from the Samples and Macroscopic Examination

As part of the research plan, preliminary screening tests were carried out from March to May 2025, using only samples impregnated with fresh and waste sunflower oil. Determinations were made regarding in which fungal suspension, prepared at three different pH levels (3, 5, and 7) on Scots pine and spruce wood, the biofilm formation developed more markedly and which pH level was more effective. It was observed that on

the test samples exposed to the outdoor environment, a more effective and denser biofilm coating formed, particularly at the pH 5 level (Fig. 2 and 3). As shown in Fig. 2, the densest biofilm layer was observed on spruce samples treated with an *A. melanogenum* suspension at pH 5, following impregnation with fresh sunflower oil and heating to 160°C in spruce wood. Waste sunflower oil resulted in a lower biofilm formation performance. The literature reports that reheating used oil leads to the formation of certain substances which decompose and slow down the curing process (Dmitrenkov *et al*. 2021). According to the report by Oparanti *et al*. (2025), various chemical reactions occur, particularly at temperatures between 170 and 200°C, which alter the composition and quality of the oil. A thesis on the subject stated that “oils are a complex substrate and many of the compounds constituting oils have not been tested as a carbon and energy source for the growth of *A. melanogenum*.” The same study also reported that “while some compounds, such as highly reactive fatty acids, can inhibit growth; glycerol, oleic acid, and linoleic acid serve as a carbon and energy source for growth, highly reactive linolenic acid led to cell densities too low to be accurately measured” (Van Nieuwenhuijzen, 2018). The low yield in waste oil may have been due to these reasons.

As is evident from Figure 3, the densest biofilm layer on the Scots pine wood samples was observed on the samples that were exposed to the *A. melanogenum fungal* suspension at pH 5 and 7, following impregnation with fresh sunflower oil at 160°C. Similar to the spruce wood samples, very little biofilm formation occurred on the wood samples impregnated with waste sunflower oil. Based on the overall findings, it was concluded that the most effective fungal suspension was the one with a temperature of 160°C and a pH level of 5-6.

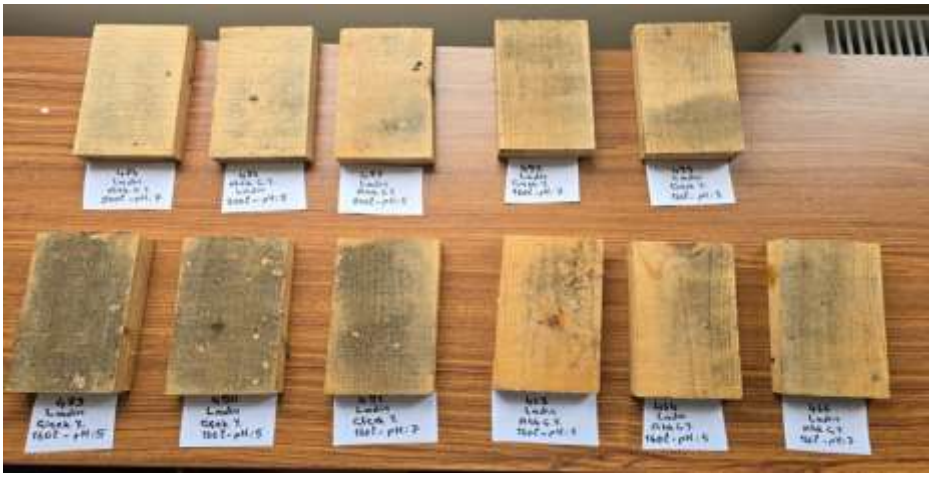

Fig. 2
A. melanogenum biofilms on spruce wood impregnated with fresh and waste sunflower oil.

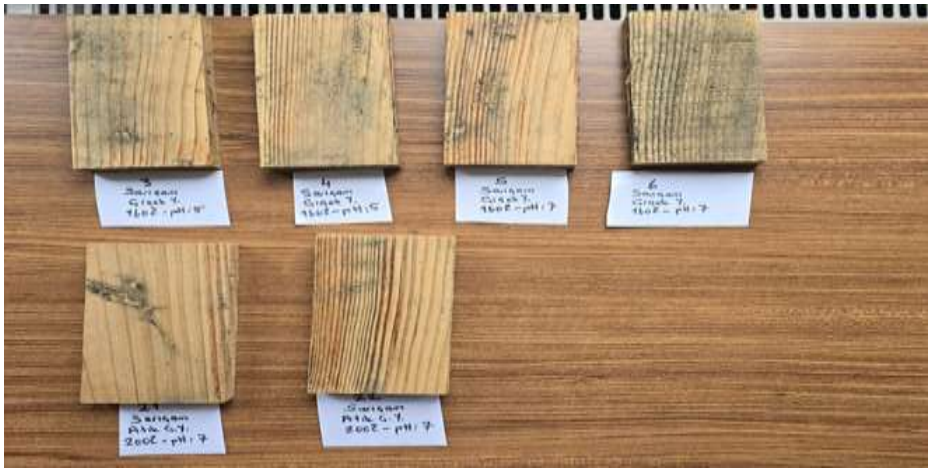

Fig. 3
A. melanogenum biofilms on Scots pine wood impregnated with fresh and waste sunflower oil.

Swabs taken from the wood samples were inoculated onto a nutrient medium and examined under a microscope. After this process, it was observed that the biofilm layer was predominantly formed by the fungus *A. melanogenum*. This result was evaluated as an indication that the relevant fungus was functioning under

suitable environmental conditions. The wood samples from which the swabs were taken and the development of the *A. melanogenum* fungi from these samples on the petri dish are shown in Figures 4-9.

According to the preliminary test results and based on the knowledge that many bacteria grow faster at pH 7, pH 6 solutions were used in subsequent studies to prevent potential bacterial degradation of the wood samples.

Fig. 4
Scots pine, fresh sunflower oil, 160°C, pH:5

Fig. 5
Scots pine, fresh sunflower oil, 160°C, pH:7

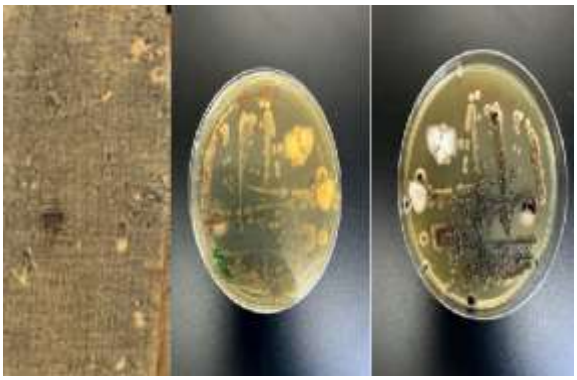

Fig. 6
Spruce, fresh sunflower oil, 160°C, pH:5

Fig. 7
Spruce, fresh sunflower oil, 160°C, pH:7

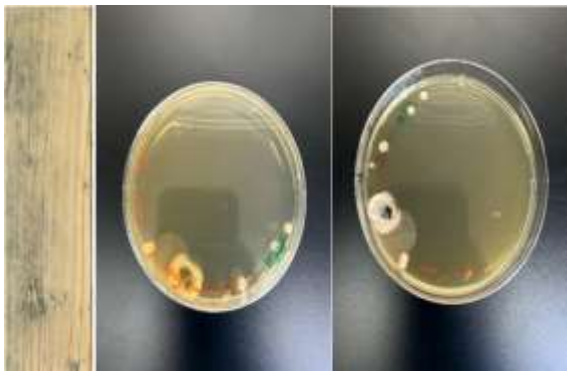

Fig. 8
Scotch pine, waste sunflower oil, 160°C, pH:5

Fig. 9
Spruce, waste sunflower oil, 160°C, pH:5

Oil Retention

The average weight percent gain (WPG), thickness swelling (TS) and retention values for Scots pine and spruce wood samples impregnated at 160°C with a 100% concentration of soybean oil, sunflower oil, waste sunflower oil, and raw linseed oil are given in Table 2. The highest weight percent gain (85%) and retention rate (398 kg/m3) were observed in Scots pine wood samples impregnated with waste sunflower oil; and the lowest weight percent gain (16%) and retention rate (68 kg/m3) were observed in spruce wood samples impregnated with raw linseed oil. A comparison between the Scots pine and spruce wood samples revealed higher retention values in the pine. This was attributed to the anatomical structure of spruce, which makes it less permeable to impregnation than pine. In addition, it was found noteworthy that the lowest

retention occurred in the spruce wood samples impregnated with raw linseed oil. Additionally, the highest weight gain and the highest retention amount were observed in the Scots pine wood samples impregnated with waste vegetable oil. On the other hand, the small losses in sample thickness are thought to be due to the shrinkage-enhancing effect of the hot oil application (Table 2).

Table 2

Values obtained from oil impregnation

Species	Oil	Retention (kg/m³)	WPG (%)	TS (%)
Scots Pine	Soybean	296	61	0,46
	Sunflower	307	58	0,44
	Waste Sunflower	398	85	-0,98
	Raw Linseed	311	63	-0,03
Spruce	Soybean	94	23	0,71
	Sunflower	92	23	0,27
	Waste Sunflower	127	31	0,08
	Raw Linseed	68	16	0,73

Temiz *et al.* (2008) found that a high oil retention level is necessary for the effectiveness of vegetable oil treatments. In their study, Baar *et al*. (2021) impregnated European beech with hemp oil and thermally modified it at 200°C, finding retention values of 331-385 kg/m³. In a study by Humar and Lesar (2013) on beech wood samples impregnated with different types of oils, retention values ranging from 348 to 383 kg/m³ were obtained. In another study where European aspen (*Populus tremula*) and downy birch (*Betula pubescens*) wood were impregnated with three different types of oil, retention values were observed to range from 67.6 kg/m³ to 371.2 kg/m³ (Ahmed *et al.* 2017).

The natural weathering test on wood samples impregnated with oils was carried out in the coastal region of Trabzon, Turkey (40°81'85" N, 39°79'10" E). The fungal strain *Aureobasidium melanogenum* was used as the biofilm-producing fungus. Wood samples impregnated with oils and treated with the fungal strain on their surface were exposed to outdoor weather conditions for a 3-month period (June-July-August-2025) (Fig. 10). Some macroscopic examinations with the naked eye were carried out on wood surfaces where a biofilm layer formed. The factors considered here are macroscopic observation elements, such as whether the biofilm has a homogeneous density and thickness, and whether it covers the wood surface completely so that the wood texture (annual rings or wood fibers) is no longer visible. Accordingly, a near-ideal biofilm formation is observed on some of the spruce samples; these are the samples that were impregnated only with oil and inoculated with the fungal strain (Fig.11). In the Scots pine samples, however, the biofilm formation does not completely cover the wood texture and possesses a more partial structure (Fig. 12).

Fig. 10.
The natural weathering test site.

Fig. 11.
Spruce test samples after 3 months.

Fig. 12
Scots pine test samples after 3 months.

Physical Tests

Table 3 shows the equilibrium moisture content (EMC) values and the compressive strength (CS) values parallel to the grain for Scots pine and spruce wood samples impregnated with a 100% concentration of sunflower oil, waste sunflower oil, soybean oil, and raw linseed oil at 160°C.

Table 3

Compressive strength and EMC values of the samples

Species	Oil	Compressive strength (N/mm²)	Control (N/mm²)	EMC (%)	Control (%)
Scots Pine	Soybean	34,3	57,3	4.8	9,9
	Sunflower	42,4	40,4	6,6	10,7
	Waste Sunflower	46,9	49,1	5,9	9,4
	Linseed	37,0	48,9	7,4	8,7
Spruce	Soybean	34,9	35,6	4,6	7,0
	Sunflower	33,1	33,0	7,9	9,4
	Waste Sunflower	34,4	33,9	6,9	10,4
	Linseed	35,6	38,8	7,0	11,1

When Table 3 is examined, it is generally observed that the compressive strength values in Scots pine samples decreased somewhat compared to the control. In contrast, it can be stated that there is no significant difference in compressive strength between the spruce wood test and control samples. The highest compressive strength value is seen in the Scots pine wood soybean oil control group samples (57.3 N/mm²),

followed by the Scots pine wood test samples impregnated with waste flower oil (46.9 N/mm²). The lowest compressive strength value was observed in the spruce wood sunflower oil control group samples (33.0 N/mm²). The compressive strength values for other variations fluctuated between these lower and upper limits. When the control samples were evaluated among themselves, the compressive strength values of the Scots pine samples were found to be higher than those of the spruce samples.

In the literature, the average compressive strength of spruce wood is reported as approximately 28 - 30.5 N/mm² (Bozkurt 1971; Çetin and Gündüz 2017), while the average compressive strength of Scots pine wood is stated as approximately 49.70 N/mm² (Çetin and Gündüz 2017). Accordingly, it can be concluded that no significant strength loss occurred in the wood samples that were impregnated with oils and subjected to a three-month outdoor test after the application of the fungal strain.

In some cases, high oil loading leads to reductions in mechanical resistance (Tomak and Yıldız, 2012). Indeed, Olsson *et al*. (2001) impregnated Scots pine wood with raw linseed oil to achieve 25%, 75%, and 105% weight gain and reported that at 75% and 105% weight gain values, reductions in mechanical resistance occurred and microstructural changes took place. In these loadings, cracks appeared in the tracheid cell wall. This formation has been attributed to the mechanical oil loading applied to the cell wall increasing the internal pressure in the cell wall. This type of pressure causes micro-cracks in the cell wall layers. Additionally, the micro-cracks occurring in the S1 layer reduce the resistance values. Similarly, Tomak (2011) also stated that he detected reductions in the compressive strength parallel to the grains of wood impregnated with vegetable oils. Additionally, the micro-cracks that occur in the S1 layer reduce the strength values.

According to Table 3, the EMC values of the Scots pine test samples impregnated with sunflower oil, waste sunflower oil, soybean oil, and raw linseed oil varied between 4.8% and 7.4%. In the Scots pine control samples, however, the EMC values range from 8.7% to 10.7%. Accordingly, it can be stated that a decrease in EMC values was detected in the Scots pine test samples compared to the control samples. On the other hand, it can be seen that the DRM values of the spruce wood test samples range from 4.6% to 7.9%, while those of the control samples range from 7% to 11.1% (Table 3). Therefore, it can be said that there was also some decrease in the spruce wood test samples compared to the control. Similar results have also been reported in the relevant literature. Wang and Cooper (2005a, 2005b) reported that they obtained a 30-50% reduction in EMC, 20-40% water absorption values, and 40% anti-shrink efficiency (ASE) values in spruce wood samples they impregnated with soybean oil, palm oil, and wax. In a study where Scots pine sapwood samples were impregnated with tall oil formulations, the tall oil applications were shown to reduce the water uptake of pine sapwood (Hyvonen *et al.* 2006).

Surface Characterization Tests

Fig.13 and Fig.14 show the color change (ΔE) of Scots pine and spruce, respectively, after impregnation with various oils at 100% concentration and 160°C. Here, the control group refers to wood samples that were impregnated with oil but not coated with the fungal suspension on their surface. On the other hand, the variations labeled as Type-1,2,3 describe some pre-treatments applied to the wood surface before the fungal suspension was coated. Since these pre-treatments are under a patent process, they are only presented here as numbered entries. “Untreated” refers here to the samples that are oil-impregnated and inoculated with fungus (without pre-treatment), while “control” refers to the samples that are only oil-impregnated (Fig. 13-14).

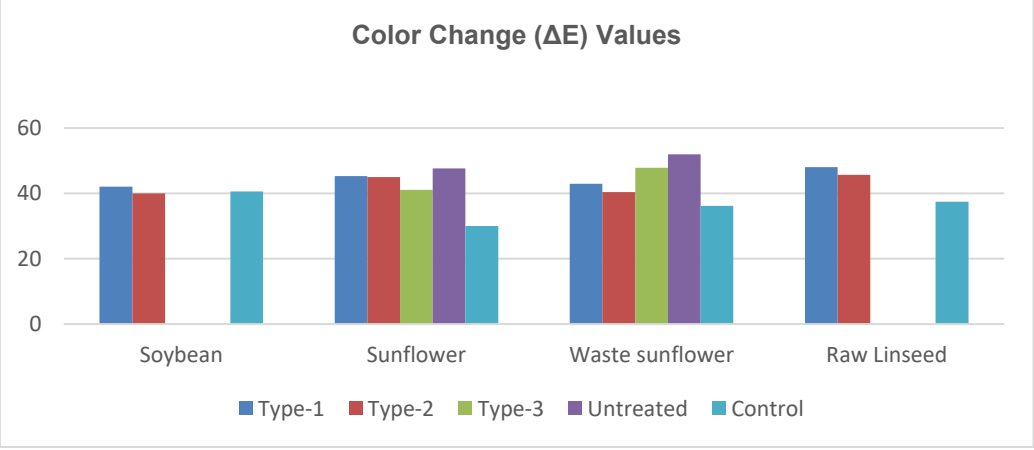

Fig.13.
Color change in Scots pine samples.

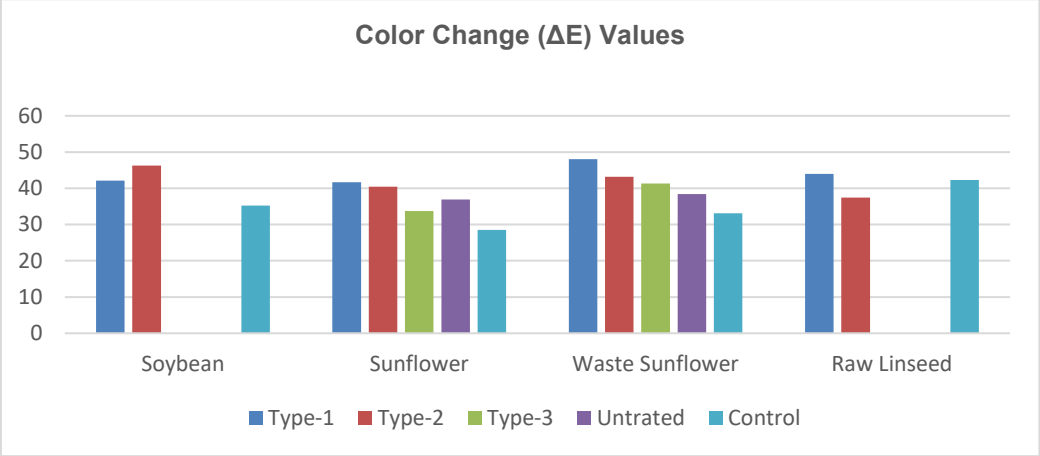

Fig.14.
Color change in spruce samples.

An examination of the tables reveals that the highest color change value was recorded for non-pretreated Scots pine wood samples impregnated with waste flower oil (52.00). Conversely, the lowest color change value was found in the control group of spruce wood specimens impregnated with flower oil (28.5). The color change values of the control groups were generally found to be lower than those of the test groups. On the other hand, it can be concluded that the oil impregnation treatment significantly changes the wood's color. The higher degree of color change observed in the test samples suggests that a dark biofilm layer has formed. In one study, it was reported that the color change on wood surfaces with biofilm was much lower than the control, which was attributed to the formation of a dark biofilm layer (Poohphajai *et al.* 2021). In an another study investigating color change and dimensional stability in wood using linseed oil and shellac, no significant differences were detected in the L, a, and b values compared to the control samples (Liu *et al.* 2020).

Fig.15 and Fig.16 show the average gloss (GU) values of Scots pine and spruce, respectively, after impregnation with various oils at 100% concentration and 160°C. The terms “untreated” and “control” retain their meanings from the previous figures. $G^{\perp}$ denotes gloss perpendicular to the fibers, and G denotes gloss parallel to the fibers.

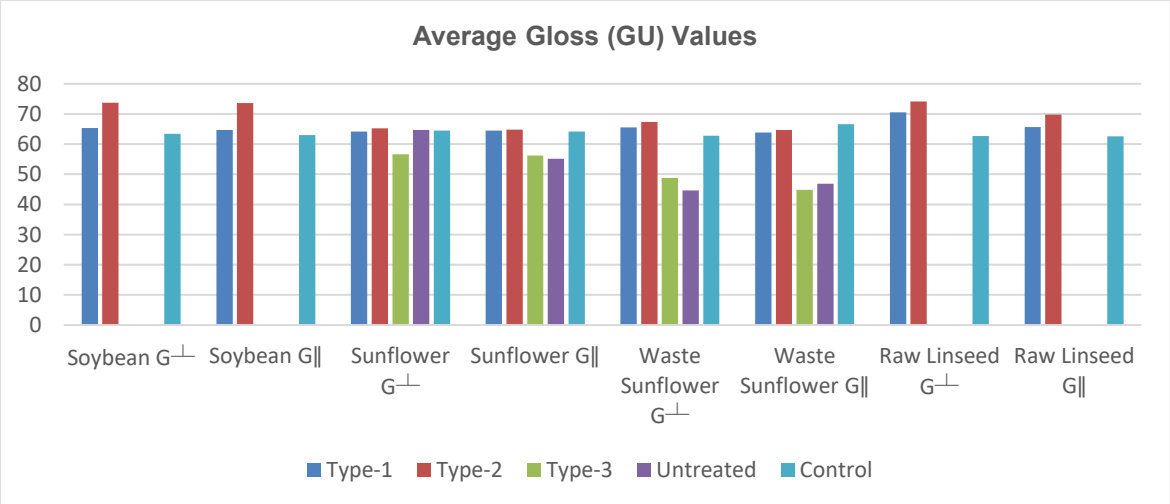

Fig.15.
Gloss values in Scots pine samples.

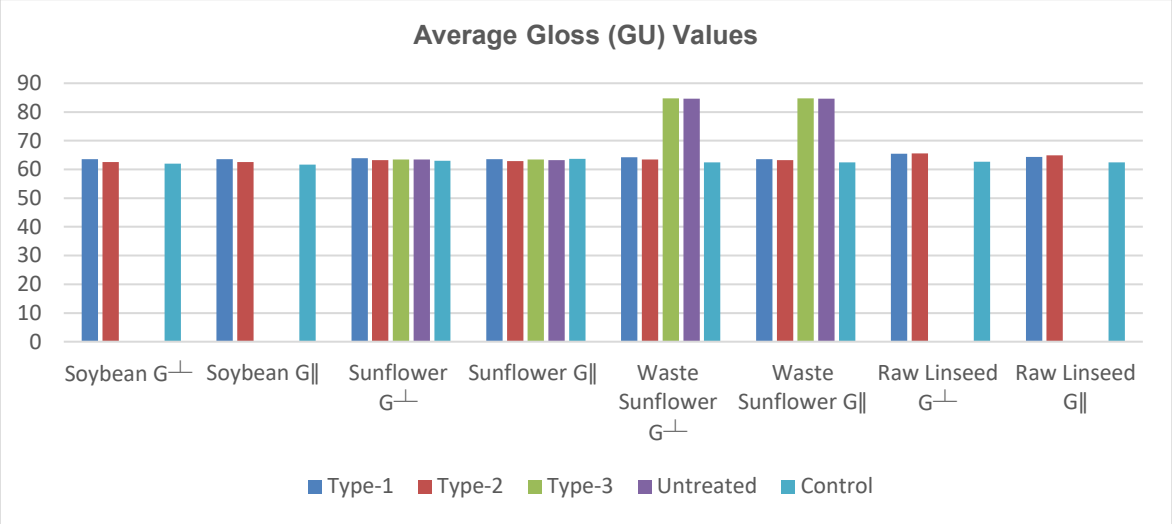

Fig.16.
Gloss values in spruce samples.

As can be seen from the figures, the highest surface gloss values were detected in spruce wood samples impregnated with waste sunflower oil (84.6-84.7 GU). The samples in this group were classified as high gloss. Conversely, the lowest surface gloss values were found in Scots pine wood samples impregnated with waste sunflower oil (44.6-48.8 GU). The samples in this group were classified as silky matte. The findings indicate that Scots pine test specimens impregnated with sunflower oil and waste sunflower oil exhibit a lower gloss grade than the control. Conversely, the gloss values for specimens treated with soybean oil and linseed oil are observed to be nearly identical to the control and can be evaluated as silky matte (Fig. 15). For the spruce wood specimens, it was observed that all test specimens, except those impregnated with waste oil, exhibited a gloss value similar to the control, classified as silky matte. The waste sunflower oil-treated specimens, on the other hand, were categorized in the high gloss group. Based on these findings, it can be concluded that the gloss values of the test specimens subjected to outdoor weathering exhibited a reduction, even if it was a low-rate one. In a related study, it is stated: 'The decrease in wood surface gloss is associated with the wear of the wood surface and the accompanying process of surface erosion. As a result, light scattering on the surface increases, which is recorded as a decrease in the gloss value" (Poohphajai *et al.* 2021).

Table 4 shows the surface roughness parameter values for Scots pine wood samples impregnated with a 100% concentration of sunflower oil, waste sunflower oil, soybean oil, and raw linseed oil at 160°C.

Table 4

Surface roughness parameter values of the Scots pine wood samples

Oil Type	Treatment Type	Surface Roughness Parameters (µm)		
		R_a	R_y	R_z
Soybean	Type-1	8,0	56,7	48,9
	Type-2	14,5	110,2	84,4
	Control	5,7	40,4	35,4
Sunflower	Type-1	7,7	60,4	45,4
	Type-2	7,8	56,0	47,0
	Type-3	9,9	77,1	62,8
	Untreated	9,3	69,8	54,7
	Control	4	35,4	27,4
Waste Sunflower	Type-1	9,9	66,5	55,9
	Type-2	8,7	63,4	52,7
	Type-3	5,7	46,8	37,1
	Untreated	6,4	60,8	43,3
	Control	6,0	33,3	33,2
Raw Linseed	Type-1	6,8	47,7	41,9
	Type-2	9,8	69,0	54,4
	Control	2,4	22,3	17,4

In general, it can be seen that all test specimens inoculated with the fungus suspension exhibited a significant increase in roughness parameters relative to the control specimens. A comparison with the Scots pine control specimens indicates that the pre-treatments and the application of the fungal suspension led to an increase in the surface roughness values of the test sample. Conversely, for many variations in spruce, it was not possible to obtain measurements due to the overly rough surfaces of both the test and control specimens. Among the variations for which measurements were obtained, the Ra, Ry, and Rz values of spruce wood were found to be significantly higher than those of Scots pine (ranging from 11.2 to 18.6 for R_a; 72 to 130.2 for R_y; and 62 to 89.2 for R_z). It is generally reported that softwood surfaces tend to be rougher than hardwood surfaces. Furthermore, the proportion of earlywood to latewood in the annual ring is one of the factors influencing surface roughness (Aydın and Çolakoğlu 2003). In a study conducted on the subject, it was reported that the biofilm layer initially increased the surface roughness to some extent, but in subsequent stages, the roughness values remained stable compared to the control (Poohphajai *et al.* 2021).

CONCLUSIONS

Protective biofilm formation on wood is an emerging, environmentally friendly and sustainable wood preservation technique. In the study, biofilm formation was investigated on Scots pine (*Pinus sylvestris*) and Eastern spruce (*Picea orientalis*) wood samples impregnated with various vegetable oils such as sunflower oil, waste sunflower oil, raw linseed oil and soybean oil. The fungal strain *Aureobasidium melanogenum* was used as the biofilm-producing fungus. Wood samples impregnated with oils and treated with the fungal strain on their surface were exposed to outdoor weather conditions for a 3-month period (June-July-August-2025). Some macroscopic examinations with the naked eye were carried out on wood surfaces where a biofilm layer formed. a near-ideal biofilm formation is observed on some of the spruce samples; these are the samples that were impregnated only with oil and inoculated with the fungal strain. In the Scots pine samples, however, the biofilm formation does not completely cover the wood texture and possesses a more partial structure.

The equilibrium moisture content values of the test samples treated with four different oils were generally found to be lower compared to the control samples. It can be concluded that no significant strength loss occurred in the wood samples that were impregnated with oils and subjected to a three-month outdoor test after the application of the fungal strain. The color values (ΔE) of the control groups were generally found to be lower than those of the test samples. On the other hand, when both wood types are considered, the gloss values recorded at a high gloss level in the control samples reached a silky matt level in the test samples with a few exceptions. In numerous variations of spruce wood, measurements were unattainable due to the excessive roughness of the sample surfaces. A comparison with the Scots pine control samples indicates that the oil treatment increased the surface roughness of the test specimens. The preliminary results obtained can be considered promising.

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REFERENCES

Ahmed SA, Moren T, Sehlstedt-Persson M, Blom A (2017) Effect of oil impregnation on water repellency, dimensional stability and mold susceptibility of thermally modified European aspen and downy birch wood. J Wood Sci 63:74-82.

Aydın İ, Çolakoğlu G (2003) Odun yüzeylerinde pürüzlülük ve pürüzlülük ölçüm yöntemleri (Roughness and roughness measurement methods on wood surfaces). Kafkas Üniversitesi Artvin Orman Fakültesi Dergisi 1-2:92-102.

Baar J, Brabec M, Slavik R, Cermak P (2021) Effect of hemp oil impregnation and thermal modification on European beech wood properties. European Journal of Wood and Wood Products 79:161-175.

Bozkurt AY (1971) Önemli bazı ağaç türleri odunlarının tanımı teknolojik özellikleri ve kullanış yerleri (Description, technological properties and uses of the wood of some important tree species). IÜ Yayın No. 1653 O.F. Yayın No. 177, İstanbul

Castenmiller CJJ (2004) Surface temperature of wooden window frames under influence of solar radiation. Heron 49:339-348.

Catley BJ (1971) Role of pH and nitrogen limitation in the elaboration of the extracellular polysaccharide pullulan by *Pullularia pullulans*. *Appl Microbiol 22*:650-654.

Catley BJ (1980) The extracellular polysaccharide, pullulan, produced by *Aureobasidium pullulans*: A relationship between elaboration rate and morphology. *J Gen Microbiol 120*:265-268.

Çetin F, Gündüz G (2017) Türkiye'deki bazı ağaç türü odunlarının mekanik özellikleri üzerine yapılan araştırmaların değerlendirilmesi (A Review of studies on the mechanical properties of wood from various tree species in Turkey). Bartın Orman Fakültesi Dergisi 19(1):161-181.

DIN 4768 (1990) Determination of values of surface roughness parameters Ra, Rz, Rmax using electrical contact (Stylus) instruments, concepts and measuring conditions. Deutsches Institut für Norming Online at: https://www.dinmedia.de/en/standard/din-4768/1552484

Dmitrenkov A, Nikulina N, Nikulin S, Vostrikova G (2021) Ways to improve the properties of natural wood when using waste vegetable oils. IOP Conf. Series: Earth and Environmental Science 875 012063

Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clinical microbiology reviews 15:167-193.

González-Laredo RF, Rosales-Castro M, Rocha-Guzmán NE, Gallegos- Infante JA, Moreno-Jiménez MR, Karchesy JJ (2015) Wood preservation using natural products. Madera y Bosques 21:63-76.

Gün İ, Ekinci FY (2009) Biyofilmler: yüzeylerdeki mikrobiyal yaşam (Biofilms: microbial life on surfaces). Gıda 34(3):165-173.

Humar M, Lesar B (2013) Efficacy of linseed- and tung-oil-treated wood against wood-decay fungi and water uptake. Int Biodeterior Biodegradation 85:223-227.

Hyvonen A, Piltonen P, Niinimaki J (2006) Tall oil/water - emulsions as water repellents for Scots pine sapwood. Holz als Roh- und Werkstoff 64:68-73.

Lacroix C, LeDuy A, Noel G, Choplin L (1985) Effect of pH on the batch fermentation of pullulan from sucrose medium. *Biotechnol Bioeng 27*:202-207.

Liu M, Tu X, Liu X, Wu Z, Lv J, Varodi A (2020) A comparative study on the effects of linseed oil and shellac treatment on the hygroscopicity, dimensional stability, and color changes of Chinese ash wood. BioResources 15(4):8085-8092.

Mai C, Kües U, Militz H (2004) Biotechnology in the wood industry. Appl Microbiol Biotechnol 63:477-494.

Müjdeci GN, Bozdemir MT, Özbas, ZY (2024). Experimental modeling and optimization of pullulan production by Aureobasidium pullulans AZ-6. *Polymer Bulletin 81*(10):9139-9180.

Olsson T, Megnis M, Varna J, Limdberg H (2001) Measurement of the uptake of Linseed oil in pine by the use of an X-Ray microdensitometry technique. Journal of Wood Science 47:275-281.

Oparanti SO, Obebe EO, Fofana I, Caferi R A (2025) State-of-the-Art review on the potential of waste cooking oil as a sustainable insulating liquid for green transformers. Appl Sci 15(14), 7631:1-32.

Poohphajai F, Sandak J, Sailer M, Rautkari Belt T, Sandak A (2021) Bioinspired living coating system in service: evaluation of the wood protected with biofinish during one-year natural weathering. Coatings (11-701): 2-14

Sailer M (2023) A productive biofilm for wood preservation extension. Online at: efaidnbmnnnibpcajpcglclefindmkaj/https://www.xyhlo.com/wp-content/uploads/3.8-004-Regge-Hout-fact-sheet pilot_def.pdf.

Sailer M (2009) TNO internal report. EAWP biofilm development.

Sailer MF, Van Nieuwenhuijzen EJ, Knol W (2010) Forming of a functional biofilm on wood surfaces. Ecologial Engineering 362:163 -167.

Schoeman MW, Webberb JF, Dickinson DJ (1999) The development of ideas in biological control applied to forest products. International Biodeterioraton & Biodegradation 43:109-123.

Sheppard DC, Howell PL (2016) Biofilm exopolysaccharides of pathogenic fungi: lessons from bacteria. The Journal of Biological Chemistry (291) 24:12529-12537.

Teaca CA, Roşu D, Mustata F, Ruşu T, Roşu L, Roşça I, Varganici CD (2019) Naturel biobased products for wood coating and protection against degredation: a review. Bioresources 14(2):4873-4901.

Temiz A, Panov D, Terziev N, Hafren J (2008) Research on silicones and oils as hydrophobic agents. In. Proceedings of V Congreso Nacional de Proteccion de la Madera, San Sebastian (Spain), pp. 75-80.

Tomak ED (2011) Masif odundan bor bileşiklerinin yıkanmasını önlemede yağlı ısıl işlemin ve emülsiyon teknikleri ile emprenye işleminin etkisi (The effect of oil heat treatment and impregnation with emulsion techniques on preventing the leaching of boron compounds from solid wood). PhD Diss, Karadeniz Technical University, Trabzon, Türkiye

Tomak ED, Yıldız ÜC (2012) Bitkisel yağların ahşap koruyucu bir madde olarak kullanılabilirliği (Usability of vegetable oils as a wood preservative). Artvin Çoruh Üniversitesi Orman Fakültesi Dergisi 13(1):142-157.

TS ISO 13061-17 (2019) Odunun fiziksel ve mekanik özellikleri – Küçük kusursuz odun numuneleri için deney yöntemleri – Bölüm 17: Liflere paralel basınç altında nihai gerilimin belirlenmesi (Physical and mechanical properties of wood — Test methods for small clear wood specimens Part 17: Determination of ultimate stress in compression parallel to grain).

Uzundağ D, Yılmaz EŞ, Baba H (2020) Biofilm formation mechanism in fungi. Anatoloian Journal of Botany 4(2):116-120.

Van Nieuwenhuijzen EJ (2018) A study of natural fungal-based wood finishes, PhD Diss, Eindhoven University of Technology, Eindhoven, Netherlands.

Van Nieuwenhuijzen EJ, Sailer M, Va der Huvel ED, Rensing S, Adan OCG, Samson RA (2019) Vegetable oils as carbon and energy source for *Aureobasidium melanogenum* in batch cultivation. Microbiology Open (8):1-12.

Var AA (2001) Ahşap malzemede su alımının parafin vaks / bezir yağı karışımıyla azaltılması (Reducing water uptake in wood materials with paraffin wax / linseed oil mixture). Süleyman Demirel Üniversitesi Orman Fakültesi Dergisi A (2):97-110.

Wang J, Cooper P (2005a) Effect of oil type, temperature and time on moisture properties of hot oil treated wood. European Journal of Wood and Wood Products 63(6):417-422.

Wang J, Cooper P (2005b) Properties of hot oil treated wood and the possible chemical reactions between wood and soybean oil during heat treatment. 36. IRG Annual Meeting, Banglore, IRG-WP 05-40304

Xyhlo Biofinish (2023) Online at: xyhlo.com/en/