Determination of Decay, Larvae Resistance, Water Uptake, Color, and Hardness Properties of Wood Impregnated with Honeybee Wax

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The aim of the study was to determine the effect of honeybee wax impregnation on the antifungal, larvicidal, water uptake, color, and mechanical properties of wood. Wood samples (poplar, Scots pine, beech, and lime) were impregnated with melted honeybee wax under vacuum. The wax-impregnated samples were exposed to the wood-decay fungi *Trametes versicolor* and *Neolentinus lepideus* for 8 weeks. The larvicidal effect of the beeswax was tested against European old house borer (*Hylotrupes bajulus* L.). Water uptake, color measurements, and surface hardness were also tested. According to the obtained findings, a 34.6% mass loss was seen in the poplar control wood, and only 3.9% mass loss was found in the 100% beeswax-impregnated samples. The results showed that *H. bajulus* larvae could digest honeybee wax with wood when beeswax surface treatment was applied. Additionally, an average of 30% larvae mortality rate was achieved on beeswax-treated wood surfaces, compared to a 2.5% rate on the controls. However, when wood was deeply treated with beeswax, larval mortality reached 100%. In the water uptake test, beeswax-treated samples showed water repellent efficiency. The lowest water uptake (24.2%) was obtained in poplar wood treated with 100% beeswax, compared to 92.6% in the poplar control in 96 h immersion time. With the beeswax treatment, *a** and b* color values increased, while the *L** values decreased.

Keywords: Honeybee wax; Impregnation; Decay; Hylotrupes bajulus; Water uptake; Color properties; Hardness

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INTRODUCTION

Waxes have been used as a coating and surface material for many years (Lozhechnikova *et al*. 2017). Recently, the use of biocides has been limited due to American and European Union legal regulations (Kartal *et al.* 2006). Therefore, interest in the usage of waxes for wood protection has started to increase in terms of its ability to improve wood's absorption properties and dimensional stability. Wax treatment has also been reported to slow down the photo-degradation of wood (Lesar *et al.* 2011). Waxes benefit wood protection because, being natural and non-toxic, they do not cause harm to environmental systems or human health. The cell lumens of the wood impregnated with water-repellent waxes are filled with wax, thus increasing the wood's resistance to rot. Therefore, fungal degradation of the wood impregnated with hydrophobic wax is slowed (Lesar and Humar 2011).

It has been reported that treatment with waxes also protects against termites, though it cannot completely stop the damage (Scholz *et al.* 2010a). Waxes also increase the mechanical properties of wood. The hardness, compression resistance, bending strength, and impact bending strength values of wood impregnated with different wax types increase (Krupa and Luyt 2001; Esteves *et al*. 2014). Waxes, including beeswax, are widely used in the conservation and consolidation of wooden works (Timar *et al* 2010; Hutanu *et al*. 2013)

Waxes are natural and renewable materials. The melting temperature of beeswax depends on the geographical region, but it is generally between 61 and 67 °C (Gaillard *et al.* 2011). Waxes also show high plastic properties at low temperatures. It has been reported that their density varies between 0.958 and 0.970 g/cm^3, while thermal conductivity is 0.25 W/mK (Morgan *et al.* 2002). There are approximately 300 different components in beeswax. It mostly consists of 14% hydrocarbons, 12% free fatty acids, 35% mono esters, 14% diesters, 3% triesters, 4% hydroxy monoesters, 8% hydroxy polyester, 1% monoester acids, and 2% polyester acids (Marquez *et al*. 2019).

Beeswax has a wide variety of applications, from basic honeycomb production in the beekeeping industry to the cosmetic industry. It is used for various purposes in woodworking, including to polish parquet varnish and in paint. Beeswax is used to make metallic containers and bottle caps, small sculptures, and trinkets. It is also used in the production of candles, perfume, and cosmetic lipsticks (Bogdanov 2009). Beeswax is widely used in shoemaking, water-resistant yarn production, waterproofing tents and other materials, medical purposes, and in many other fields. It is also reported that wax is used to produce various types of ointment drugs for human health, as well as in the production of facial creams and dentistry (Tulloch 1980; Mladenoska *et al.* 2012).

The aim of the present study was to investigate the effect of wax impregnation on the biological, mechanical, and physical properties of certain wood species with low durability. According to the author's knowledge, the effects of beeswax impregnation on wood's ability to withstand old house borer, as well as its effects on wood's color and hardness properties, have not been tested in the literature.

EXPERIMENTAL

Wood Samples

Poplar (*Populus alba* L.), lime (*Tilia grandifolia* Ehrh.), Scots pine (*Pinus sylvestris* L.), and beech (*Fagus orientalis* L.) wood species were supplied from Düzce Province, Turkey. Wood specimens were prepared from sapwood in dimensions of 50 × 10 × 5 mm^3 for fungal tests and to observe color and hardness properties. Samples were sized 50 × 25 × 15 mm^3 for larvae test, and 50 × 10 × 5 mm^3 (longitudinal × radial × tangential) for water uptake (WU). Oven dry densities of poplar, lime, Scots pine, and beech wood species were recorded as 0.43, 0.47, 0.52, and 0.58 g/cm^3, respectively. The ring widths were measured with an approximate average of 5 mm for each wood species used in the study.

Impregnation of Wood Samples

Beeswax was obtained from Aktarix Bitkisel Ürünler Company, Antalya, Turkey. Its melting point was 61°C. Six replicates were used for each species in wax impregnation. All samples specimens were dried at 103 ± 2 °C for 24 h, and the dried weights (G_0) were recorded before the impregnation process. The impregnation process was completed in a desiccator. The desiccator was put on a heater held at 120 °C. The test specimens, except the controls, were placed into the melted beeswax solution in the desiccator and kept under a vacuum of 0.079 MPa for 60 min. After impregnation, the remaining beeswax was rapidly wiped away with a paper towel. Next, the beeswax-impregnated samples were weighed (G_1). The rate of weight gain (WPG) of the samples was calculated using Eq. 1. All samples were conditioned at 20 ± 2 °C and 65 ± 2% relative humidity (RH) for two weeks after treatment. The relative change in weight was calculated as follows:

$$\triangle G\ (\%) = (G_1 - G_0) / G_0 \times 100 \qquad (1)$$

Antifungal Test

The decay resistance of beeswax-treated poplar, lime, Scots pine, and beech wood specimens against white rot *Trametes versicolor* (TV) and brown rot *Neolentinus lepideus* (NL) was tested according to the EN 113 (1996) standard. Six replicates were used for each species in antifungal testing. Malt extract agar (MEA) medium (Merck, Darmstadt, Germany) (3%) was used to grow fungi cultures. The media were sterilized at 121 ± 2 °C and 1.1 atm for 20 min. Wood blocks were sterilized in a UV-featured biohazard safety cabined (JSR Research Inc., Gongju, Republic of Korea) for 90 min so that the beeswax did not leach from the wood samples at high temperature. The media were transferred to petri dishes. After inoculation, the dishes were kept at 26 ± 2 °C and 80% RH. When the media surfaces were completely colonized by the test fungi, beeswax-treated and untreated wood samples were placed into the petri dishes. The wood samples were exposed to white rot (*T. versicolor*) and brown rot (*N. lepideus*) in an incubator at 26 ± 2 °C and 80% RH for 8 weeks. *T. versicolor* was obtained from RISH, Kyoto University, Japan. *N. lepideus* was isolated from decayed wood in a log depot of Bolu province in Turkey. After the fungal exposure, exposed wood samples were cleaned from the fungal mycelium using a brush. Cleaned samples were dried at 55 °C for 48 h and reweighed (control samples were dried overnight at 103 ± 2 °C). Then, percent mass losses were calculated using the weights recorded before and after the fungal tests (Eq. 2). Decay resistance tests were conducted in the Forest Biology and Wood Preservation Laboratory of Düzce University (Düzce, Turkey). The weight loss (Eq. 2) was determined as follows,

$$\text{WL} = [(M_1 - M_0) / M_1] \times 100 \qquad (2)$$

where M_1 is the weight of wood (g) after wax treatment and before the fungal test and M_0 is the weight (g) of the wood after the fungal test.

Larvae Test

The larvae tests were conducted according to EN 46-1 (2016) and EN 47 (2016) standards. Surface treatment was applied according to EN 46 -1 (2016), while EN 47 followed for the deep impregnation process. Scots pine (four replicates) test specimens were cut into dimensions 25 × 15 × 5 mm^3. For the control and beeswax-treated samples, a gap was created in one side of the specimens for placement of the larvae, while the other surfaces were covered with paraffin wax. Newly hatched *Hylotrupes bajulus* (Coleoptera: Cerambycidae) larvae obtained from the end of cultivation were used for the larvae test according to the standard EN 46-1 (2016). A total of 10 larvae were inserted between glass and wood surfaces for one wood block. All wood blocks included controls, and 80 larvae were used in the experiment. Wood blocks were kept at 26 ± 2 °C and 80% relative humidity. After four weeks, the glasses were removed on the wood block surfaces, and the living and dead larvae were recorded (classified as not tunneled, starting to tunnel, alive, and tunneled) under a microscope. Then, the test continued until the end of the 16 weeks.

After testing, all wood blocks were cut and the amount of live and dead larvae was determined. Larvae mortality rates were calculated by using Eq. 3.

Four replicates were used for the deeply impregnation. Six larvae were inserted on the hole opened on the wood. A total of 48 newly hatched larvae were used, which includes control samples according to the EN 47 standard (2016). Wood blocks were kept under the same conditions with surface treatment samples in a conditioner. All wood blocks were cut after 16 weeks and the amount of live and dead larvae was recorded. Larvae mortality rates were calculated by using Eq. 3,

$$\text{Mortality} = (N_f / N_t) \times 100 \quad (3)$$

where the total larvae number before testing is N_t and the number of dead larvae after testing is N_f.

Water Uptake Test

A total of 48 samples from poplar, lime, Scots pine, and beech were used for WU tests. Control samples were dried to a constant weight at 103 ± 2 °C; their dry weights were re-measured to the nearest 0.01 g (A_0). All specimens were conditioned at 20 ± 2 °C and 65 ± 2% RH for 2 weeks before the WU test. The weights after impregnation were used as A_0 for WU tests, while oven-dried weights were used for the control samples. The beeswax-treated and control samples were immersed in water with 20 ± 1 °C, and a heavy stone was put on them to ensure that they remained below the water surface. The weights of the test and control samples were measured at the end of water immersion periods of 1, 2, 4, 8, 16, 32, 48, 60, 72, and 96 h (A_1). At the end of each period, the samples were removed from the water container and wiped off with a paper towel. The WU was calculated with following Eq. 4:

$$WU = [(A_1 - A_0) / A_0] \times 100 \quad (4)$$

The WRE of the beeswax used in the study was calculated using Eq. 5 for each test separately,

$$WRE = [(A_C - A_1) / A_C] \times 100 \quad (5)$$

where, A_C is the water uptake of the control sample, and A_1 is the water uptake of the test sample at the end of the specified periods.

Determination of Color Measurement

Ten replicates were used for control and beeswax treated samples. Red/green color tone (a^*), lightness (L^*), and the yellow/blue color tone (b^*) of wax-treated and untreated specimens was determined using a CS-10 colorimeter (Hangzhou CHNSpec Technology Co., Ltd., Hangzhou, China), a CIE 10° standard observer; and a CIE D65 light source, with 8°/diffused illumination, according to ASTM standard D2244-16 (2016). A CIELAB system, characterized by the three-axis L^*, a^*, and b^* was used (Ayata 2019). The total color difference (ΔE^*), Δa^*, ΔL^*, and Δb^* were calculated using Eqs. 6, 7, 8, and 9:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (6)$$

$$\Delta L^* = L^*_{\text{Wax-treated}} - L^*_{\text{untreated}} \quad (7)$$

$$\Delta b^* = b^*_{\text{Wax-treated}} - b^*_{\text{untreated}} \quad (8)$$

$$\Delta a^* = a^*_{\text{Wax-treated}} - a^*_{\text{untreated}} \quad (9)$$

Determination of Shore D Hardness

Shore D hardness (stand: model Ld-J Loyka; Shenzhen Omena Technology Co., Ltd., Guangdong, China) was loaded with 5 kg using ASTM D2240-15 (2010) standard. Ten tests were performed for untreated and wax-treated samples.

Statistical Analysis

Minimum, maximum, and average values, as well as variance analysis, homogeneity groups were given for color and Shore D hardness tests on samples that were wax-treated and untreated. The SPSS 17 program (Sun Microsystems, Inc.; Santa Clara, CA, USA) was used in Statistical Analysis

SEM Analysis

Scanning electron microscopy (SEM) analyses were performed at Düzce University Scientific and Technological Researches Application and Research Center (Düzce, Turkey). The SEM images were taken using a Quanta FEG 250 instrument (FEI Europe B.V., Eindhoven, Netherlands). The wood samples were mounted onto aluminum stubs with double-sided carbon tape, and the mounted specimens were coated with 10 nm gold film using a sputter coater (Desk V-Standard; Denton Vacuum, LLC, Moorestown, NJ, USA) before analyses. Surface morphologies of the samples were investigated with an SEM Quanta FEG 250 (FEI Europe B.V., Eindhoven, Netherlands), which used an electron acceleration voltage of 10 keV. The images showed the radial section of the woods.

RESULTS AND DISCUSSION

Weight Gain

As shown in Fig. 1, the highest ΔG was recored in beeswax-impregnated poplar samples, while the lowest was recorded in beech. It seemed that ΔG was related to the densities of wood species. When wood densities decreased, the ΔG value increased, as shown in Fig. 1.

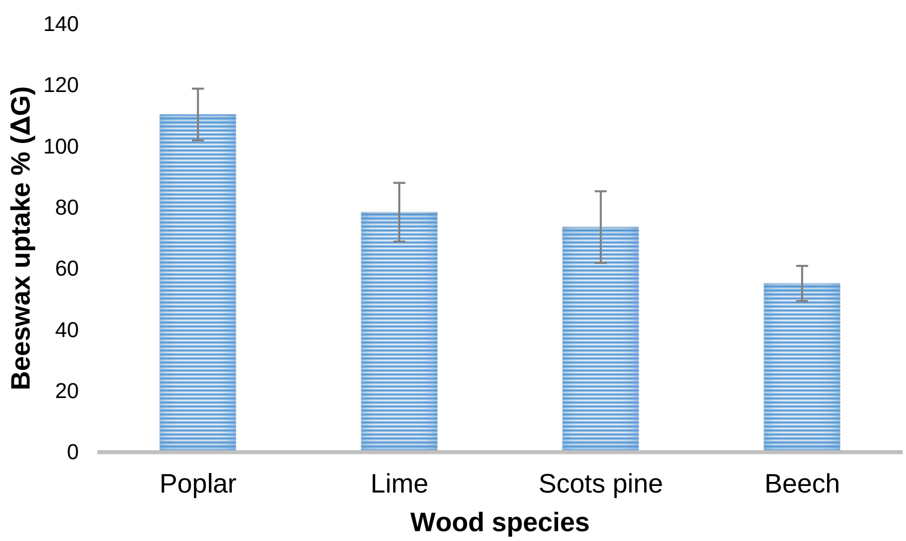

Fig. 1. Weight gain rate of the wood specimens (note: error bars indicate standard deviation)

Fungal Test

Antifungal properties of the beeswax-impregnated samples are given in Fig. 2. The highest mean weight losses were detected in control samples of the woods. Poplar control samples exposure to *N. lepideus* and *T. versicolor* gave higher weight losses by 34.6% and 33%, respectively, compared to the other wood species. When wood species were impregnated with beeswax at the concentration of 100%, weight losses decreased in all wood species. Only 3.9% mass loss occurred in poplar wood impregnated with 100% honeybee wax in impregnated specimens. In general, weight loss in both the control and the beeswax-impregnated specimens in terms of the decomposition of fungal species gave similar results. Melted wax was filled into the cell lumens of the wood impregnated with beeswax, thus giving water-repellent properties to beeswax-impregnated wood (Németh *et al*. 2015). For this reason, fungal development is prevented in wood with reduced moisture content.

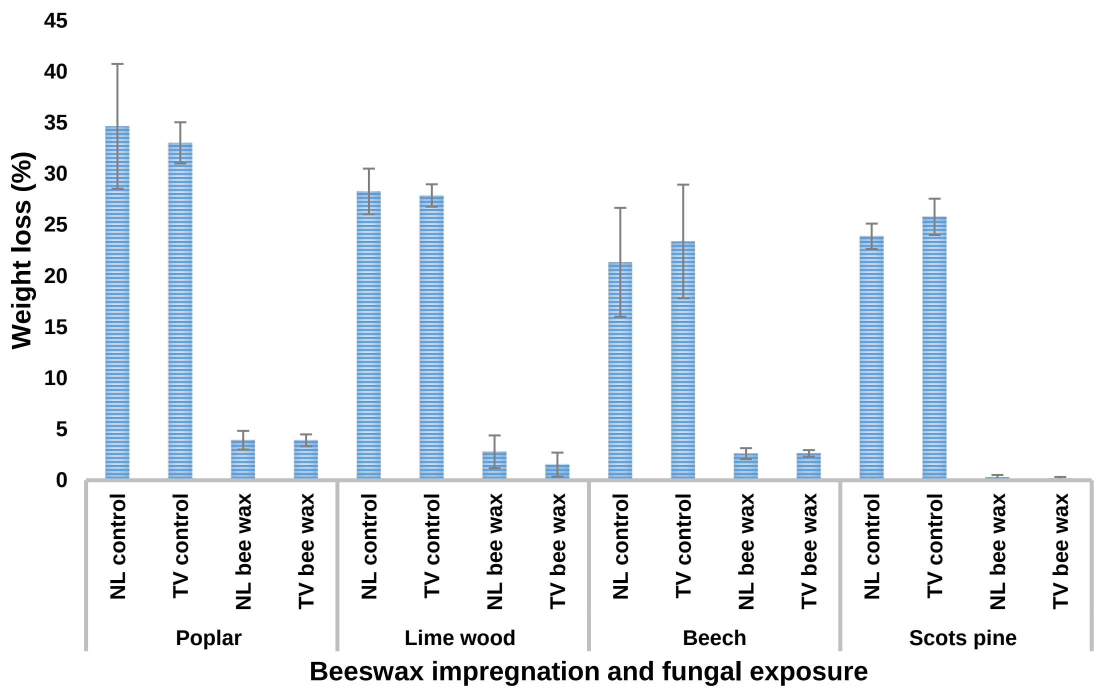

Fig. 2. Antifungal properties of beeswax impregnation (note: NL: *Neolentinus lepideus*, TV: *Trametes versicolor*, error bars indicate standard deviation)

Figure 3 shows SEM images of poplar wood impregnated with honeybee wax and exposed to wood decay fungi. According to the SEM images, there was a dense fungal hyphae in the trachea of wood species that were not impregnated with honeybee wax, while hyphae were not detected in the trachea of the impregnated wood species. In addition, a large amount of wax was observed in the trachea of the lumens in the images of impregnated samples. It is understood that wood cell lumens filled with the beeswax prevent the spread of fungal hyphae (Nemeth *et al.* 2015). It can also be said that fungi cannot develop under such circumstances, as there is no moisture in cell lumens filled with beeswax.

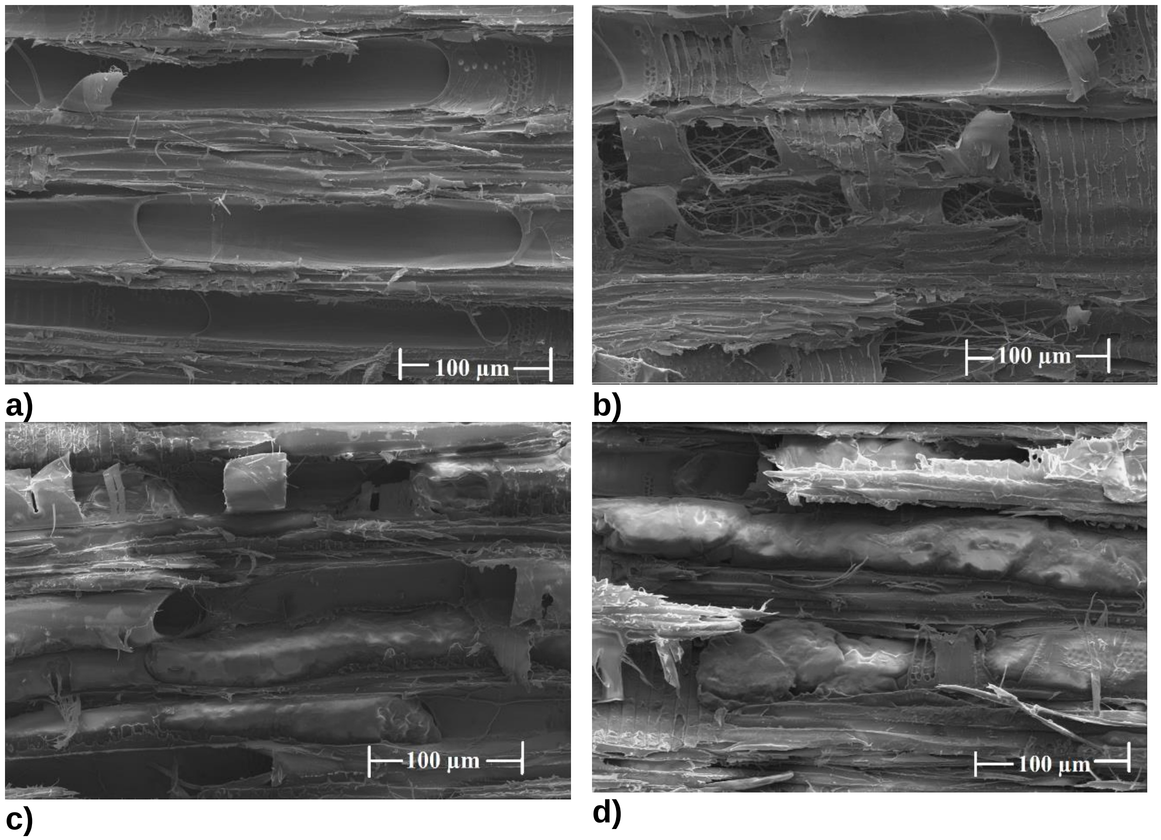

Fig. 3. Scanning electron microscopy images of control and beeswax-treated poplar wood: a) un-impregnated control, b) un-impregnated and exposed to decay fungi, c) beeswax-impregnated, and d) beeswax-impregnated and exposed to decay fungi

Larvae Test

Larvae mortality rates of beeswax-treated and control woods after four and 16 weeks are shown in Table 1. As shown, mean larvae mortality rate was recorded as 2.5% in the control woods. When wood species were surface treated with beeswax, the mean percentage larvae mortality rate reached 30%. Almost all the larvae in the control woods were alive and tunneled. The highest mortality rate on the surface observed was 70% in the wood sample treated with 1.52 g of beeswax, and the lowest was observed in the sample surface treated with 1.02 g of beeswax. Thus, as the beeswax amount inceased, the larvae mortality increased. As a result of examinations under the microscope, it was observed that some larvae digested the beeswax and discarded it in the frass form. In addition, as a result of the microscope observations, it was observed that the larvae mostly died before they passed the wax layer, and the larvae that reached the wood continued feeding. According to these findings (surface treatment), beeswax impregnation protected wood against *H. bajulus* but did not completely stop the damage. Similar findings have also been reported for termites by Scholz *et al*. (2010a). Nemeth *et al.* (2015) detected insect damage on the surface of beeswax-impregnated beech wood. They reported that wood-boring beetles could digest beeswax together with wood because beeswax has a low biocide effect on insects. However, the current study showed that when Scots pine samples were deeply impregnated with beeswax, the larval mortality rate was obtained as 100% (Table 1). According to these findings, beeswax can be used effectively against *H. bajulus* when wood deeply treated.

Table 1. Larvae Mortality Rates on Scots pine Wood Surfaces Treated with Beeswax

Surface Treatment							
Wood	Dead Larvae on Surface After 4 Weeks				Mean Mortality After 4 Weeks (%)	Mean Mortality After 16 Weeks (%)	Amount of Beeswax (g)
	Not Tunneled	Started to Tunnel	Alive and Tunneled	Mortality (Surface) %			
Control	0	0	10	0	2.5	2.5	
	0	1	9	10			
	0	0	10	0			
	0	0	10	0			
Beeswax-treated	7	0	3	70	30	30	1.52
	0	0	10	0			1.06
	1	0	9	10			0.82
	4	0	6	40			0.85

Deeply Treatment						
Live and Dead Larvae After 16 Weeks						
	Live larvae	Dead larvae	Larvae mortality rate (%)	Larvae mortality rate (%)	Retention (Kg/m^3)	Average Retention (Kg/m^3)
Control	5	1	16.6	12.4		
	6	0	0			
	4	2	33.3			
	6	0	0			
Beeswax-treated	0	6	100	100	48.5	47.3
	0	6	100		47.8	
	0	6	100		44.9	
	0	6	100		47.9	

Water Uptake of Wood Species Impregnated with Beeswax

Mean water uptake (WU) for control and beeswax-impregnated poplar, lime, Scots pine, and beech wood samples with immersion time is shown in Figs. 4 through 7. Generally, it was seen that water uptake values for beeswax-impregnated wood were much lower than for un-impregnated control samples. Water uptake values for controls and beeswax-impregnated samples increased rapidly from the start of immersion until approximately 48 h of immersion. After that time, mean water uptake increase rates decreased notably, and they remained nearly constant except in the poplar control wood. The water-uptake increase rate for beeswax-treated wood samples was much lower than that of control woods during the immersion time. The highest WU (92.6%) was found in the poplar control wood, and the lowest was seen in the beech control with a 96-h immersion time. Beeswax-impregnated poplar (24.6%) and beech (24.2%) wood showed the lowest WU in the impregnated samples, although poplar wood's corresponding control had the highest WU at the end of the immersion time. According to obtained results, beeswax impregnation provided the high-water repellent efficiency (WRE). Even under 96 h of immersion, 276.4% water repellent efficiency was obtained in beeswax-impregnated poplar wood, while 114.1% WRE was obtained in beeswax-treated beech.

Water uptake is related to wood species density (Ding *et al*. 2012). Because poplar wood used in the study had the lowest density, it gave the highest WU and WRE values. Resins, paraffins, and waxes are hydrophobic and water repellent substances (Zhang *et al.* 2007), as well as insoluble in water. When wood is impregnated with wax, it locates in the cell lumen and forms a thin layer at the wood's surface (Ding *et al.* 2012; Ren *et al.* 2016). It reduces swelling of the wood (Ding *et al.* 2012). Moreover, beeswax impregnation increases the surface contact angle between wood and water. Thus, beeswax impregnation improved the hydrophobic properties of the wood surfaces. Li *et al*. (2020) concluded that beeswax impregnation remarkably enhanced the dimensional stability, water absorption, and surface hygroscopicity of wood.

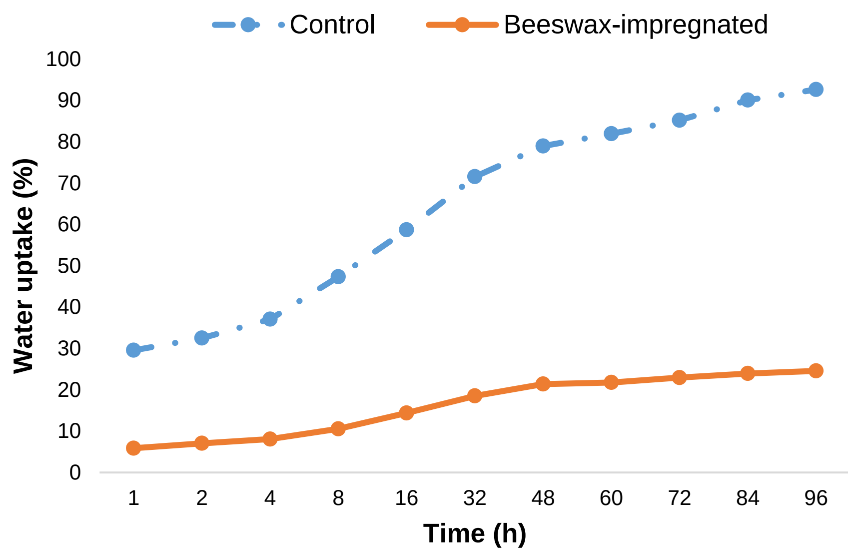

Fig. 4. The WU of poplar wood treated with beeswax at different water-holding periods

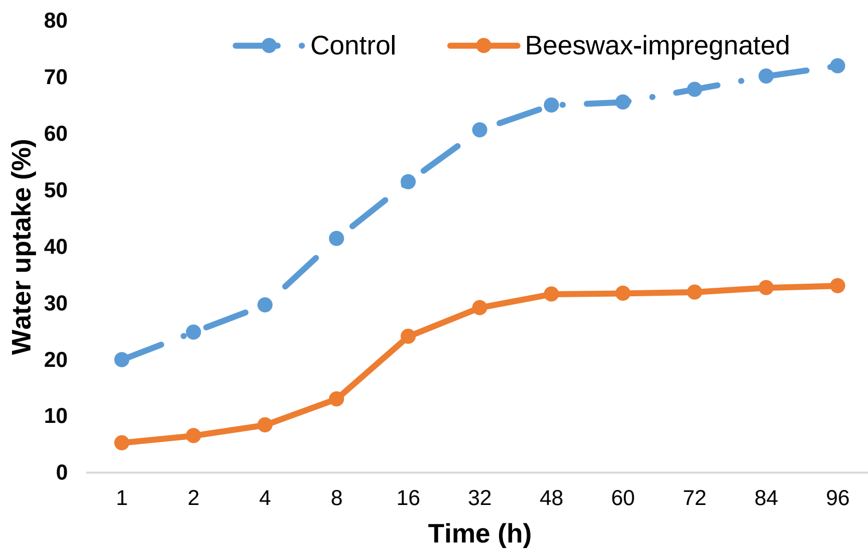

Fig. 5. The WU of lime wood treated with beeswax at different water-holding periods

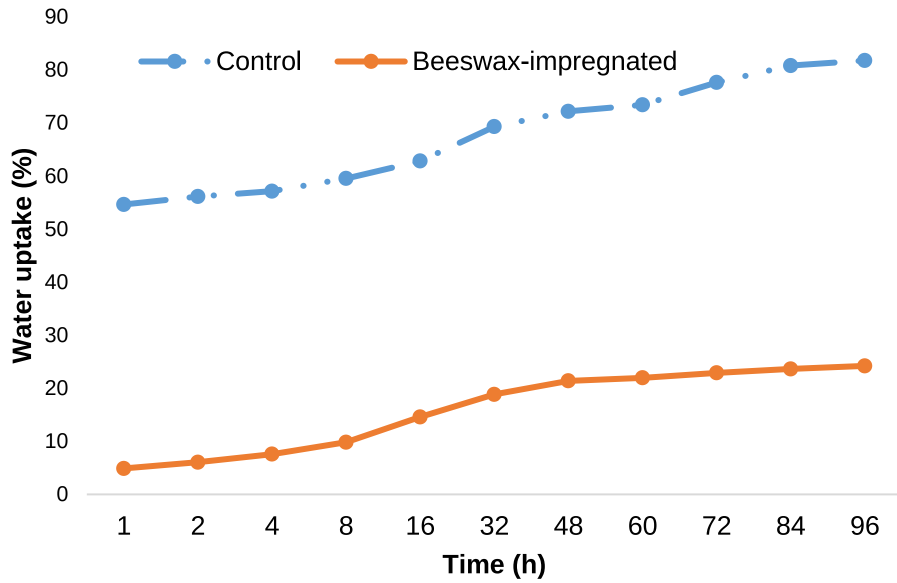

Fig. 6. The WU of Scots pine wood treated with beeswax at different water-holding periods

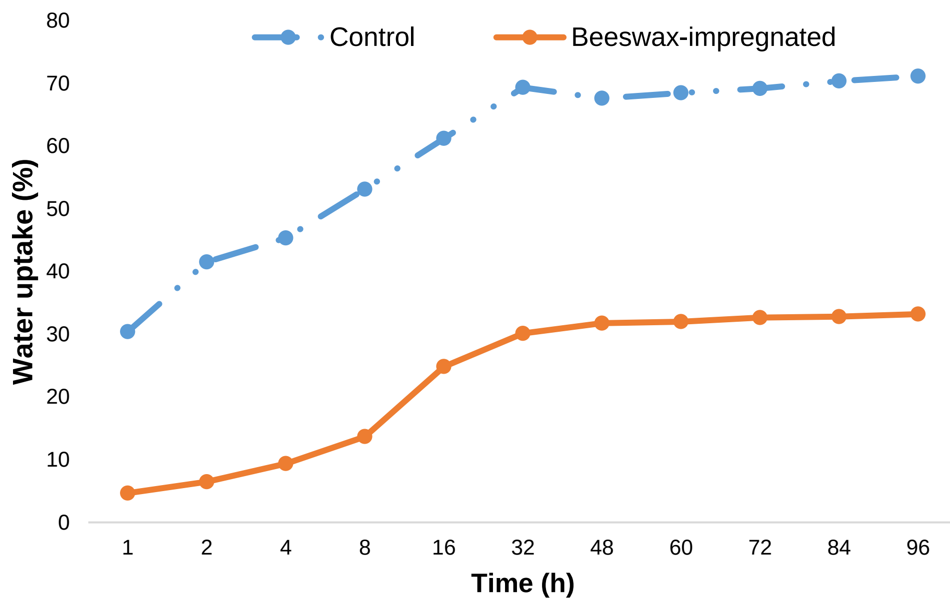

Fig. 7. The WU of beech wood treated with beeswax at different water-holding periods

Color and Hardness Changes

Table 2 shows the results of variance analysis for ΔL^*, Δa^*, Δb^*, and Shore D. According to wood type (A), impregnation (B), fungi type (C), and interaction (AB, AC, BC, and ABC) for L, a^*, b^*, and Shore D were significant at 95% confidence level ($P \leq 0.05$).

Table 2. Results of the Variance Analysis of Color Parameters (*a**, *b**, and *L**) and Shore D Hardness Before and After Fungi Tests

Test	Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Sig.
Lightness (L*)	Wood Type (A)	3740.402	3	1246.801	3348.925	0.000*
	Impregnation (B)	14422.286	1	14422.286	38738.466	0.000*
	Interaction (AB)	2480.285	3	826.762	2220.694	0.000*
	Fungi Type (C)	157.401	2	78.700	211.390	0.000*
	Interaction (AC)	2035.495	6	339.249	911.228	0.000*
	Interaction (BC)	334.585	2	167.292	449.350	0.000*
	Interaction (ABC)	496.231	6	82.705	222.147	0.000*
	Error	80.417	216	0.372		
	Total	1154727.021	240			
Red Color Tone (a*)	Wood Type (A)	433.263	3	144.421	1738.234	0.000*
	Impregnate (B)	124.805	1	124.805	1502.138	0.000*
	Interaction (AB)	18.479	3	6.160	74.138	0.000*
	Fungi Type (C)	4.149	2	2.075	24.969	0.000*
	Interaction (AC)	1009.590	6	168.265	2025.217	0.000*
	Interaction (BC)	138.722	2	69.361	834.821	0.000*
	Interaction (ABC)	107.870	6	17.978	216.385	0.000*
	Error	17.946	216	0.083		
	Total	14163.582	240			
Yellow Color Tone (b*)	Wood Type (A)	2225.411	3	741.804	2619.957	0.000*
	Impregnate (B)	460.984	1	460.984	1628.136	0.000*
	Interaction (AB)	807.803	3	269.268	951.019	0.000*
	Fungi Type (C)	912.383	2	456.191	1611.210	0.000*
	Interaction (AC)	1283.680	6	213.947	755.632	0.000*
	Interaction (BC)	299.827	2	149.914	529.476	0.000*
	Interaction (ABC)	403.511	6	67.252	237.525	0.000*
	Error	61.157	216	0.283		
	Total	149969.412	240			
Shore D Hardness	Wood Type (A)	10200.846	3	3400.282	1246.328	0.000*
	Impregnate (B)	670.004	1	670.004	245.581	0.000*
	Interaction (AB)	786.679	3	262.226	96.116	0.000*
	Fungi Type (C)	3219.733	2	1609.867	590.075	0.000*
	Interaction (AC)	233.467	6	38.911	14.262	0.000*
	Interaction (BC)	469.433	2	234.717	86.032	0.000*
	Interaction (ABC)	573.833	6	95.639	35.055	0.000*
	Error	589.300	216	2.728		
	Total	323979.000	240			
* Significant according to α ≤ 0.05						

Table 3 shows the statistical analysis results according to the wood species control, beeswax treatment, and fungi type for *L**, *a**, *b**, and Shore D. When wood was impregnated with 100% beeswax, the highest *L** value of percentage change was found in beech wood with 26.8%, while the lowest was seen in Scots pine with 10.6%. It was seen that all *L** values in wood species decreased after the beeswax treatment. Fungal degradation differed according to wood species in terms of the *L** value. Red color tone (*a**) values in all wood species increased after beeswax impregnation compared to un-treated control specimens. The differences between beeswax-treated and untreated samples for red color tone values were found to be significant ($P \leq 0.05$). The positive increase in the "*a**" value indicated that beeswax impregnation contributed to increasing the red color value. The highest "*a**" value of percentage change for control samples was observed in poplar wood at 49%, while the lowest was in Scots pine at 23.7% after wax treatment. When *b** values were examined, it was observed that *b** values increased in all beeswax-treated and fungal-exposed specimens compared to control specimens. Positive increases in beeswax-treated samples indicated that beeswax impregnation also contributed to a yellow color tone. Beeswax-treated lime wood showed the highest *b** value of percentage change, while Scots pine and poplar wood showed an almost similar percentage change when compared to un-treated control specimens. As shown in Table 3, Shore D hardness values differed according to wood species. Shore D hardness values increased in poplar and lime wood (low-density wood species) after beeswax treatment. As expected, fungal exposure negatively affected the hardness values in all wood species.

Table 3. Statistical Analysis Results for Color Parameters (*a**, *b**, and *L**) and Shore D Hardness Before and After Fungi Tests

Wood Species	Treatment	Fungi Species	Lightness (*L**)			Red Color Tone (*a**)		
			Mean	*HG*	*SD*	Mean	*HG*	*SD*
Beech	Control	Control (untreated)	66.97	L	0.57	7.99	E	0.28
		Trametes versicolor	81.97	A*	0.33	4.78	N	0.10
		Neolentinus lepideus	75.01	G	0.14	6.89	HI	0.30
	Beeswax	Control (wax-treated)	48.96	S	0.83	14.16	A*	0.43
		Trametes versicolor	48.18	T**	0.83	4.08	P	0.44
		Neolentinus lepideus	54.21	R	0.72	4.44	O	0.29
Poplar	Control	Control (untreated)	76.19	F	0.66	3.59	R**	0.38
		Trametes versicolor	78.44	E	1.13	5.09	M	0.16
		Neolentinus lepideus	79.10	D	0.71	5.51	L	0.04
	Beeswax	Control (wax-treated)	58.71	P	0.31	7.05	FGH	0.29
		Trametes versicolor	58.27	P	0.34	7.31	F	0.36
		Neolentinus lepideus	63.39	N	1.09	6.74	J	0.20
Scots pine	Control	Control (untreated)	79.74	C	0.16	4.62	NO	0.16
		Trametes versicolor	68.91	K	0.20	10.84	D	0.10
		Neolentinus lepideus	69.45	J	0.43	11.30	C	0.40
	Beeswax	Control (wax-treated)	71.28	H	0.31	6.06	K	0.15
		Trametes versicolor	62.55	O	1.32	11.97	B	0.66
		Neolentinus lepideus	64.68	M	0.31	11.45	C	0.18
Lime	Control	Control (untreated)	81.78	A	0.09	4.59	NO	0.13
		Trametes versicolor	78.38	E	0.18	6.92	GHI	0.13
		Neolentinus lepideus	80.85	B	0.34	5.16	M	0.18
	Beeswax	Control (wax-treated)	70.41	I	0.30	7.75	E	0.12
		Trametes versicolor	63.32	N	0.16	7.18	FG	0.30
		Neolentinus lepideus	66.78	L	0.70	6.41	J	0.27
Wood Type	Treat-ment	Fungi species	Yellow Color Tone (*b**)			Shore D Hardness (N/mm^2)		
			Mean	*HG*	*SD*	Mean	*HG*	*SD*
Beech	Control	Control (untreated)	20.87	J	0.34	52.80	A*	2.53
		Trametes versicolor	20.67	JK	0.18	34.60	G	0.84
		Neolentinus lepideus	29.27	C	0.25	45.10	D	0.99
	Beeswax	Control (wax-treated)	22.94	I	0.41	49.70	B	0.95

		Trametes versicolor	18.30	N	0.40	45.10	C	0.99
		Neolentinus lepideus	19.30	M	0.14	44.10	C	0.32
Poplar	Control	Control (untreated)	19.10	M	0.77	31.50	H	2.27
		Trametes versicolor	20.37	KL	0.95	25.40	J	1.26
		Neolentinus lepideus	21.04	J	0.28	21.30	K**	0.82
	Beeswax	Control (wax-treated)	25.96	F	0.67	33.20	G	1.75
		Trametes versicolor	26.52	DE	0.76	25.50	J	0.53
		Neolentinus lepideus	25.95	F	0.94	28.50	I	0.53
Scots pine	Control	Control (untreated)	19.97	L	0.47	39.20	E	4.83
		Trametes versicolor	33.89	B	0.20	29.10	I	0.74
		Neolentinus lepideus	29.63	C	1.00	31.00	H	0.82
	Beeswax	Control (wax-treated)	25.16	G	0.44	36.80	F	2.30
		Trametes versicolor	35.47	A*	0.80	31.50	H	1.08
		Neolentinus lepideus	33.81	B	0.18	28.40	I	0.52
Lime	Control	Control (untreated)	14.98	O**	0.16	40.40	DE	1.17
		Trametes versicolor	26.06	EF	0.26	27.90	I	1.85
		Neolentinus lepideus	20.97	J	0.60	31.00	H	1.56
	Beeswax	Control (wax-treated)	24.59	H	0.13	43.70	C	1.83
		Trametes versicolor	25.10	G	0.20	41.80	D	1.32
		Neolentinus lepideus	26.98	D	0.27	41.10	D	0.88

HG: Homogeneity group
SD: Standard deviation
* Highest value
** Lowest value

Table 4 shows the total color changes observed due to wax treatment and fungal exposure in varying wood species. The highest total color change (Δ*E**) was found in Scots pine and beech control and beeswax-treated samples exposed to fungi. It was thought that because of their light color tone, poplar and lime wood were not affected during the fungal degradation. In fungal-exposed specimens, higher total color change values were recorded in control woods than in beeswax-treated ones because beeswax protected wood against fungal degradation.

When the total color change values for beeswax-treated specimens were examined, the highest color change (Δ*E**) was found in beech and poplar wood, *i.e.* 19.15 and 19.12, respectively. The total color change of Scots pine was the lowest by 10.04, compared to the other wood species. The total color change was found negative for Δ*L** and positive for Δ*a**, Δ*b**, and Δ*E** in all wood species.

The complicated chemical composition of beeswax caused color change in wood. Its chemical composition consists of 15 different compounds that are divided into three main groups: fatty acids, esters, and saturated hydrocarbons. There are some small quantities of dyes and essential oils in beeswax, and these small quantities determine the beeswax color. Natural beeswax color can range between white and light brown. However, a good color is the color of lemon (Istrefi *et al*. 2017).

It was stated that impregnating wood with wax plays an important role in increasing the hardness of the wood (Esteves *et al*. 2014). Investigations confirmed an increase in hardness of up to 189% (lateral) as well as 86% (longitudinal) for wax-impregnated beech (*Fagus sylvatica* L.) wood (Scholz *et al*. 2010a,b).

Table 4. Total Color Changes (Δ*L**, Δ*a**, Δ*b**, and Δ*E**)

Wood Species	Treatment	Fungal Species	Δ*L*	Δ*a*	Δ*b*	Δ*E*
Beech	Control (un-impregnated)	*Trametes versicolor*	15.01	-3.21	-0.20	15.36
		Neolentinus lepideus	8.04	-1.10	8.40	11.70
	Beeswax (impregnated)	*Trametes versicolor*	-0.78	-10.09	-4.64	11.18
		Neolentinus lepideus	5.25	-9.73	-3.64	11.68
Poplar	Control (un-impregnated)	*Trametes versicolor*	2.25	1.50	1.27	3.37
		Neolentinus lepideus	2.91	1.91	1.94	4.10
	Beeswax (impregnated)	*Trametes versicolor*	-0.44	0.26	0.56	1.24
		Neolentinus lepideus	4.68	-0.32	-0.02	4.79
Scots pine	Control (un-impregnated)	*Trametes versicolor*	-10.83	6.22	13.92	18.70
		Neolentinus lepideus	-10.29	6.68	9.66	15.63
	Beeswax (impregnated)	*Trametes versicolor*	-8.73	5.90	10.31	14.76
		Neolentinus lepideus	-6.60	5.39	8.65	12.16
Lime	Control (untreated))	*Trametes versicolor*	-3.41	2.33	11.08	11.82
		Neolentinus lepideus	-0.93	0.57	5.98	6.10
	Beeswax (impregnated)	*Trametes versicolor*	-7.09	-0.56	0.51	7.14
		Neolentinus lepideus	-3.63	-1.33	2.39	4.60
Beeswax Treatment						
Wood Species			ΔL	Δa	Δb	Δ*E*
Beech			-18.01	6.17	2.07	19.15
Poplar			-17.48	3.46	6.86	19.12
Scots pine			-8.46	1.44	5.18	10.04
Lime			-11.38	3.16	9.61	15.23

CONCLUSIONS

1. Water uptake properties decreased, while antifungal, and color properties were increased in wood impregnated with honeybee wax.
2. Scanning electron microscopy images indicated that beeswax-impregnated samples were more durable against fungi decay compared to un-impregnated control samples.
3. Deep beeswax treatment showed a larvicidal effect on *H. bajulus*.
4. The surface hardness of the poplar and lime wood species (low-density woods) increased after beeswax impregnation.
5. Fungal exposure negatively affected surface hardness of the beeswax-impregnated wood.

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