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Research Article
Evaluation of Hydrophobic-hydrophilic Properties and Anti-adhesive Potential of the Treated Cedar Wood by Two Essential Oil Components Against Bioadhesion of *Penicillium expansum* Spores

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Abstract
Objective: The important role of the physicochemical parameters in the phenomenon of microorganisms adhesion on materials is very well established in the scientific literature. The objectives of this study were to investigate the treatment impact of two compounds on the physicochemical surface properties of cedar wood and their anti-adhesive effects against the of *Penicillium expansum* spores. **Methodology:** The physicochemical characterization, in term of hydrophobicity/hydrophilicity and electron acceptor/donor properties of the cedar samples, before and after treatment for 15 min by 1.8-cineol and β-ionone, was carried out by the contact angle measurements throughout the sessile drop technique. The anti-adhesive potential of these essential oil components, vis-a-vis of *P. expansum* spores on the cedar surface, was also evaluated, after 10 h of contact, with the Environmental Scanning Electron Microscopy (ESEM) and by using MATLAB software program. **Results:** The obtained results revealed that the impact of the treatments on the cedar surface were very indicative. In fact, the initially hydrophobic character of the cedar surface ($\theta w = 89\pm0.12°$; $\Delta Giwi = -67.93$ mJ m^{-2}) was significantly decreased on the samples treated with β-ionone ($\theta w = 46.5\pm0.4°$; $\Delta Giwi = -7.52$ mJ m^{-2}). The treatment with 1.8-cineol made the cedar surface qualitatively and quantitatively hydrophilic ($\theta w = 39.9\pm0.6°$; $\Delta Giwi = 8.35$ mJ m^{-2}). Furthermore, the ESEM images analyzed by the MATLAB software program showed a very important reduction of spores adhesion rates on the treated surface of cedar wood with β-ionone (21.84%) and 1.8-cineol (9.34%). **Conclusion:** It therefore, appears very clearly in this study that the physicochemical properties of cedar wood were significantly influenced by both treatments and their anti-adhesive potential against *Penicillium expansum* spores was demonstrated.

Key words: Cedar wood, essential oil components, hydrophobicity, anti-adhesive activity, *Penicillium expansum* spores, environmental scanning electron microscopy

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Wood is a very appreciated and used material in several fields diverse and varied. However, its heterogeneous chemical composition and its important hygroscopic nature make it vulnerable to microbial biodegradation[1,2]. The vulnerability of wooden materials vis-a-vis of fungi depends on both of their structure and chemical composition than of the fungal species belonging to various taxonomic groups[3-6].

Under certain conditions of temperature and humidity, the bioadhesion of microorganisms, their growth and formation of biofilms on the lignocellulosic materials lead to huge damage by weakening the structure and by affecting the esthetic quality of the wood[6-10]. Although, several studies have been reported on the study of wood decay by molds as well as the damages caused by the latter, very little study has focused on the initial adhesion of spores of these fungi on wooden materials[11-13].

The bioadhesion, first step of the phenomenon of biofilm formation on materials, is governed by physicochemical surfaces properties described by the fundamental theory of thermodynamic and well established in the scientific literature[14-18]. During this step, the Lifshit Van der Waals forces, acid/base of Lewis and electrostatic interactions influence considerably the initial approach and the attachment of microorganisms, including spores to materials.

Several methods such as plasma[19,20], heat treatment[21], copper amine[22], plant triglycerides[23], fatty acids[24], as well as plant extracts[25] are used for wooden surface treatments. However, although recently some studies have reported the efficiency of essential oils (that have a very significant antibacterial and antifungal potential on both planktonic[26-28] and sessile[29-31] forms of microorganisms) in wood preservation, no studies to our knowledge have been investigated on the anti-adhesive potential of the major components of essential oils.

Thus, the present study reports the impact of two major components of essential oils on the physicochemical surface properties of cedar wood in term of hydrophobicity/hydrophilicity (the wetting behavior and interfacial free energy). In addition, an environmental scanning electron microscope analysis was also performed to investigate the anti-adhesive effect of these compounds on the spores of *Penicillium expansum*.

The aim of this study was to contribute to the preservation of cedar wood, which is widely used in the monuments of the medina of Fez and to evaluate the anti-adhesive effect of β-ionone and 1.8-cineol for an application as anti-adhesive and biocide agents against the biodegradation of cedar wood caused by mold fungi.

MATERIALS AND METHODS

Preparation of the cedar wood surface: The substratum of this study was the cedar wood (*Cedrus atlantica*), it is widely used in the construction of houses in the old medina of Fez (Morocco). Each cedar wood sample has a length of 3 cm, a thickness of 0.4 cm and a width of 1 cm. The roughness of all wood specimens was set at 1 μm using a rugosimeter (Model: SJ-301, 2011, Mitutoyo, Japan). Then, each sample was washed 6 times with distilled water before being autoclaved at 120°C for 15 min.

Chemicals: The effect of essential oil components on the physicochemical surface properties and their anti-adhesive activity on cedar wood was evaluated using 1.8-cineol (99%) and β-ionone (pure $\geq$95%) purchased from sigma-aldrich.

Cedar wood surface treatment: On the surface of cedar wood samples, prepared, such as mentioned above, a volume of 10 μL of essential oil components was deposited for 15 min. After a good drying and adsorption of the tested essential oil components (1.8-cineol and β-ionone) on the cedar samples surfaces, at room temperature, the contact angle measurements were directly performed on the samples.

Contact angle measurements and calculation of the interfacial free energy: The physicochemical properties of the cedar wood surface were characterized by the contact angles measurements through the sessile drop technique using a goniometer apparatus[32-34]. The initial contact angle of each liquid was measured after drop stabilization on the solid sample surfaces. For the determination of the interfacial free energy of the solid surface (treated and untreated samples), three liquids are recommended[35]. They consist of two polar liquids (water and formamide) and one apolar liquid (diiodomethane) with known surface tension characteristics (Table 1). Therefore, contact angles measurments on each wood samples were made using these pure liquids. Then, all parameters of the surface physico-chemical characteristics (the Lifshitz-Van Der Waals component (γ^{LW}), the electron donor or Lewis base (γ^{-}) and the electron acceptor or Lewis

Table 1: Surface tension properties of pure liquid used to measure contact angles[36]

	Surface energy parameters (mJ m^{-2})		
Liquids	γ^{LW}	γ^{+}	γ^{-}
Water (H_2O)	21.8	25.5	25.5
Formamide (CH_3NO)	39	2.3	39.6
Diiodomethane (CH_2I_2)	50.5	0	0

acid (γ^+)) allowing to determine the surface free energy of each sample (ΔGiwi) were calculated by the Young's equation[36]:

$$\gamma_L(\text{Cos}\theta + 1) = 2(\gamma_S^{LW}\gamma_L^{LW})^{1/2} + 2(\gamma_S^{+}\gamma_L^{-})^{1/2} + 2(\gamma_S^{-}\gamma_L^{+})^{1/2} \quad (1)$$

where the terms (S) and (L) denote solid surface and liquid phases, respectively.

Lewis acid-base component (γ_S^{AB}) is obtained by:

$$\gamma_S^{AB} = 2(\gamma_S^{-}\gamma_S^{+})^{1/2} \quad (2)$$

Moreover, the degree of hydrophobicity of each sample surface was evaluated by applying the approach of Van Oss[36]. According to this approach, the degree of hydrophobicity of a given material (i) Is expressed as the free energy of interaction between two entities of that material immersed in water (w): ΔGiwi. This parameter has been calculated through the surface tension components of the interacting entities, according to the following formula:

$$\Delta\text{Giwi} = -2\gamma_{iw} = -2\left[\begin{array}{l}((\gamma_i^{LW})^{1/2} - (\gamma_w^{LW})^{1/2})^2 + \\ 2\left((\gamma_i^{+}\gamma_i^{-})^{\frac{1}{2}} + (\gamma_w^{+}\gamma_w^{-})^{\frac{1}{2}} - (\gamma_i^{+}\gamma_w^{-})^{\frac{1}{2}} - (\gamma_w^{+}\gamma_i^{-})^{\frac{1}{2}}\right)\end{array}\right] \quad (3)$$

The values of the surface tension parameters for the three pure liquids used in this study are shown in Table 1.

Microorganism, growth conditions and harvesting spores: *Penicillium expansum* was isolated from cedar wood decay and identified in our laboratory[37]. Growth was obtained at 25°C using Malt Extract Agar. After 7 days of incubation, the spores of *P. expansum* were then harvested by scraping the culture surface in KNO_3 (0.1 M). The spore suspension was concentrated by centrifugation at 10,000 g for 15 min at 4°C until a concentration of 10^7–10^8 spores mL^{-1} (counted with a hemacytometer).

Antiadhesion essay and environmental scanning electron microscopy (ESEM) analysis: After the treatment of the cedar wood samples by the studied essential oils components (1.8-cineol and β-ionone), the samples (untreated and treated) were immersed in a *Penicillium expansum* spore suspension. Then after 10 h of incubation at 25°C, the samples were rinsed with distilled water to remove the spores, which had not adhered. The samples were placed in sterile petri dishes and sent for an analysis by the ESEM, a Quanta 200 model with a tungsten filament.

RESULTS AND DISCUSSION

The physicochemical characterization of the cedar wood surface untreated and treated by the essential oil components, 1.8-cineol and β-ionone, has been carried out by the sessile drop technique. Hydrophobicity, surface energy as well as the electron donor/acceptor characters were evaluated with the contact angle data and calculations were done using the approach of Van Oss *et al.*[38].

According to Van Oss *et al.*[38] and Vogler[39] when the value of the water contact angle exceeds 65°, the surfaces are characterized as hydrophobic and hydrophilic when inversely the value of the contact angle of water is less than 65°. Moreover a positive value of the surface free energy (ΔGiwi) means that the surface is hydrophilic and a negative value indicates that it is hydrophobic.

The surface free energy gives a quantitative indication of the hydrophobicity of the substrate surface, while the contact angle with water permits a qualitative assessment of hydrophobicity.

Cedar wood surface wettability behavior before and after treatment: Table 2 shows the averaged results of contact angles measurements with the three pure liquids on the untreated samples surfaces (control) of the cedar wood and those treated for 15 min by β-ionone and 1.8-cineol along with their standard deviations.

Thus, as it can be seen in Table 2, the mean value of the contact angle between the drop of water and the untreated cedar sample was θw = 89±0.12°. This value reflects a strong qualitative hydrophobicity (contact angle >65°) of the initial state of the cedar surface.

The liquid contact angles on the wooden surfaces, in particular with water which were reported in the previous studies in the literature were spread over a wide range of values. Indeed, they may vary from 54.5° on the surface of beech wood[21] to 81° on the surface of oak[40], or from 18° on the teak[40] compared to 86°on the cedar wood[25]. The latter value is also close to that found in this study and also reflects the hydrophobic character of the untreated cedar sample surfaces.

Table 2: Contact angle measurements on cedar wood surface before and after treatment with β-ionone and 1.8-cineol

	Liquids contact angles (θ°)		
Cedar wood	Water	Formamide	Diiodomethane
Untreated	89.0±0.12	38.5±1.0	28.9±0.7
Treated with β-ionone	46.5±0.40	28.0±1.2	13.9±0.6
Treated with 1.8-cineol	39.9±0.60	28.8±1.8	21.6±0.8

Thus, in the light of these contact angle values reported in literature, this large variation of angles could be imputed to the types of species. Depending on the species, the angles formed by the drops of water with each of these wooden surfaces were very low and therefore qualitatively hydrophilic, or very high and thus qualitatively hydrophobic.

However, for the samples treated with the β-ionone and 1.8-cineol, a very significant decrease of the water contact angle values compared to the control samples with $\theta w = 46.5°$ and $\theta w = 39.9°$, respectively was noticed. This clearly indicates a hydrophilic character of these treated sample surfaces by the both essential oils components. The treatment with 1.8-cineol showed a lower value and therefore more hydrophilic than by the β-ionone.

Impact of the essential oil components on the cedar wood interfacial free energy: The values of the surface tensions characteristics obtained by means of the contact angles of the three liquids are given in Table 3.

On the one hand, the values of γ^{LW} were increased slightly and those of γ^{+} were almost zero for the treated samples compared to controls, but on the other hand, there was an important and very significant increase of the γ^{-} values.

Among the intermediate parameters in the calculation of the free energy of interaction, the surface tension γ^{-} (or Lewis base parameter) also allows to assess the hydrophobicity of a surface. Indeed, when the value of γ^{-} exceeds 27.9 mJ m^{-2}, the surface is characterized as hydrophilic and inversely, the surface is hydrophobic when the value is less than[35] 27.9 mJ m^{-2}.

On this basis, it can be clearly seen (Table 3) that the surface treated by the β-ionone remains hydrophobic (γ^{-} = 27.52 mJ m^{-2}), while the second treatment makes the surface hydrophilic (γ^{-} = 36.31 mJ m^{-2}).

The calculation results of the free energy of interaction are reported in Table 4.

Table 3: Surface tension parameters (Lifshit Van Der Waals (γ^{LW}) and the Lewis acid (γ^{+}) and base (γ^{-}) parameters) of the cedar samples before and after treatments

	Surface tension components (mJ m^{-2})		
Cedar wood	γ^{LW}	γ^{+}	γ^{-}
Untreated	44.55±0.3	3.03±0.2	0.28±0.06
Treated with β-ionone	49.22±0.3	0.24±0.06	27.52±0.41
Treated with 1.8-cineol	47.18±0.2	0.18±0.08	36.31±1.88

Table 4: Interfacial free energies of the untreated and treated wood by essential oil components

Cedar wood	ΔGiwi (mJ m^{-2})
Untreated	-67.93
Treated with β-ionone	-7.52
Treated with 1.8-cineol	8.35

The observed results in Table 4 revealed that the initial surface properties of the cedar were significantly modified after the treatments. The value of the surface free energy obtained for the untreated cedar wood was negative (ΔGiwi = -67.93 mJ m^{-2}), in fact, this negative value of ΔGiwi means that this wood surface have less affinity for water. This reflects the hydrophobic character of the untreated cedar surface (Table 4).

If the analysis of the water contact angle value on the treated surface by the β-ionone also allowed a hydrophilic assessment of the surface, it is especially the ΔGiwi which is decisive for the hydrophobic/hydrophilic characterization of a surface.

However, although the treatment by β-ionone always maintains the hydrophobic character of the samples surface (ΔGiwi = -7.52 mJ m^{-2}), the comparison of the results of ΔGiwi between the control samples and those treated by β-ionone showed that this treatment contributed to reduce considerably the hydrophobicity of this surface. Indeed, more the value of ΔGiwi is negative and more the degree of hydrophobicity becomes important.

Unlike the wetting behavior observed above for the both treatments with the water contact angles which were very significantly below the limit of 65°, the value of ΔGiwi in the case of the samples treated with 1.8-cineol was positive (ΔGiwi = 8.35 mJ m^{-2}).

In comparison with other studies reported in the literature, the wetting behavior and surface free energy of wooden materials are more or less affected according to the types of treatments, the species of wood and the estimation methods.

It had already reported the initial hydrophobic character of the cedar surface in previous study[18], where on the one hand, the important modification of the physical-chemistry of cedar surface after the single and combined adhesion of spore of two *Penicillium* species on cedar had seen. On the other hand, the treatment of cedar surface with *Thymus vulgaris* extracts obtained by maceration and sonication[25] also induced a very significant modification of the initial physicochemical properties. Indeed, the hydrophobic cedar wood became very hydrophilic with values of ($\theta w = 29.7°$; ΔGiwi = 17.78 mJ m^{-2}) and ($\theta w = 18.2°$; ΔGiwi = 30.62 mJ m^{-2}) for the treated samples with the extracts obtained, respectively by maceration and ultrasound.

Several studies showed that the heat treatment contributes to reduce significantly the wettability of the wooden surfaces. Indeed, Gerardin *et al.*[21] reported that the value of the contact angle with water increased considerably on the surface of the pine wood after the heat treatment (from 55.4-81.3° after heat treatment). Similarly, the surface of beech wood, naturally hydrophilic, became suddenly very hydrophobic after the heat treatment at a temperatures between 130 and 160°C (from 0-90° after heat treatment)[41].

Poaty *et al.*[42] also reported similar results compared to black spruce wood after treatment in 50% Ar–50% CF_4 plasmas. Indeed, the results that they found showed a progressive increase of the contact angle on the surface of the black spruce wood according to the exposure time to the plasma treatment. Thus, the water contact angle values increased from 80° (for untreated wood) to 109.5 and 128.8°, respectively, after 5 and 45 min of exposure to the plasma at a distance of 7.8 cm which resulted in the strengthening of the hydrophobicity of black spruce wood.

Anti-adhesive activities of *Cedrus atlantica* wood treated with the essential oil components: The images of Fig. 1 show the results of the achieved anti-adhesion test. The analysis of the samples by ESEM allowed an assessment in terms of spores adhesion on the surface of the treated and untreated cedar samples.

Thus, the analysis of the adhesive behavior as well as the adhesion rate of the spores on the various samples allowed to observe a very strong adhesion on the untreated wood (control) (Fig. 1a). Moreover, looking more closely, the *P. expansum* spores are arranged in clusters throughout the occupied surface of the sample: Reflecting the ability of this strain to adhere on cedar wood surface. This finding is in agreement with those found by El Abed *et al.*[43] and Moulay *et al.*[44], which have both studied on the same support and with fungal spores.

The comparison with the images of Fig. 1b and c, corresponding to the samples treated with β-ionone and 1.8-cineole, respectively, showed clearly a significant difference with the control. Indeed, a very low spore adhesion was observed for the both treatments. However, the adhesive behavior of spores that have adhered on both surfaces differs depending on the used compounds. On one side (Fig. 1b), the spores were totally dispersed over the entire surface and appeared largely individually. But on the other side (Fig. 1c), spores were more grouped than in Fig. 1b.

The percentage of the cedar surface covered by the *P. expansum* spores, depending on treatments, was evaluated by image analysis using the MATLAB software program.

As shown in Fig. 1d, the spore adhesion rates were 56.22% for the untreated sample and 21.84 and 9.34% for those treated with β-ionone and the 1.8-cineol, respectively.

The adhesion of filamentous fungi on material surfaces is usually explained in the literature by their genetic potential

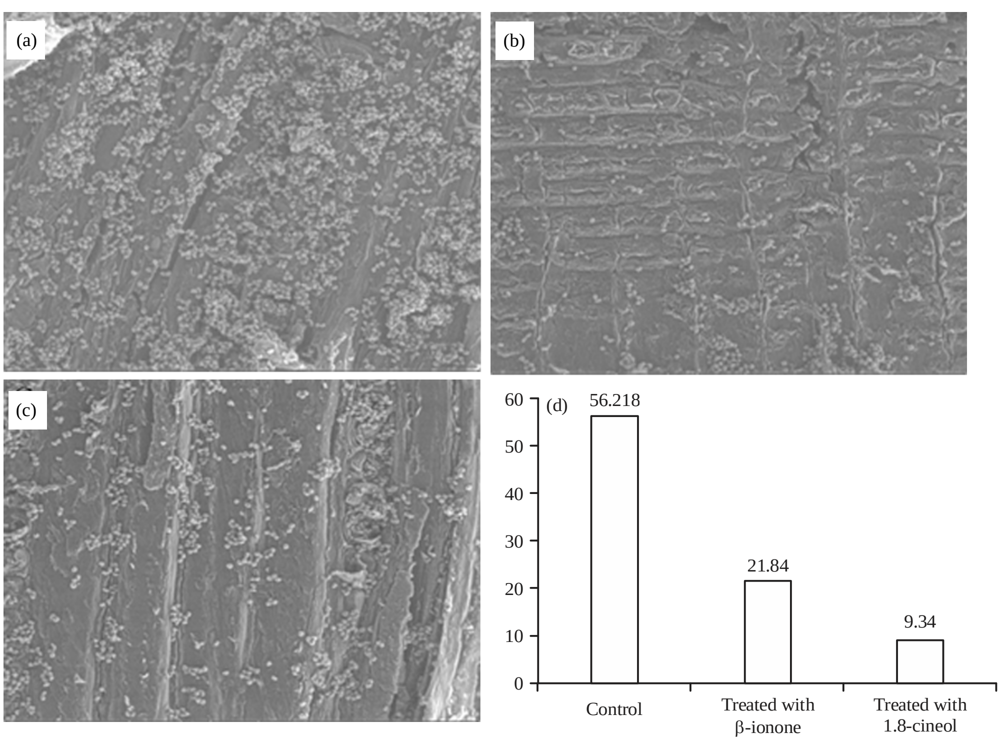

Fig. 1(a-d): Electro micrographs of (a) cedar wood surface before, (b) after treatment with β-ionone, (c) 1.8-cineol and (d) the spore adhesion rates and the involvement of proteins called hydrophobins, which allow fungi to attach on materials and to cross this 1st step leading to the biofilm formation.

However, the importance of the physicochemical properties, in the adhesion phenomenon is very clearly established in the scientific literature. In addition, it is also important to note that these forces, described by the thermodynamic approach are involved in this 1st phase and may be repulsive or attractive before the attachment of microorganisms to the surfaces.

The very important inhibition observed for the spore adhesion on cedar could be explained by the interaction of the physicochemical properties between the two surfaces: those of spores and materials (for both treatments). It is also generally admitted that the hydrophilic interactions are repulsive, while those hydrophobics are attractives. On this basis, the results obtained in this study, in relation to the adhesion rate of the spores, are understandable according to treatments. Indeed, the spores of *P. expansum* being hydrophilic and thus electron donor[13], their adhesion on a very hydrophobic cedar surface and more acceptor than electron donor was quite expected. As well as hydrophilic-hydrophilic interactions between a cedar surface (made hydrophilic by the 1.8-cineol) and that of the spores was found repulsive and has considerably decreased the adhesion. The sharp decrease of the cedar wood hydrophobicity following the treatment with β-ionone could explain its result compared to the adhesion rate.

On the other hand, it is also very important to consider the bioactive potential of essential oils in general and of major components used in this study. Several studies have shown their antimicrobial activities[45,46] and even their use in preservation of lignocellulosic materials against the biodeterioration by the microorganisms[47].

Salem *et al.*[47] reported a good essential oil activity extracted from the leaves of *Eucalyptus camaldulensis* against the growth of 5 fungi (*Alternaria alternata*, *Fusarium subglutinans*, *Chaetomium globosum*, *Niger Aspergillus* and *Trichoderma viride*) involved in the biodeterioration of three commercial wood (*Pinus sylvestris*, *Pinus rigida* and *Fagus sylvatica*). The major component of their essential oil was eucalyptol (1.8-cineol) with a proportion of 60.32%.

Similar results already shown in recent study, in terms of anti-adhesion against fungal spores on cedar. This previous study showed that the anti-adhesive effect of the *Myrtus communis* extract obtained by sonication was best at a concentration[44] of 20-5 mg mL^{-1}. But, the spores inhibition rates on the cedar surface sample are significantly more interesting in the present study.

CONCLUSION

The wettability behavior and the surface free energy of the cedar wood samples surfaces, before and after treatment by the two essential oil components, were evaluated, in this study, by the contact angle method and the Van Oss and Vogler approaches. The results showed that the physicochemical properties of the cedar wood surface were considerably modified in terms of hydrophobicity. In this study, degree of modification depends on the used molecules. Furthermore, the treatment of wood by these essential oil components contributed significantly to reduce the adhesion rate of *Penicillium expansum* spores on the cedar surface. The choice of the mold spore form in this study is very important and contributes to preventive fight against the biodeterioration of wooden materials. Finally, 1.8-cineol and β-ionone can be considered for an anti-adhesive and antifungal application against the biodegradation of the wood by molds.

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