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Anti-adhesive effect of carvacrol on surface treatment of *Cedrusatlantica* wood against fungal spore

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Abstract

The physicochemical properties of the cedar wood surface was investigated before and after bio-adhesion of *Thielaviahyalocarpa* spore by the contact angle method and the environmental scanning electron microscopy (ESEM) was used to evaluate the ability of the spores adhesion as well as the antiadhesive potential of the carvacrol molecule. The obtained results showed the initial hydrophobic character of untreated cedar samples ($\theta w = 72.87°$; $\Delta Giwi = -62.49$ mJ/m^2) with low electron donor/acceptor properties. The bio-adhesion of *T. hyalocarpa* spores had considerably modified the wood surface properties. The cedar surface became hydrophilic ($\theta w = 53.93°$; $\Delta Giwi = 2.91$ mJ/m^2) with important electron donor properties. Furthermore, the ESEM images showed the ability of *T. hyalocarpa* spores to adhere on cedar surface and an important inhibitory potential of the treated cedar surface by carvacrol against the spore bio-adhesion. Thus, the carvacrol molecule, an essential oil component, could be considered in the formulation of anti-adhesive agents for the preservation of wooden materials.

1. Introduction

The hygroscopic nature of wood and its heterogeneous and organic chemical composition make it vulnerable to biodeterioration by insects, termites and microorganisms such as fungi, which are known for their massive production of extracellular enzymes [1-2]. The spores produced by these fungi allow the survival of the species in extreme environmental conditions as well as the dispersal and growth of the species on new surfaces.

The microorganism's adhesion and the biofilm formation on wooden surfaces present an increasing interest to researchers in recent decades [3-4]. The involvement and the importance of physicochemical properties during the phenomenon of microbial adhesion on the material surfaces is well established in the scientific literature [5-10]. These physicochemical properties govern the microbial adhesion process and involve three types of interactions: those Lifshitz-van der Waals, acid/base of Lewis and electrostatics.

The wood, an appreciated and widely used material, is subjected to different types of treatments to preserve it from microbial biodeterioration among others.

Several promising techniques have been investigated for the preservation of wood in recent years. In fact, among these methods, the heat treatment is a technique used for several decades and results in a modification of surface properties of the heat treated wood [11-12]. Furthermore, the impregnation of the wood cell walls by nano-compounds is also a promising technique used for wood's preservation in order to improve its durability [13-14]. However, although some works have recently reported the use of essential oils and plant extracts [15], none to our knowledge, has shown the potential of the essential oils major compounds as anti-adhesive agents against the fungal spores adhesion on materials.

Thus, the present study investigated the cedar wood surface properties in terms of wetting behavior, electron acceptor-donor properties and interfacial free energy. Then, the bio-adhesion impactof *Thielaviahyalocarpa* spores on the cedar surface properties was evaluated. In addition, the inhibitory potential of the treated cedar wood surface by carvacrol, an essential oil's major component of medicinal and aromatic plants, was also investigated against the adhesion of *T. hyalocarpa* spores.

2. Material and Methods

2.1. Fugal Strain, Growth Conditions and Harvesting Spores

The studied fungal strain, *Thielaviahyalocarpa,* was isolated from cedar wood decay and identified in our laboratory [16]. This strain was cultured at 25°C on Malt extract agar. Then, the spores were harvested by scraping a 7 days fungal culture surface in KNO_3 solution (0.1M). The obtained spore suspension was concentrated by centrifugation at 10,000g for 15min at 4°C until a concentration of 10^7-10^8 spores/ml (counted with a hemacytometer).

2.2. Cedar Wood Samples: Preparation and Cleaning

The substratum used in this study was the cedar wood (*Cedrusatlantica*) which is widely used in the construction of houses in the old medina of Fez. The cedar wood was cut into pieces which had the following dimensions: length = 3cm, thickness = 0.4cm and width = 1cm. The roughness of the wood pieces was set to 0.8µm by using a rugosimeter (Model: SJ-301; 2011; Mitutoyo; Japan). Then, each cedar sample was washed six times with distilled water then autoclaved at 120°C for 15 min.

2.3. Impact of the Fungal Spores Bio-adhesion on the Cedar Surface Properties

The untreated wood samples, prepared such as mentioned above, were immersed in 10ml of the spore suspension. The spore bio-adhesion on the sample surfaces was carried out by sedimentation [4; 17-18]. After 10h of incubation at 25°C, non adhered spores to cedar surface were rinsed three times with sterile distilled water, by moving slowly in a Petri dish [19-20]. Then, the sampleswere left for drying overnight at room temperature in sterile Petri dish. The contact angles measurements were performed on the wood samples with the three liquids (Water, Formamide and Diiodomethane). Each sample was repeated three times.

2.4. Contact Angle Measurements and Calculation of Surface Free Energy and Surface Tension Components

The physicochemical properties of the cedar wood surface were characterized by the contact angles measurements through the sessile drop technique using a goniometer apparatus (GBX Instruments, France) [21]. The initial contact angle of each liquid was measured after drop stabilization on the solid sample surfaces. To determine the interfacial free energy of the solid surface (treated and untreated samples), three liquids are recommended [22]. They consist of two polar liquids (Water and Formamide) and one apolar liquid (Diiodomethane) with known surface tension characteristics (Table 1). Therefore, contact angles measurements on each wood sample 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 acid (γ^+)) allowing to determine the surface free energy of each sample (ΔGiwi) were calculated by the Young's equation [23-24]:

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

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

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

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

Moreover, the degree of hydrophobicity of each sample surface was evaluatedby applying the van Oss approach [24]. 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:

$$\Delta Giwi = -2\gamma_{iw} = -2\left[((\gamma_i^{LW})^{1/2} - (\gamma_w^{LW})^{1/2})^2 + 2\left((\gamma_i^+\gamma_i^-)^{\frac{1}{2}} + (\gamma_w^+\gamma_w^-)^{\frac{1}{2}} - (\gamma_i^+\gamma_w^-)^{\frac{1}{2}} - (\gamma_w^+\gamma_i^-)^{\frac{1}{2}}\right)\right] \qquad (3)$$

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

2.5. Essential Oil Component

The molecule used in the present study for the cedar wood surface treatment in order to inhibit the spores bio-adhesion of *T. hyalocarpa* strain was carvacrol (pure ≥ 99%), an essential oil component. In fact, it is the major component of Thyme and Oregon essential oils. The molecule was purchased from Sigma-Aldrich.

Table 1: Surface tension properties of pure liquid used to measure contact angles [24].

Liquids	Surface Tension components (mJ m^{-2})		
	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

2.6. Cedar Wood Surface Treatment

On the surface of cedar wood samples, prepared such as mentioned above, a volume of 10µl of carvacrolwas deposited for 15min. After a good drying and adsorption of the tested essential oil component at room temperature, the cedar samples (treated and untreated) were immersed in the concentrated spore suspension and incubated for 10h at 25°C. Then, after the incubation time, the immersed samples were removed and rinsed three times with sterile distilled water, as mentioned previously, and were left for drying overnight at room temperature. The untreated and treated samples were send for ESEM analysis.

2.7. Environmental Scanning Electron Microscopy Analysis

The untreated and treated cedar samples were imaged, after the bio-adhesion of *T. hyalocarpa* spores, by using environmental scanning electron microscopy (ESEM) a Quanta 200 model equipped with a tungsten filament (FEI Company, US). All samples were observed and their analysis provided valuable information on the spore adhesion of the studied strain and on the anti-adhesive potential of the treated cedar surface with carvacrol.

3. Results and discussion

3.1. *Physicochemical Characterization of Cedar Wood Surface*

The study of physico-chemical surfaces properties, in terms of hydrophobic-hydrophilic characteristics and electron donor-acceptor properties, have already been reported in several works in the scientific literature [5-10]. Indeed, van Oss et al. [23] and Vogler [25] suggested that the plane surface of a solid material can be defined as hydrophilic or hydrophobic according to his wetting behavior *vis-à-vis* of water as well as its interfacial free energy of interaction. In fact, when the water contact angle (WCA) value, on a material's plane surface, is greater than 65°, the material surface is qualitatively characterized as hydrophobic. Inversely, it is defined as hydrophilic when the WCA value is less than 65°. Furthermore, the degree of hydrophobicity of materials is naturally linked to the magnitude of the interfacial free energy. Thus, depending on whether the interfacial free energy is positive or negative, the surface of the material is quantitatively characterized as being hydrophilic or hydrophobic, respectively [23].

The averaged values of the contact angle measures, performed with the three liquids on the surfaces of cedar wood samples, before and after bio-adhesion of the *T. hyalocarpa* spores, as well as the interfacial free energy of interactionare reported in Table 2. The surface tension components (Lifshitz-van der Waals forces, and the electron donor-acceptor properties) of these samples are also reported (Table 3).

Table 2: Contact angle (θ) measurements and surface free energy (ΔGiwi) of cedar wood surface before and after bio-adhesion of *T. hyalocarpa*spores

Samples	Contact angles (°)			ΔGiwi (mJ m^{-2})
	θ_w	θ_F	θ_D	
Untreatedwood (0h)	72.87±0.64	35.6±1.14	12.47±0.15	-62.49
*T. hyalocarpa*spores	41.90±0.63	45.10±0.19	55.00±0.55	26.86
Wood surface after spore adhesion (10h)	53.93±0.67	49.9±0.89	23.63±0.15	2.91±0.57

Thus, as can be seen from this table, the WCA value on the cedar surface was 72.87° (Table 2). This reflects the low affinity of water molecules with the untreated cedar wood surface. The wetting behavior of material surfaces *vis-à-vis* of water is defined according to the degree of affinity of their constituent molecules with those

of water. Thus, according to the measured value of the WCA on the cedar surface, the latter could be qualitatively considered as hydrophobic (θw> 65°).

Table 3: The Lifshitz–van der Waals (γ^{LW}), the electron donor (γ^{-}) and electron acceptor (γ^{+}) properties of the *T. hyalocarpa* spore surface and the cedar wood surface (before and after spore bioadhesion)

Samples	Surface Tension components (mJ m^{-2})		
	γ^{LW}	γ^{+}	γ^{-}
Untreatedwood (0h)	49.51±0.03	0.88±0.09	3.75±0.15
*T. hyalocarpa*spores	31.50±0.31	0.50±0.03	44.90±0.68
After spore adhesion (10h)	46.53±0.05	0.29±0.05	32.81±0.34

Furthermore, the obtained results for the untreated cedar samples showed very low values for the polar surface tension components. Indeed, the electron acceptor properties (or Lewis acid) were about 0.88 mJ/m^2, while those of electron donor (or Lewis base) were 3.75 mJ/m^2 (Table 3). Thus, although the Lewis acid/base properties were low, the surface cedar was more electron donor than acceptor.

The negative value of the interfacial free energy (ΔGiwi = -62.49 mJ/m^2 < 0) obtained for the untreated wood was characteristic of hydrophobic surfaces (Table 2).

The results found in this study are consistent with those reported in our previous works on the physicochemical surface properties of cedar wood [4; 26-27]. Indeed, the hydrophobic character of the wood (both qualitatively, through the WCA value on cedar samples, and quantitatively, through the magnitude of ΔGiwi) has already been found with values of (θw = 89±0.12°; ΔGiwi = -67.93 mJ/m^2) [26], or (θw = 74.7±0.78°; ΔGiwi = -61.8 ± 0.98 mJ/m^2) [27].

Unlike to the obtained results in the present work for cedar wood, Gérardin et al. [28] have reported hydrophilic surface properties of the untreated Beech (θw = 54.5°; ΔGiwi = 58.6 mJ/m^2) and Pine woods (θw = 55.4°; ΔGiwi = 54.8 mJ/m^2). Similar results were also reported for Teak, Mango and Pine woods with WCA values of 18; 50 and 46°, respectively [29]. However, the authors of these works had also found that the surfaces of the wooden species of Ash, Oak and Cloves have formed very high WCA about68; 81 and 91°, respectively [29].

Several parameters such as hygroscopicity, porosity, grain size, heterogeneous chemical composition, age and species could influence the evaluation of the physicochemical surface properties of the wooden materials.

3.2. Impact of the T. hyalocarpa Spore's Bioadhesion on Cedar Wood Surface Properties

The obtained results (Table 2 and 3) revealed that the initial surface properties were significantly modified after the spore adhesion on the cedar surface. Indeed, the wetting behavior, which was initially characteristic of hydrophobic surfaces for the untreated cedar, became hydrophilic after the bio-adhesion of *T. hyalocarpa* spores. This is revealed by a WCA value in the order of θw = 53.93° (Table 2).

It can also be noted (in Table 3) that the surface tensions components of Lifshitz-van der Waals and Lewis acid (γ^{LW} = 49.51 mJ/m^2; γ^{+} = 0.88 mJ/m^2) of the cedar surface decreased after the bio-adhesion of spores (γ^{LW} = 46.53 mJ/m^2; γ^{+} = 0.29 mJ/m^2). On the other hand, the spore adhesion increased the electron donor property of the cedar surface. In fact, γ^{-} increased from 3.75 mJ/m^2 (for the control) to 32.81 mJ/m^2 after the spore bio-adhesion (Table 3). Thus, the treated sample by spores became almost monopolar basic. In the light of these results, it can be seen that the surface tension value of γ^{-} exceeds 27.9 mJ/m^2. This is characteristic of hydrophilic surfaces and confirms the results found from the WCA after the bio-adhesion of *T. hyalocarpa* spore on cedar samples. Furthermore, the positive value of the interfacial free energy (ΔGiwi = 2.91 mJ/m^2) confirmed the last conclusion on the hydrophilic property of the cedar surface after the bio-adhesion.

The impact of the bio-adhesion of fungal spores, especially those of *Penicilliumexpansum* and *Penicilliumcommun*, on the cedar wood surface properties was investigated previously [17-18].

Similar findings of microbial adhesion impact on the material surface properties have already been reported in the scientific literature. Indeed, Ksontini et al. [30] have shown in their work that the bio-adhesion of *Bacillus cereus*, with hydrophilic surface properties (θw = 52.6°; ΔGiwi = 3.37 mJ/m^2), significantly decreased the initially very hydrophobic property of the silicone surface (θw = 99.3°; ΔGiwi = -68.28 mJ/m^2) just after 3 hours

of adhesion (θw = 68.9°; ΔGiwi = -18.3 mJ/m^2). The authors also found that the silicone surface became hydrophilic after 7h of contact with *B. cereus* (θw = 58°; ΔGiwi = 2.34 mJ/m^2). And after 24h, the bio-adhesion of *B. cereus* has led to a strengthening of the hydrophilicity on the silicone surface (θw = 47.3°; ΔGiwi = 23.7 mJ/m^2).

The surface properties of another material were also significantly modified after the bio-adhesion of this same *B. cereus* strain [31]. In fact, they showed that the stainless steel had lost its initial hydrophobic properties (θw = 125.9°; ΔGiwi = -57.6 mJ/m^2) just after 2 hours of contact with *B. cereus* (θw = 41.3°; ΔGiwi = 39.6 mJ/m^2).

On the other hand, the bio-adhesion of the *T. hyalocarpa* spores on the cedar surface also induced a strong increase of the electron donor property. The involvement of the latter in the microbial bio-adhesion phenomenon on substrates is well known in the scientific literature. Indeed, several authors have reported the important role of the Lewis acid/base properties in the adhesion phenomenon of microorganisms on materials [31-34].

Contrary to the Lifshitz-van der Waals interactions, the contribution of the acid-base interactions to the interfacial free energy can be attractive, repulsive or zero. Furthermore, the contribution of the polar interactions (acid-base) is the most important in the evaluation of the free energy of interaction [24].

Bernardes et al.[35]and Ksontini et al.[31]also found an increase in the electron donor character due to the microbial adhesion on the surfaces of their materials.

The physicochemical properties of micro-organisms depend on the chemical composition and the morphology of their surfaces. But they are also influenced by different physical parameters of their environment such as temperature[36] and pH [37], but also by the nature of the available nutrients[38-39]and their growth phase [39].

Indeed, the Lewis base component (γ^-) of the surface is often due to the presence of different chemical groups to the surface of these microorganisms, and which are mostly negatively charged, such as carboxylate (COO^-), phosphate (PO_4^{-3}) or SO_3 groups [40-41]. These chemical groups belong to the main macromolecules which constitute the external membranes of microorganisms (lipopolysaccharides, lipoproteins and phospholipids).

Therefore, we could suggest that the important increase in the electron donor property, found in the present study, would be due to the negatively charged molecules of the surface of *T. hyalocarpa* spores after their bio-adhesion on cedar surface. In addition, several authors have reported the production of hydrophobic proteins called hydrophobins by filamentous fungi (Ascomycetes and Basidiomycetes) [4;42]. These proteins are localized on the aerial structures of hyphae and spores [42-43] and are involved in their attachment to the hydrophobic surfaces [43-44].

3.3. Anti-adhesive Potential of the Carvacrol on Cedar Wood Surface Against T. hyalocarpa Spores

The analysis of ESEM electromicrographs allowed to observe the bio-adhesion of *T. hyalocarpa* spores on the untreated cedar surface (Fig. 1A) and the anti-adhesive potential of the treated cedar wood surface by carvacrol, an essential oil compound, against the bio-adhesion of the studied spore (Fig. 1B).

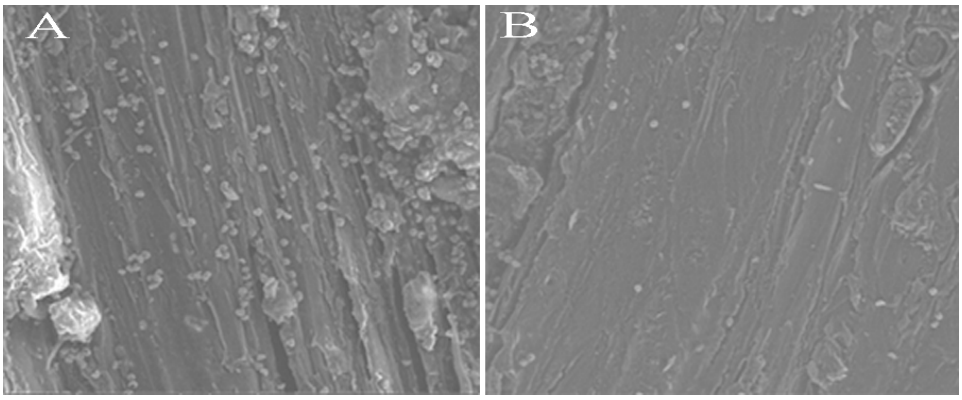

Figure 1: electro-micrographs of the bio-adhesion of *T. hyalocarpa* spores on the cedar wood surface before (A) and after (B) treatment with carvacrol.

Indeed, as it can be seen, there was an important adhesion of *T. hyalocarpa* spores on the untreated cedar surface (Fig. 1A). This reflects the ability of spores of this strain to adhere to the cedar wood. The spores are dispersed over the entire surface of the sample and some are grouped in the parts where the roughness is most

important.Similar results, on the adhesion of spores of *Aspergillusniger* and *Penicilliumexpansum* on the same substrate, were reported by El Abed et al. [4]. The authors had suggested the involvement of the Lewis acid-base interactions in the adhesion of the spores of their two strains which were also showed to have strong electron donor properties of their surfaces. The adhesion rates of their spores on cedar surface varied according to strains (62% for *A. niger* spores and only 30% for those of *P. expansum*) [4].

Moreover, the cedar surface treatment by carvacrol had an important anti-adhesive effect against the *T. hyalocarpa* spores. Indeed, unlike to the control sample, the second ESEM image showed only few spores on the treated cedar surface (Fig. 1B). The inhibition of *T. hyalocarpa* spores was almost complete.

We have recently shown the impact of the wood treatment by carvacrol on its physico-chemical surface properties [26]. The carvacrol had modified the initial hydrophobic character of cedar surface to hydrophilic character, both qualitatively ($\theta w = 42.2° < 65°$) and quantitatively ($\Delta Giwi = 11.29$ mJ/m^2> 0), and with important electron donor properties ($\gamma - = 36.82$ mJ/m^2) [26].

Although the used compound in this study have not been subject to many investigations in terms of anti-adhesive activities, we have recently highlighted the anti-adhesive potential of two others essential oil compounds against the *Penicilliumexpansum* spores on the cedar wood surface. Indeed, the treated cedar surface by the β-Ionone showed a spore inhibition rate of 61.15% when that of the treated sample with the 1,8-cineole was 83.39% compared to the spore adhesion rate on the untreated cedar samples [20].

This modification would be due to the chemical composition of this used essential oil component. Indeed, the hydroxyl group of carvacrol is more hydrophilic due to its hydrogen bonding. Likewise, the significant increase of the electron donor property could be due to the pairs of electrons of its oxygen atom. This strengthens the γ-character of the treated cedar surface with carvacrol.

It is generally accepted that hydrophobic interactions are attractive while hydrophilic ones are repulsive. In the light of this report, we could understand the significant anti-adhesive effect, of the hydrophilic and electron donor surface, of the treated wood by carvacrol against *T. hyalocarpa* spores, which are also hydrophilic and electron donor.

However, the significant anti-adhesive potential of carvacrol, which is one of the main components of the thyme and oregon essential oils, could also be due to its antimicrobial activity. Indeed, several studies have shown the efficiency of carvacrol for the inhibition of fungal and bacterialgrowth [45-47].

Conclusion

The bio-adhesion impact of T. hyalocarpa spores on the cedar wood surface physicochemical properties was investigated in this study in terms of wetting behavior, electron donor and acceptor properties as well as interfacial free energy. The latter were considerably influenced by the spores adhesion on the untreated wood surface. The results revealed that the initially hydrophobic cedar surface became hydrophilic after the studied spores adhesion. On the other hand, the involvement of the Lewis acid/base interactions in the adhesion process was also shown through the increase of the electron donor properties. Likewise, the ability of T. hyalocarpa spores to adhere on the cedar wood surface was confirmed by the ESEM images which also showed the important inhibitory potential of the treated cedar surface by carvacrol against the bio-adhesion of T. hyalocarpa spores. This work is the first, to our knowledge, which reports the anti-adhesive activity of the carvacrol molecule against fungal spores on materials. These results could contribute to preserve the cedar wood against fungal decay. Likewise, they will also contribute to promote the use of essential oil compounds of the medicinal and aromatic plants as antifungal and anti-adhesive agents against microorganisms.

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