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THE EFFECTS OF ACQ AND WATER GLASS ON THE COLOR CHANGE AND DECAY RESISTANCE OF CARBONIZED BAMBOO

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ABSTRACT

In this study, samples of bamboo and carbonized bamboo were impregnated with alkaline copper quaternary (ACQ) and water glass, the resulting differences in color and resistance to decay by *Gloeophyllum trabeum* were evaluated. The results showed that the impregnated bamboo and carbonized bamboo greatly reduced their lightness (L^*). The red-green color index (a^*) first decreased and then increased, while the yellow-blue color index (b^*) first increased and then decreased. The total chromatic aberration (ΔE) was largest for bamboo and carbonized bamboo impregnated with ACQ and allowed to decay. Carbonized bamboo impregnated with ACQ and water glass and bamboo impregnated with ACQ reached level I (strong decay resistance). The decay resistance of bamboo and carbonized bamboo was as follows: ACQ impregnated > water glass impregnated > CK. Scanning electron microscopy further confirmed that the bamboo and carbonized bamboo were impregnated with ACQ had fewer hyphae, the maintained intact structure, and good decay resistance.

KEYWORDS: Moso bamboo, impregnation, rot fungi, colorimetry, micromorphology.

INTRODUCTION

Moso bamboo is a large woody bamboo with the highest ecological, economic, and cultural value of all bamboo types, but it is susceptible to decay by rot fungi (Xu et al. 2013a). Bamboo forests are important in southern China, covering an area of 6.16 million ha. Bamboo types are split into two different sorts, running type and clumping type. Typically Moso bamboo

(*Phyllostachys edulis*) and Lei bamboo (*Phyllostachys violascens*), accounted for 77.71% of the total area of bamboo forests in China and were estimated to contribute 75% of the total carbon captured in phytolith (PhytOC) sequestration in Chinese bamboo (Wang et al. 2013, Huang et al. 2014, Yen 2016). Moso bamboo has the highest ecological, economic, and cultural value of all bamboo types. The edible shoots of Moso bamboo are a popular delicacy, and its timber has excellent physical and mechanical properties. It is widely used in pulp and paper, musical instruments, floor decoration, furniture, charcoal and traditional food packaging materials (Jianchao et al. 2014). Carbonized bamboo is a common building material, used primarily for flooring and furniture, including cabinets. It's darker in color and softer than natural bamboo. Carbonizing turns the bamboo a carmel to dark brown color, and because the fibers themselves changed color, a surface scratch won't expose lighter material underneath it. Carbonized bamboo is considered and environmentally-friendly material - a bamboo stand matures in about 3~5 years or so and will regenerate without replanting. The color allows for more decorative possibilities and can be mixed with lighter, natural bamboo. However, the high content of carbohydrates in cells makes them vulnerable to insects and fungi, resulting in decreases in weight and fiber strength. With the continuous expansion of the development and utilization of bamboo, preservative treatments of bamboo have received increasing attention. Preservative treatment is the best way to prolong the service life of bamboo, enhance its utilization, and protect forest resources (Wei et al. 2013, Moller and Mild 2018). Although wood rot fungi cause damage to biomass materials such as wood and bamboo, there is a lack of research on color changes of bamboo and carbonized bamboo (Chuang et al. 2008, Feng et al. 2017). Color is not only one of the important surface properties of bamboo, but also one of the important indicators to characterize the visual physical quantity. Color affects customer buying behavior. Research could reveal the inherent chemical mechanisms of how wood rot fungi affect bamboo and carbonized bamboo resists decay and color change.

Many preservatives are related to environmental pollution, and some of them may be harmful to human health. For example, chromated copper arsenate (CCA) can expose the human body to arsenic, which has led to restriction of its use (Lee et al. 2001). Therefore, CCA has been largely replaced by copper-rich second-generation alkaline copper quaternary (ACQ) preservatives (Pankras et al. 2012). To achieve the decay resistance of bamboo material, a more environmentally acceptable, traditional method is to heat the bamboo material at a high temperature. The modern process involves placing bamboo in a high-pressure carbonization boiler at 200-300°C for steam carbonization and then further processing it into industrial products (Zhang et al. 2020). Another method is to treat bamboo by impregnating it with ACQ (Jin et al. 2015).

Water glass (sodium silicate) is one of the materials used in inorganic coatings. At present, a series of investigations regarding the properties of water glass coatings has been performed, such as using water glass to prevent the growth of fungi. The antifungal effect of water glass with an increase in sodium oxide was observed to be significant (Li et al. 2020).

In this study, ACQ and water glass were used to impregnate bamboo and carbonized bamboo, and the changes in color and decay resistance were evaluated. These research results

will provide a corresponding scientific basis for decay resistance applications and process improvements of bamboo and its products.

MATERIAL AND METHODS

Plant material and reagents

In this study, Moso bamboo was collected at Xiasi, a town of Kaili city. The bamboo was 5 years old, 70-100 mm in diameter and 6-11 mm in wall thickness. A complete bamboo tube was cut at 0.5 m from the ground, the green and yellow bamboo was removed and samples of 20 × 20 × 5 mm (L×R×T) were processed. The samples were dried to constant weight after they were numbered. The bamboo samples were placed in a high-temperature kiln, and heated with high pressure steam until its color changed. The steam pressure was 0.2-0.5 MPa (temperature is 133-159°C) and the carbonization time was 4 h to obtain carbonized bamboo. Totally 15 bamboo samples and 15 carbonized bamboo samples were prepared.

We referred to the Chinese national standard GB/T 13942.1: 2009 (Durability of wood. Part 1: Method for laboratory test of natural decay resistance) to prepare nutrient solution medium from river sand and wood chips. The control sample for the decay resistance test was sapwood of *Pinus massoniana,* purchased from the Guiyang Forest products market, and processed with a saw into samples of size 20 × 20 × 10 mm (L×R×T). Brown rot fungus *Gloeophyllum trabeum* was provided by the College of Forestry, Guizhou University.

Water-soluble environmentally friendly type D alkaline copper quaternary (ACQ-D) preservative was purchased from Shenzhen Lvtai Environmental Protection Technology Co., Ltd., with an active ingredient mass fraction of 15.0% (w/w) including didecyldimethyl ammonium chloride (DDAC), a copper compound (calculated ratio Cu/O% as 4.87/10.52). Finally, the solution was diluted with distilled water to a concentration of 1% (w/w).

Water glass ($Na_2O{\cdot}nSiO_2$) (SiO_2 26.15%, Baume degree at 20°C 45° Bé, molarity 3.2 M, Na_2O 8.2%) was purchased from Changzhou Zidan Building Material Technology Co., Ltd. An experimental preservative was prepared by mixing 5 kg of water glass with 10 kg of water.

Main experimental equipment

A high-temperature kiln (developed in-house) with dimensions of 300 × 300 × 600 cm (length × width × height) was heated by steam and had a drying capacity of 1 $t{\cdot}h^{-1}$ and a temperature range of 55-350°C. We also used a biochemical incubator (Herocell 180), Shanghai Rundu Biotechnology Co., Ltd., an automatic colorimeter (NR10QC) at 8°/d (8 degree illuminating diffuse reflectance reception), Shenzhen 3nh Technology Co., Ltd., and a scanning electron microscope (SU8010), HITACHI, Japan.

Impregnation process conditions

The ACQ and water glass were evacuated and pressurized (first under a vacuum at -0.085 MPa for 30 min, then at 1.0 MPa for 30 min) and impregnated into bamboo, carbonized bamboo and *P. massoniana* sapwood samples. The impregnated samples were dried in an oven at 48 ± 2°C to constant weight (moisture content in samples were 0%), and then the colorimetric parameters were measured.

Decay resistance test

G. trabeum was inoculated into a Petri dish (90 mm diameter) containing 4% (w/w) maltose and 2% (w/w) agar. Once the hyphae covered 2/3 of the Petri dish, a 5 mm punch was used to punch holes to obtain three blocks containing fungi, which were immediately placed into 500 mL of sterilized river sand and sawdust-nutrient medium in a culture flask. The inoculated culture flask was placed in a biochemical incubator at a temperature of 28 ± 2°C and a relative humidity of 75-85% for approximately 10 days. When the surface of the culture medium in the flask was covered with hyphae, samples were added, and they were infected by the fungi. The mass loss rate was calculated using Eq. 1. Tab. 1 shows the classification of sample decay resistance based on the mass loss rate caused by fungal attack. This method was performed in accordance with the national standard (GB/T13942.1-2009):

$$\text{Mass loss rate (\%)} = (m_1 - m_2) / m_1 \times 100 \quad (1)$$

where: m_1 is the dry mass prior to the decay resistance test (g), and m_2 is the dry mass after the decay resistance test (g).

Tab. 1: Classification of sample resistance based on the mass loss caused by fungal attack.

Mass loss (%)	Decay resistance	Decay resistance class
0-10	Strong decay resistance	I
11-24	Ordinary decay resistance	II
25-44	Slightly decay resistance	III
> 45	Not decay resistance	IV

Color measurement methods

An automatic colorimeter was used to determine the color of bamboo and carbonized bamboo. The test light source was a D65 standard light source with a correlated color temperature of 6504 K. CIE 1976 ($L^*a^*b^*$) uniform color-space and color-difference formula was implemented (Mclaren 2008), where L^* is lightness, a^* is the red-green chromaticity index, and b^* is the yellow-blue chromaticity index. The total chromatic aberration (ΔE) was calculated using Eq. 2:

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

where: $\Delta L^* = L_t^* - L_0^*$, $\Delta a^* = a_t^* - a_0^*$, and $\Delta b^* = b_t^* - b_0^*$. L_0, a_0, and b_0 represent samples before the decay resistance test. L_t, a_t, and b_t represent samples after the decay resistance test. Chroma was calculated using Eq. 3, Hue angle (H^o) was calculated using Eq. 4:

$$C = (a^{*2} + b^{*2})^{1/2} \quad (3)$$

$$H^o = \tan^{-1}(b^*/a^*) \quad (4)$$

Micromorphology of bamboo and carbonized bamboo

The bamboo and carbonized bamboo were dried and pasted on the sample carrier. After ion sputtering gold plating, longitudinal sections of the samples were photographed and observed by Scanning electron microscopy (SEM).

Statistical analysis

Microsoft Excel 2013 was used to establish the original data table of the test results of bamboo and carbonized bamboo. Statistical analysis was performed using SPSS Statistics Version 21.0 software (Chicago, IL, USA). Dunn's test of multiple comparisons ($P < 0.05$) was performed to assess the statistically significant differences between the mean values (means) of three replicates. Origin 9.1 (Origin Lab, Northampton, MA, USA) was employed to construct the graphs.

RESULTS AND DISCUSSION

The color difference between the surfaces of bamboo and carbonized bamboo

Fig. 1 shows the specific colorimetric index changes of each sample. Fig. 1a shows that the L^* value of the bamboo was higher than that of the carbonized bamboo, and the L^* values of bamboo and carbonized bamboo were lower after impregnation with ACQ and water glass. The value of L^* continues to decrease after impregnated samples were allowed to decay, mainly because the content of cellulose and hemicellulose (white) in bamboo was higher than that of lignin (dark brown). *G. trabeum* can secrete cellulase and hemicellulase, resulting in a decrease in the content of cellulose and hemicellulose, while the content of lignin was relatively higher, so the L^* values of the samples were reduced (Wang and Ren 2008). Fig. 1b shows that the values of L^* of the two samples (bamboo and carbonized bamboo) impregnated with ACQ and its decayed samples first decreased and then increased. However, the values of a^* for the two samples impregnated with water glass all increased, and the values of a^* for the samples from impregnated with ACQ to its decayed samples first decreased and then increased. As Fig. 1c shows, the b^* values underwent little change for the two samples impregnated with ACQ, but they significantly decreased for samples impregnated with ACQ and allowed to decay. The b^* values of the bamboo and carbonized bamboo, impregnated with water glass, and those of the two bamboo and carbonized bamboo, impregnated with water glass and allowed to decay, first increased and then decreased. The color of the bamboo changed from bright light yellow (before impregnating) to dark blue-brown (after impregnating and decay), and the color of the carbonized bamboo changed from yellowish brown (before impregnating) to orange-brown (after impregnating and decay) (Lu et al. 2006, Xu et al. 2013b).

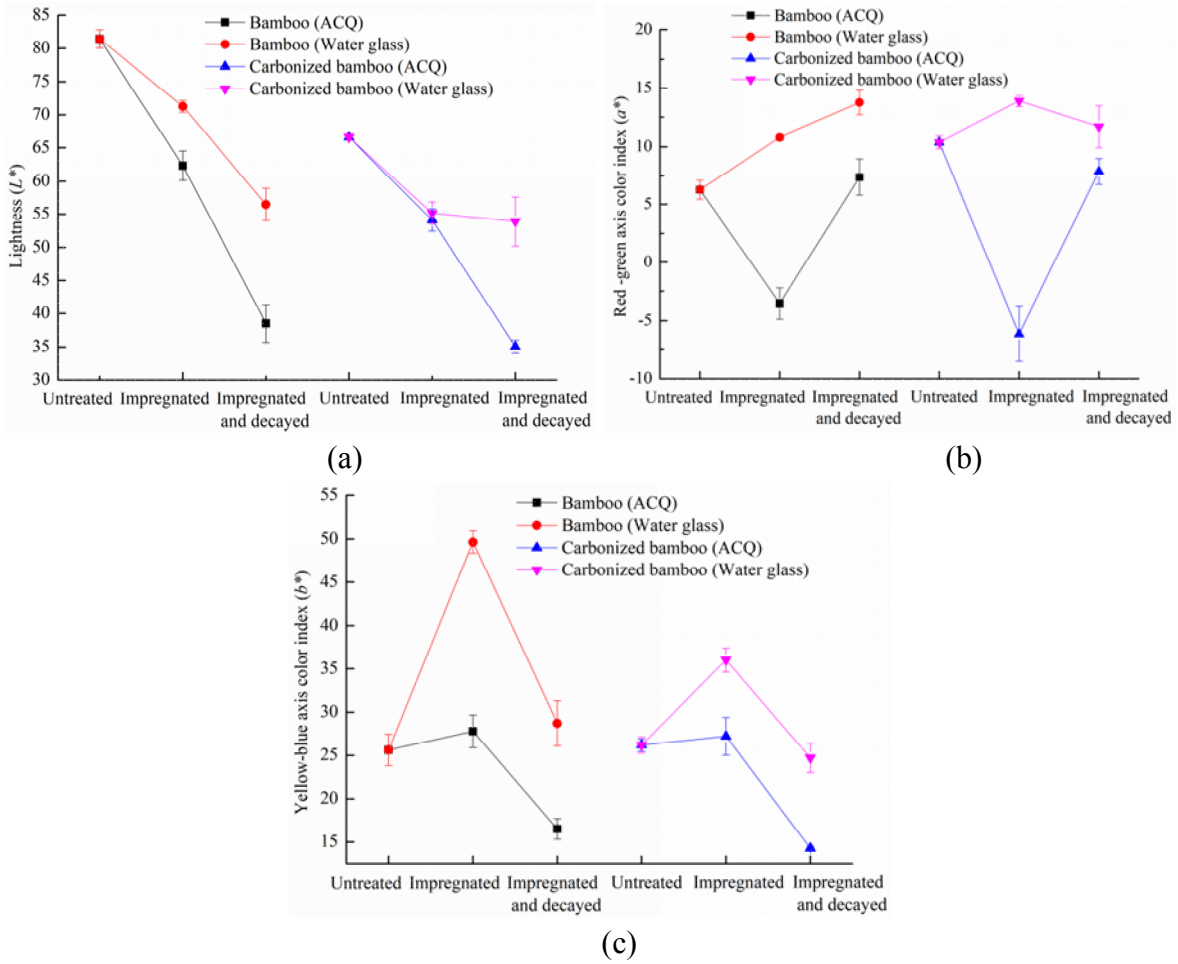

Fig. 1: Lightness (L) (a), red-green color index (a*), (b) yellow-blue color index (b*), (c) of samples before and after impregnation treatments and decay.*

Tab. 2 shows that the C and H^o values of the bamboo and carbonized bamboo samples before and after impregnation and decay were quite different (Alexander et al. 1976). The ΔE of the bamboo impregnated with ACQ and decay was the largest, and the ΔE of the bamboo impregnated with ACQ was the smallest. There was little difference in ΔE between bamboo (impregnated water glass) and bamboo (impregnated water glass and decayed). Carbonized bamboo has the largest ΔE after impregnation with ACQ and decay. The impregnation treatment has little effect on the color of the bamboo, while the combination of impregnation and decay treatment has a greater impact on the color of the bamboo. This is mainly due to the large amount of C=O, C=C conjugated double bond structures, -OH, -OCH_3 and other chromophoric groups or auxochrome groups (Wang and Ren 2008). Under external conditions such as *G. trabeum* or oxygen, these chromophoric groups or auxochrome groups were prone to break and recombine chemical bonds, resulting in significant changes in the color of bamboo (Tomak et al. 2013, Wei 2015). The carbonized bamboo itself was very durable, so it had a smaller ΔE than bamboo after the same impregnation and decay. The mechanism of *G. trabeum* causing the color change of bamboo has not yet been reported. It is believed that the degradation mechanism of brown rot fungi will be detected through transcriptome, metabolome, and proteomic analysis in the later stage of this work, and the pathways and related genes that cause enzyme discoloration in brown

rot fungi and the color-developing substances in bamboo can be found, which can finally clarify the mechanism of *G. trabeum* causing the discoloration of bamboo (Hori et al. 2013, Jian et al. 2014).

Tab. 2: Color difference of bamboo and carbonized bamboo, impregnated with ACQ and water glass, before and after decay.

Samples	Chroma value (C)	Hue angle (H^o)	Total chromatic aberration (ΔE)
Bamboo	26.39 ± 1.95c	76.27 ± 0.99a	
Impregnated with ACQ	28.04 ± 1.79bc	-82.65 ± 2.97c	21.59 ± 1.96b
Impregnated with ACQ and allowed to decay	18.10 ± 2.59d	66.09 ± 2.24b	44.01 ± 2.78a
Impregnated with water glass	50.81 ± 0.32a	77.71 ± 0.21a	26.48 ± 2.10b
Impregnated with water glass and allowed to decay	31.85 ± 2.97b	64.32 ± 1.72bc	26.46 ± 3.62b
Carbonized bamboo	28.17 ± 0.86b	68.39 ± 0.61b	
Impregnated with ACQ	26.471 ± 2.66b	-76.86 ± 2.19a	13.71 ± 2.04b
Impregnated with ACQ and allowed to decay	16.32 ± 0.67c	61.20 ± 2.24c	33.86 ± 0.75a
Impregnated with water glass	38.61 ± 1.11a	68.89 ± 1.40b	15.63 ± 0.24b
Impregnated with water glass and allowed to decay	27.40 ± 2.52b	64.90 ± 2 .30bc	13.10 ± 2.96b

Note: Different letters after the data in the same column indicate significant differences between treatments ($P < 0.05$).

Decay resistance test results and analysis

Brown rot fungi can invade bamboo cell cavities and release enzymes that degrade cellulose, hemicellulose, and pectin, thereby degrading the bamboo (Kim et al. 2011). Fig. 2 shows that the mass loss rate of *P. massoniana* without impregnation treatment was 38.8% after *G. trabeum* infection for 12 weeks, followed by bamboo with 19.8% mass loss.

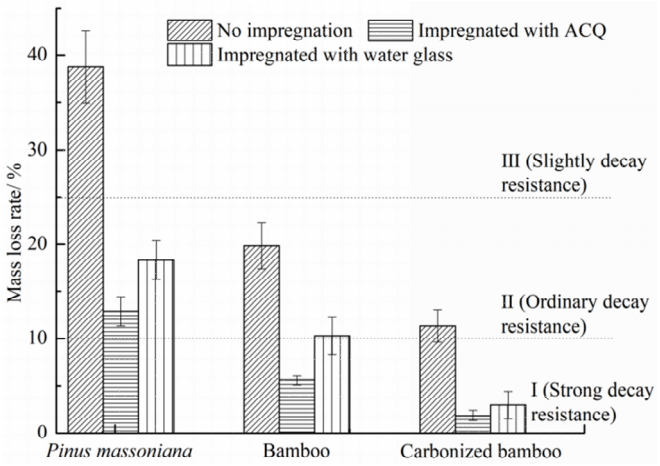

Fig. 2: Mass loss rates of bamboo and cabonized bamboo invaded by Gloeophyllum trabeum for 12 weeks.

The decay resistance of the carbonized bamboo sample was the best among the unimpregnated samples, and the mass loss rate was only 11.3%. With the same impregnated preservatives or decay conditions, the carbonized bamboo, had a lower mass loss rate and better decay resistance than bamboo. The main reason was that hard carbonized particles form a dense environment where fungi do not easily live (Pfeffer et al. 2011). The hyphae of *G. trabeum* had

difficulty entering the interior of the bamboo, thereby inhibiting the growth of the fungi (Kaur et al. 2016). Carbonized bamboo also loses a large amount of organic matter, such as starch and sugar, leading to the lack of nutrients for the growth of *G. trabeum* (Shangguan et al. 2016).

Carbonized bamboo impregnated with ACQ and water glass and bamboo impregnated with ACQ can reach level I strong decay resistance. *P. massoniana* impregnated with ACQ and water glass, bamboo and impregnated with water glass, and carbonized bamboo can reach level II decay resistance. The decay resistance of the bamboo and carbonized bamboo samples was as follows: impregnated with ACQ > impregnated with water glass > not impregnated. The decay resistance of the commercial ACQ preservative was better than that of water glass. The main reason was that the ACQ was insoluble in water, could be fixed in bamboo for a long time and was not easily lost. The copper compound and DDAC contained in ACQ were more toxic to *G. trabeum* (Tang et al. 2019). Water glass has antifungal and mothproof effects. Generally, as the content of sodium oxide in the ingredients increases (alkalinity increases), the antifungal efficacy improves. In addition, the decay resistance mechanism of impregnated water glass was mainly to form a film on the surface of the sample to physically isolate the hyphae to a certain extent (Pfeffer and Militz 2010, Wang et al. 2018). However, the high temperature and high humidity environment generated by the autoclave sterilization of the sample before the decay test, and the long term high humidity environment in the triangular flask during the decay test, damaged the film. Mass loss also occurred after the decay resistance test.

Analysis of microstructure

Analyzing the mechanism of hyphae penetrating the cell wall by SEM is very important for understanding the decay process of bamboo (Ming et al. 2007, Chang et al. 2008). The cross section of samples (Figs. 3a,b) show that the bamboo and carbonized bamboo cells were intact and that the vessel, vascular bundles, parenchyma tissue, cell walls etc. were normal. Figs. 3c,d show that there were many hyphae inside the bamboo after it was infected by *G. trabeum*, and the cell structure was destroyed. Carbonized bamboo also exhibited the same phenomenon of being damaged by hyphae. This shows that when the bamboo and the carbonized bamboo were not impregnated with preservatives, they suffered different degrees of decay. The hyphae of *G. trabeum* easily entered the interior of the bamboo from the channel with least resistance, that is, the cell walls or pit membranes of the cells with open ends. When the hyphae penetrated the cell wall, they secreted various enzymes that react with the chemical components of the bamboo material, thereby degrading the cellulose and hemicellulose and finally, forming perforations (Nguyen et al. 2018). The bamboo and carbonized bamboo in Figs. 3e,f were impregnated with ACQ, so both samples maintained good integrity and color, and the pores were clearly visible. Bamboo fibers remained basically intact. The comparison of samples impregnated with preservatives and CK samples showed that the ACQ preservative improved the decay resistance of bamboo.

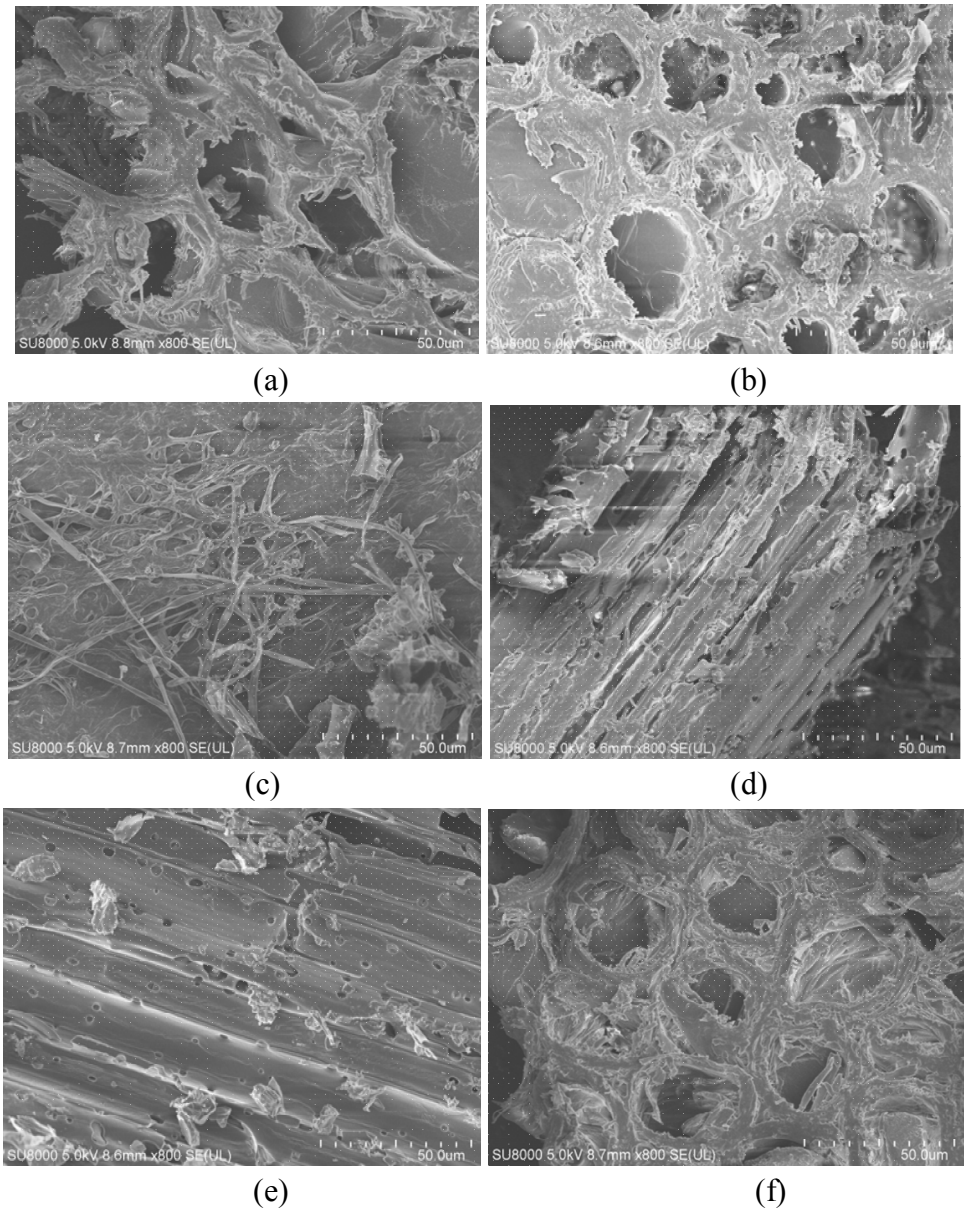

Fig. 3: Scanning electron micrographs (magnification 2000×) of bamboo and carbonized bamboo infected by Gloeophyllum trabeum: bamboo (a), carbonized bamboo (b), bamboo after decay (c), carbonized bamboo after decay (d), bamboo impregnated with ACQ after decay (e), and carbonized bamboo impregnated with ACQ after decay (f).

CONCLUSIONS

Some chromophoric groups or the auxochrome group in bamboo and carbonized bamboo degraded and reorganized in the presence of *G. trabeum* or oxygen, resulting in significant color changes. The total chromatic aberration (ΔE) of bamboo and carbonized bamboo, after both were impregnated with ACQ and allowed to decay, was larger than that of samples impregnated with water glass and allowed to decay, but the mass loss rate was smaller.

Cellulase and hemicellulose, secreted by *G. trabeum,* degraded bamboo and carbonized bamboo and resulted in mass loss. ACQ was more effective than water glass in protecting

the two materials. When bamboo and carbonized bamboo were impregnated with ACQ or water glass and allowed to decay under the same conditions, the mass loss rates were lower for carbonized bamboo than uncarbonized bamboo.

SEM images show that when bamboo was infected by *G. trabeum*, many hyphae appeared inside. However, when bamboo and carbonized bamboo impregnated with ACQ, there were fewer hyphae on the surface, the structure remained intact, and the antifungal performance was good.

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