Study on infection behavior and characteristics of poplar wood dyed by *Lasiodiplodia theobromae*

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Abstract
The technology of dyeing wood by microorganisms is a kind of pollution-free and sustainable wood dyeing technology. To achieve fast and rich dyeing of *Lasiodiplodia theobromae* on the surface of poplar wood, tyrosinase and tricyclazole were used as induction factors in this experiment. The results showed that *L. theobromae* had a better induction effect in the cross-section of poplar wood and induced with tricyclazole. The surface color of poplar ranged from light yellow dyeing to gray and brown, the chromatic aberration of the cross-section of wood was above 44.5 NBS, and the infected area was over 50%, while the dyed parts of radial and tangential sections of wood were only on the surface of the wood after 30 days of infection. The induced infection of *L. theobromae* on poplar wood had little effect on the chemical components of poplar and had good colorfastness to washing and light. Therefore, microbial dyeing of wood showed a beneficial application prospect in the field of wood dyeing.

1 Introduction

Nearly a year and a half after the outbreak of the new coronavirus (COVID-19) in December 2019 (Hotez et al. 2021; Paul et al. 2021), people are paying increasing attention to their health and the protection of their living environment (Sun et al. 2021), especially for public office supplies with frequent daily contact, such as desks and chairs, as well as beds, doors, and floors for home and office use. The extent and severity of the epidemic are deeply worrisome and have seriously threatened the safety of people’s travel and life (Curșeu et al. 2021).

The furniture and decoration materials that we use daily are mostly made of wood materials. Due to China’s protection of forest resources and the scarcity of wood resources, there is less and less precious wood that can be used to produce furniture, because of a shortage of prized timber (Barrett et al. 2013; Arunkumar et al. 2012), such as red sandalwood (Hansen 2000), scented wood (Metcalfe 1935), ebony (Thompson-Brenner et al. 2011), and siam rosewood (Shou et al. 2014). These woods are the ideal material and have the delicate texture as well as excellent strength and toughness. Researchers have worked on ways to imitate this wood (Liu et al. 2012). Li et al. (2016) impregnated poplar wood with phenol-formaldehyde resin in the presence of a surfactant as a penetrant and used the wood polymer composites (WPCs) method to prepare imitation rare woods. This method can produce wood products that partly replace precious wood (Li et al. 2016). In addition, Danihelova et al. (2015) also reported on a comparison of physical and acoustic properties between modified wood of black locust and Honduran rosewood. The sound quality of the xylophone before and after the modification of the black locust was compared, and it was found that black locust (modified by combination) could be used as a substitute for Honduran rosewood with lower quality (Danihelova et al. 2015).

However, the more widely used wood species in the market are fast-growing wood species, such as poplar, eucalyptus, and pine, which have some texture but monotonous color (Liu et al. 2020, 2021). These materials are veneered and dyed to improve their added-value and decorative effect (Nguyen et al. 2018). The dyeing method used in industry is the physical filling or chemical combination method in which the material is immersed in the dye (Liu et al. 2015). The dyes used are divided into industrial dyes such as acidic,
basic, azo, and reactive dyes (Shi et al. 2019; Hu and Yu 2014). While bringing brilliance and economic benefits, it also produces a large amount of industrial wastewater, which greatly impacts the water environment and the soil environment (Wang et al. 2017). Wastewater treatment of dyestuff and intermediates has always been a global ecological and environmental protection problem, which requires a special treatment department of dye wastewater for classification and recycling and consumes substantial funds (Hai et al. 2007; Haji et al. 2018).

Microbial dyeing technology is a new wood dyeing method developed in the last ten years; the cultivation of dyeing microorganisms has low environmental and spatial requirements, only a suitable temperature and humidity environment and sufficient nutrients, with fast growth, easy survival, and strong controllability (Kim and Choi 2015; Robinson 2012; Robinson et al. 2013). At present, there have been related studies on the application of fungi to the dyeing of wood and bamboo. Robinson et al. (2011) have inoculated two strains of \textit{Fusarium} and two strains of \textit{Arthrobotrys cuboidea} isolated from wood with red staining during culture into sugar maple (\textit{Acer saccharum} Marsh.) for 6–14 weeks to evaluate its ability to produce highly saturated and permeable stains. Robinson et al. (2011) found that both strains of \textit{A. cuboidea} produced high amounts of surface and penetrating red stain within a moderate incubation period. Vega Gutierrez et al. (2016) evaluated the potential use of fungal pigments in bamboo and compared the difference between direct infection and extraction of pigments from \textit{Scytalidium cuboideum}, \textit{Scytalidium ganodermophthorum}, and \textit{Chlorociboria aeruginosa}; results indicated that the extraction pigment using the above fungi can only be used for dyeing the surface of the bamboo, but direct inoculation of \textit{S. cuboideum} is appropriate for internal coloration (Vega Gutierrez et al. 2016). \textit{Lasiodiplodia theobromae} belongs to the imperfect fungi of sphaeopsideales and is a plant disease that is widely distributed in tropical, subtropical, and temperate regions. It is one of the main fungi causing blue stain of broad-leaved trees such as poplar, rubberwood, and Masson pine. However, the melanin secreted by \textit{Lasiodiplodia theobromae} only penetrates into the surface of wood, showing blue-gray or blue-black discoloration on the surface of wood (Irbe et al. 2018).

According to its nitrogen and sulfur content, melanin can be classified as eumelanin, alломelanin, or phaeomelanin (Prota 1988). Eumelanin is the product of a series of polymerization reactions of tyrosine catalyzed by tyrosinase, containing nitrogen atoms and no sulfur atoms. Tyrosinase is the rate-limiting enzyme of its biosynthesis (Ando et al. 2007). Tricyclazole can inhibit the activity of the enzyme encoded by the buf1 gene, resulting in the obstruction of fungal melanin synthesis, which can inhibit the formation of DHN melanin. The pigment is produced by a two-step dehydrogenation reaction of 1,3,6,8-tetrahydroxynaphthalene to scytalone (C_{10}H_{10}O_4) and 1,3,8-trihydroxynaphthalene to vermellone (C_{10}H_{10}O_3) changing from dark brown to reddish-brown (Woloshuk et al. 1980; Bourett and Howard 1992).

This study aims to imitate the color of precious wood and improve the added value of fast-growing wood. Tyrosinase and tricyclazole were used as inducers. Three sections of fast-growing poplar were dyed to study the color changes of poplar in different dyeing times and the infection behavior of \textit{Lasiodiplodia theobromae} on poplar. The micromorphology, chemical composition changes, and surface characteristics of dyed poplar were studied and analyzed to develop a sustainable and environmentally friendly dyeing technology using microorganisms for wood dyeing.

2 Materials and methods

2.1 Materials

2.1.1 Microorganism material

\textit{L. theobromae} (\textit{Pat.}) purchased from the China Forestry Culture Collection Center (CFCC 84471) and collected in a poplar forest (from Jurong, China) was used in this study.

2.1.2 Wood material

The poplar (\textit{Populus euramericana}) block was purchased from Huaqing Wood Industry Co., Ltd. The size of the test piece was processed to 50*50*20 mm, the average density was 0.55 g/cm³, and the moisture content was 6.5%.

2.1.3 Chemical and reagents

PDA (Potato Dextrose Agar) medium was a solid medium composed of potato 200 g, glucose 20 g, and agar 15–20 g with a natural pH and purchased from Beijing AoBoXing Bio-Tech co., LTD. Tyrosinase, CAS: 9002-10-2, was purchased from Shanghai Yuanye Biotechnology Co., Ltd, and tricyclazole, CAS: 41814-78-2, analytical purity standard, was purchased from Shanghai new platinum Chemical Technology Co., Ltd.

2.2 Biological dyeing process

The test pieces of cross-section, radial section, and tangential section of fast-growing poplar were prepared and the moisture content was balanced at room temperature for use, and the edges of those pieces were sealed with hot-melt adhesive. After the hot-melt adhesive was completely dried, the test pieces were put into a high-temperature sterilization...
pot at 121 °C and 0.11 MPa pressure for 30 min for sterilization and then taken out. After 7 days of cultivation in PDA medium, _L. theobromae_ was inoculated on the surface of the wood and in the induction medium (the diameter of the cake was 10 mm), and cultured in a constant temperature and humidity box at 28 °C and 85% humidity for 10 (A), 20 (B), 30 (C), and 40 (D) days, respectively. The experimental groups were divided into control, 150 ku/L tyrosinase (Ty), and 10 mg/L tricyclazole induction groups (Tr). The infected woodblocks were taken out, and the above sterilization procedure was repeated. After sterilization, the mycelium on the surface of the woodblocks was removed and dried to constant weight in a constant temperature drying oven at 40 °C. Figure 1 shows the schematic diagram of poplar induced by _L. theobromae_.

### 2.3 Instruments and equipment

The basic equipment used in the test includes: Constant Temperature and Humidity Box, #HWS-80B, Beijing Huasheng Scientific Instrument Laboratory Equipment Co., Ltd; Super Clean Workbench, # SW-CJ-1D, Beijing Huasheng Scientific Instrument Laboratory Equipment Co., Ltd; Electric constant temperature drying oven, # DHG-9075a, Beijing Huijia Technology Development Co., Ltd; pH meter, # PB-10, Sartorius Group; Rotatory viscometer, # NDJ-1, Shanghai Yoke Instrument Co., Ltd.

The testing equipment used in the test includes: Fourier transform infrared spectroscopy (FTIR): #Nicolet iS5, from Thermo Fisher Scientific at the detection wavelength of 400–4000 cm⁻¹; Optical Contact Angle Meter, # Dataphysics-OCA, produced in Germany with the contact angle measurements; scanning electron microscope, # GeminiSEM 300, from Germany at the magnification of 0–2 million times; colorimeter, # Dataflash 110, Shanghai Dingzheng Instrument Equipment Co., Ltd; Thermo-gravimetry-Differential Scanning Calorimeter (TG-DSC), #NETZSCH STA 449F3; X-ray diffraction (XRD), # Ultima IV, Rigaku Corporation, Japan.

### 2.4 Measurements

#### 2.4.1 Chromaticity value test

CIELAB uniform color space is widely used in matching coatings, building materials, and other surface pigment industries. L* is the lightness coordinate, representing the brightness of the color, a* and b* are the chromaticity coordinates. a* indicates the red-green direction, where +a* is the red direction and −a* is the green direction; b* represents the yellow–blue direction, where +b* represents the yellow direction, and -b* represents the blue direction. L* indicates lightness from 0 (black) to 100 (white). ΔE* represents the standard deviation in the L*a*b* color space, and its size can directly represent the perception gap of the human eye for the color of the object. Its formula is:

$$\Delta E^* = \left[ (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{1/2}$$  \hspace{1cm} (1)

Reflectivity and color depths of the dyed wood were tested using a Datacolor 110 spectrophotometer under illuminant D65. Each sample was measured six times in a different area and an average value was used (2):

$$K/S = \frac{(1 - R)^2}{2R} \hspace{1cm} (2)$$

where R is the reflectance of the dyed samples, K is the adsorption coefficient, and S is the scattering coefficient.

![Fig. 1 Schematic diagram of poplar induced by L. theobromae (Ty and Tr represent tyrosinase and tricyclazole induction groups, respectively)](image-url)
2.4.2 Microstructure analysis

During the infection period, fungi go through some microstructure of wood and enter into wood, grow and secrete in wood, thus changing the micromorphology of wood. A scanning electron microscope (SEM) was used to observe the microstructure of the surface and back of the wood stain to observe fungi behavior and the migration process of fungi in wood.

2.4.3 Chemical composition analysis

Fourier transform infrared spectroscopy (FTIR) was used to test the chemical composition changes before and after dyeing to analyze the effect of wood components in microbial dyeing. The size of the wood samples before and after dyeing is 200 mesh. X-ray diffraction (Segal et al. 1959) was used to test the crystallinity change during the infection of *L. theobromae* in poplar wood. The scanning angle range is 5°–90° with 5°/min of scanning speed. The calculation formula was as follows (3):

\[
\text{Crystallinity (Xc)} = \left( \frac{I_{002} - I_{am}}{I_{002}} \right) \times 100\% 
\]

where: \(I_{002}\) is the diffraction intensity of 002 crystal plane, \(I_{am}\) is the diffraction intensity of the amorphous region, \(I_{002}\) is the diffraction intensity of 20 = 22.0°, and \(I_{am}\) is the diffraction intensity of 20 = 18.0°. Differential scanning calorimetry (TG-DSC) was used to test the mass change of dyed poplar during the temperature change and the heat change during the heat absorption / exothermic process. The test steps were as follows: under the protection of nitrogen, the samples were heated from room temperature to 40 °C and kept at 40 °C for 1 min to ensure uniform temperature distribution, and then heated to 1000 °C at a heating rate of 20 °C/min.

2.4.4 Colorfastness

The dyed poplar specimens were tested in a xenon lamp aging machine for 4, 8, 16, 32, 64, and 100 h under artificial simulated sunlight. The blackboard temperature was set at 55°C ± 2 °C, the relative humidity was 65%, and the actual radiance was 40 W/m². In addition, the specimens were put into a constant temperature water bath at 80 °C for the water-resistance test for 2 h and 4 h. After light and water aging, the samples were taken out and dried at 40 °C for 24 h, and then the chromaticity value was tested.

2.4.5 Surface characteristics

Distilled water (viscosity: 0.9 mPa·s, pH: 6.8), varnish (viscosity: 90 mPa·s, pH: 7.8, solid content: 26.05%), and wood wax oil (viscosity: 3575 mPa·s, pH: 7.4, solid content: 29.74%) were used to test the contact angle of poplar wood in different dyeing types and dyeing times.

3 Results and discussion

3.1 Visual staining phenomenon

3.1.1 Chromatic aberration

Figure 2 shows the infection scene of different sections of *L. theobromae* on the 20th day. It can be seen from the figure that the cross-section of *L. theobromae* has been covered with hyphae and secreted pigment on the 20th day, while the tangential section and radial section have begun to secrete pigment. However, they exist in the center of inoculation, and the growth of mycelia on the radial section was better, which was reflected in the attachment degree and secretion of mycelia. The pigment secreted by the control and tyrosinase induction groups was black, while the pigment secreted by the tricyclazole induction group was brown. Figure 3 shows the chromatic aberration of poplar in different sections and different periods under different infection factors. The results showed that the overall chromatic aberration of the cross-section was larger than that of the other two sections after 30 days of infection, and the overall chromatic aberration of the radial and tangential sections was similar. This is because the tubes on the surface of the cross-section allow the mycelium growth and secretion, and a large amount of pigment can be deposited on the surface, while the radial and tangential sections lack this morphological structure. The pigment will deposit on the concave and convex parts of the wood surface, allowing easy removal of the pigment during sterilization and cleaning, resulting in the reduction of chromatic aberrations. The values of tricyclazole induced infection were higher than that of other groups on the whole, and the chromatic aberrations of each infection group were above 44.5 NBS after 30 days of cross-section.
infection, which indicated that fungi have a better induction effect in cross-section and tricyclazole-induced infection.

### 3.1.2 Infection patterns and color simulation

Figure 4 shows 10- and 40 days poplar infected specimens under different induction conditions. The results showed that the wood surface’s color changes after 10 and 40 days of infection. The color of the control and tyrosinase-induced groups was gray-black, while the tricyclazole-induced infection produced a red-brown to brown color on the surface of poplar wood. Compared with the dyed wood infected for 10 days, surface color uniformity and color depth were significantly improved and had a significant dyeing effect.

The color chromaticity values of different sections of poplar at different infection times and induction conditions were simulated by the color simulation tool of the colorimeter (Fig. 5). The infection color of the cross-section is deeper than that of other sections. The tyrosinase-induced infection is the most prominent in the cross-section, while the tricyclazole-induced infection is more evident in the tangential and radial sections with the reddish-brown color series. This may be because the tricyclazole inducer is on the outside of the wood when the hypha enters the cross-section vessel, leading to the position change of hypha to obtain nutrients, the inducement weakening at the later stage of dyeing, and the normal secretion of melanin and the infection of wood by *L. theobromae*.

### 3.1.3 Reflectivity and K/S values

Figures 6 and 7 show the reflectance and color depth of three sections of dyed poplar under different time and induction conditions. Figure 6 shows that compared with poplar raw materials, the reflectance of each section’s dyed surface after 10 days of dyeing is significantly reduced, and the reflectance of tangential and radial sections is significantly lower than that of the cross-section after 10 days of dyeing. However, the change of cross-section is the most significant after 10 days of dyeing. In the first ten days of dyeing, the hyphae can enter the wood through the vessel tissue more smoothly in the cross-section. Although the fungi are secreting pigment during the process, they are also consuming a lot of energy and time to invade the interior, resulting in a poor
Fig. 6 Reflectivity of different sections (a cross-section, b radial section, c tangential section) of poplar wood at different induction conditions and dyeing time (X, Y, Z: X represents the induction time, A, B, C and D represent induction culture for 10, 20, 30 and 40 days, respectively; Y represents the induction type, 1, 2, 3 represent control, tyrosinase and tricyclazole induction groups, respectively; and Z represents the induced wood block, C, R, T represent crosscutting block, radial block and tangential block, respectively)

Fig. 7 K/S values of the three sections (a cross-section, b radial section, c tangential section) of poplar wood at different induction conditions and dyeing time (X, Y, Z: X represents the induction time, A, B, C and D represent induction culture for 10, 20, 30 and 40 days, respectively; Y represents the induction type, 1, 2, 3 represent control, tyrosinase and tricyclazole induction groups, respectively; and Z represents the induced wood block, C, R, T represent crosscutting block, radial block and tangential block, respectively)
surface dyeing effect. Melanin accumulates on the wood’s surface in the tangential and radial sections due to the infection path blockage. Melanin has an excellent ability to absorb light, making the wood surface show low reflectance.

With the infection time extension, fungi secreted a large amount of pigment. Due to the limited infection space of tangential and radial sections and the mycelium aging, the chromaticity value of the wood surface was relatively stable in the later stage of dying, consistent with the chromatic aberration value result of wood infection. After 20 days of infection, the highest reflectance of the stained surface of the poplar cross-section was below 18.3%, while that of the others was below 30%. Figure 7 shows the dyeing depth of poplar under different infection times and infection types. The color depth values of tangential and radial sections after 10 days of infection had little difference but significantly increased in the cross-section. Although the color difference values of the tricyclazole-induced infection group are larger in each section, the color depth values in the cross-section are lower. It is speculated that this result is due to the hue, pigment chromaticity, and the brightness differences of induced pigment attached to the wood surface.

### 3.2 Infection degree

Figure 8 shows the three-dimensional infection morphology of *L. theobromae* infecting three sections of poplar under tyrosinase or tricyclazole induction. After 20 and 40 days of infection, the infection depth in the cross-section group increased significantly (Fig. 8a), while the infection depth in the tangential and radial sections remained unchanged, existing only in the surface staining. However, the large roughness of the cross-section showed a slightly low staining uniformity. From Fig. 8b it can be seen the measured value of the staining thickness of the tangential section and the radial section was less than 1 mm at 40 days. Due to the small staining thickness, only a rough value could be obtained after 10–20 days of staining. The tyrosinase-induced infection area of the cross-section accounts for 70% of the cross-section at 40 days; the infection depth has penetrated the thickness of the specimen by 20 mm in 20 days. The tricyclazole induced infection in the interior appears lighter, bluish-black, different from the surface infection color. This is because the wood longitudinal tissue vessel provides the path for hyphae to invade the interior of the wood, and the hyphae are less affected by the induced substances when they infect the interior of the wood.

### 3.3 Micromorphology

To further explore the growth of hyphae in the wood, the specimens infected by each section were dissected, and microscopic morphology was observed. As shown in Fig. 9, many hyphae clusters were accumulated in the tube after the cross-section surface was infected, and shriveled hyphae were found in the tube. At the same time, a large number of hyphae were found in the wood infected by the radial section. However, in the tangential section of wood, hyphae appeared in the ray cells of wood and could grow and secrete through the pores of the vessel, and the diameter of hyphae in the wood was between 5 and 10 µm.

### 3.4 Chemical composition

Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and TG-DSC were used to analyze the chemical composition of poplar wood before and after infection. As shown in Fig. 10, the infrared scanning spectra of unprocessed poplar wood and dyed wood for 20- and 40-days were compared. The results showed almost no change in the chemical composition of wood after 20 and 40 days of dyeing. The peak intensities of 2343 and 2360 cm\(^{-1}\) appeared in the dyed poplar components, attributed to the stretching vibration of the C–C triple bond.
Fig. 9 Micromorphological images of the three sections of poplar wood (Ty and Tr represent tyrosinase and tricyclazole induction groups, respectively; C, R, T represent crosscutting block, radial block and tangential block, respectively; the arrow indicates the location of the hypha)

Fig. 10 Changes of chemical components in cross-section of poplar under 10- and 40-days infection under different induction conditions (X,Y,Z: X represents the induction time, A, B, C, and D represent induction culture for 10, 20, 30, and 40 days, respectively; Y represents the induction type, 1, 2, 3 represent control, tyrosinase and tricyclazole induction groups, respectively; and Z represents the induced woodblock, C, R, T represent crosscutting block, radial block, and tangential block, respectively)

(Rassabina et al. 2020), indicating that the residual materials in the wood after L. theobromae staining of poplar wood include this component, which may be due to the pigment secreted by fungi, or other secretory products in the mycelial growth process. The typical peak intensity fluctuations exist at 3340 cm\(^{-1}\) (hydroxyl (–OH) and amino (–NH\(_2\)) structure), 1029 cm\(^{-1}\) (aromatic C–C structure or the stretching vibrations of C–O–C), and 686 cm\(^{-1}\) in-plane C–H bending deformation and out of plane C–H deformation (Girdthep et al. 2018). The peak intensities of these bands are enhanced, suggesting heterocyclic compounds with aromatic rings in the secreted products. A combination of the results showed that the induced infestation of poplar by L. theobromae did not change its chemical composition only by filling fungal secretions in the wood. Therefore, it can be inferred that the mechanical properties of dyed wood will be little affected.

According to the X-ray diffraction pattern, the relative intensity of the diffraction peak at the corresponding position on the XRD scanning pattern of wood before and after dyeing was calculated (as shown in Fig. 11). The crystallinity of each sample (from raw to B\(_1\)C to D\(_3\)C) before and after dyeing was 38.2%, 36.7%, 37.0%, 38.7%, 33.5%, 33.3%, and 34.8%. The crystallinity of dyed wood decreased slightly in the tyrosinase-induced and tricyclazole-induced groups and decreased by 3.4–4.9% after 40 days of dyeing.
The TG-DSC test showed that the weight loss rates of dyed poplar and untreated wood were 79.46–82.75% after 40 days, and the highest weight loss rate was raw wood, which may be caused by the lack of pigment inside the wood or the unavoidable difference of specimens (Fig. 12). DSC curve showed that an endothermic peak appeared at about 300 °C, which can be ascribed to the wood’s glass transition at this temperature. Two endothermic peak areas appeared in raw wood, and the second endothermic peak area in the other group was not obvious. This phenomenon may be due to the
fungus' secretion composition complexity and the inconsistent phase transition temperature.

3.5 Fastness to light/water

The colorfastness of poplar wood dyed by *L. theobromae* was also tested. The continuous lightfastness test for 4–8–16–32–64–100 h and the water fastness test for 2 and 4 h were arranged for each group of dyed poplar wood. According to human visual perception, the chromatic aberration perception can be divided into the following levels: 0–0.5 NBS, 0.5–1.5 NBS, 1.5–3 NBS, 3–6 NBS, 6–12 NBS, and 12 NBS or more, corresponding to very slight, slight, perceptible, recognizable, obvious, and very obvious.

The results of the light fastness test in Fig. 13 showed that the chromatic aberration values of each group increased gradually with the extension of light time. The chromatic aberration of poplar wood infected in cross-section was below 3 NBS, while poplar wood dyed for 40 days was less than 1.5 NBS after 100 h of light resistance. In the tangential and radial sections, the chromatic aberration of untreated poplar was close to 6 NBS at 100 h, while that of dyed poplar was below 3 NBS. Compared with the tangential and radial sections, the largest stain depth of fungi in the transverse section and the bigger chromatic aberration, while melanin has good light tolerance, improves the poplar's tolerance under the light.

Before and after the test, the chromatic aberrations were collected after boiling at 80 °C for 2 h and 4 h. As shown in Fig. 14, the color difference values of unprocessed poplar were larger than those of dyed groups, and the tricyclazole-induced group was slightly larger than that of normal staining and tyrosinase-induced groups. The tangential and radial sections of dyed samples had little difference from the
unprocessed group, while the cross-section showed marked color differences. The chromatic aberrations of water resistance of the dyed piece became smaller after the dyeing time was prolonged, and the color difference value of water resistance of the test piece after 40 days of dyeing was basically below 3NBS, and the difference between the chromatic aberrations for 2 h and 4 h of water resistance was small, showing good color fastness for water.

### 3.6 Wettability

The secondary processing of dyed poplar needs surface coating or veneering treatment; thus, the dyed wood needs to have superior surface characteristics. By testing the contact angle of dyed poplar with distilled water, varnish, and wood wax oil (Fig. 15), compared with unprocessed poplar wood, the contact angle increased in the poplar wood dyed for 20 days and 40 days, but there was no significant difference between dyeing times. In the contact angle test with distilled water as the solvent, the slight difference of contact angle after 20 days of dyeing was observed, while in varnish solvent, the contact angle of each group’s cross-section has almost no change. However, in the tangential and radial sections, the contact angles of the normal dyeing and tyrosinase-induced groups were slightly higher than that of the tricyclazole-induced group. This result was also observed with natural coating as an experimental solvent. This is because the hyphae of *L. theobromae* can enter the interior of wood without inducer or tyrosinase during normal growth. On the contrary, under the induction of tricyclazole, *L. theobromae* secretes a large amount of pigment and inhibits the growth of hyphae, the hyphae and pigment form a blockage in the tube during the dyeing process, impeding the entrance of liquid into the wood. From the typical dynamic contact angle curves of three solvents in the cross-section of poplar wood, it was found that the molecular weight, viscosity, and surface tension of wood wax oil and varnish were much higher than that of distilled water, so the contact angle of wood wax oil and varnish on the surface of poplar wood tended to be stable and much higher than that of distilled water.

### 4 Conclusion and outlook

In this experiment, different inducers were used to induce *L. theobromae* and apply it to poplar wood for microbial dyeing. The infection of different sections and times on poplar wood was studied. After dyeing, the fungal growth, chromatic aberrations, infection behavior, chemical composition, colorfastness, and surface characteristics of poplar wood were studied. This study found that poplar induced by *L. theobromae* on tyrosinase and tricyclazole showed different colors (black and brown), and the infection of *L. theobromae* on poplar was directional, and the infection path showed a three-dimensional structure. The longitudinal infection mainly invades the interior of the wood through the tube hole of the catheter, while the transverse infection mainly invades the interior of the wood through the tube hole and wood ray tissue. The induction in cross-section and tricyclazole induced of *L. theobromae* had a better induction effect.
The chromatic aberrations of cross-section after 30 days of infection were above 44.5 NBS, and the infected area was more than 50%. The fungi infestation had less effect on the chemical composition of poplar wood and showed good colorfastness to washing and light.

As an environment-friendly and sustainable dyeing technology in line with the national green development strategy, microbial dyeing of wood is nowhere near enough in terms of the current dyeing effect and efficiency. Further research needs to achieve a high-efficiency and high-quality dyeing process with faster infection, greater dyeing depth, and richer colors. This might provide more choices and optimization means in the future strain screening, strain cultivation, and wood infection process, to achieve the high-value utilization and direction induced dyeing of microbial dyed wood.

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Author contributions YL: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing—original draft, writing-review & editing, visualization. YG: data curation, validation, formal analysis, investigation, data curation, writing—original draft, visualization. ZMY: conceptualization, methodology, validation, resources, writing—review and editing, supervision, project administration, funding acquisition. YZ: methodology, software.

Availability of data and material The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest We declare that we do not have any commercial or associative interest that represents a in connection with the work submitted.

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