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ORIGINAL PAPER

Stain capacity of three fungi on two fast-growing wood

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Abstract We investigated the stain of fast-growing wood (*Cunninghamia lanceolate*, *CL*; *Paulownia*, *PT*) inoculated with three fungi (*Arthrinium phaeospermum*, *AP*; *Vibrio anguillarum*, *VA*; *Aspergillacea*, *AS*) to explore the new wood dyeing ways and the better combination of wood and fungi for dyeing. Only *AP* could dye on *CL* and *PT*. Especially for *CL*, its percentage of internal spalting, percentage of external spalting and dyeing depth were the highest (48%, 15% and 5.06 mm, respectively). Surprisingly, the bigger weight loss occurs on *PT*. The results showed that the dyeing effect of *AP* dyeing *CL* was the best, and the wood color change was obviously (Orange to dark red). *AP* could produce more pigments than the other two fungi (*VA*; *AS*), *CL* was more suitable for fungus staining than *PT*, indicating that *AP* could offered a new potential market and a chance for areas to earning higher income for *CL*. This research paves the way for improving color change was obviously (Orange to dark red). *AP* could produce more pigments than the other two fungi (*VA*; *AS*), *CL* was more suitable for fungus staining than *PT*, indicating that *AP* could offer a new potential market and a chance for areas to earn higher income for *CL*.

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Introduction

The demand of environmentally friendly wood dyestuff has been higher year by year owing to the economic development and the improvement of living standards, especially the people's awareness of environmental protection has been constantly enhanced. Therefore, many scientists have paid their attention to the researches on environmentally friendly wood dyestuff all over the world. Among these wood dyestuff, direct dyes, acid dyes and VAT dyes are usually employed to dye wood. However, these chemical dyes can cause serious air pollution, limiting the application of dyestuff in living space. Therefore, the pigment of environmentally friendly has great potential in the future. Among them, fungus pigment is regarded as a perfect wood dyestuff due to its environmental friendliness, which has attracted extensive research interest.

There is a long history of fungus pigment as a wood dye (Blanchette and Biggs 1992). Until now, fungus pigments have been used as a special dye to increase the value-added of wood, which depends on the colonization and stain wood ability of fungi (Robinson et al. 2007a, b; Robinson and Laks 2010a, b, c). How to approach the idealized technology of spalted wood is the goal of researchers in recent years. Therefore, exploring effective pigmented fungi and wood is to find the optimal environment of pigmented fungi.

Spalting is mainly divided into three parts: the stain fungi producing the stain substance, the bleaching formed by the white rot fungus, and the zone lines formed by the fungal interaction between the funguses (Robinson 2012).

The dyeing and the zone lines are widely used in wood dyeing. In terms of fungal staining, *Scytalidium cuboideum* and *Scytalidium ganodermophthorum* are relatively effective fungus, which can produce red pigment and yellow pigment, respectively (Vega Gutierrez 2016). When spalted fungi is inoculated on wood, it can obtain nutrition in wood rays or other internal tissues of wood, thus dyeing wood. However, spalted fungi have white rot ability for wood (Liese 1970; Worrall et al. 1997; Richter and Glaeser 2015). Existing studies have shown that the longer the incubation time of dyeing fungi is, the better the dyeing effect of wood is, but the higher the risk of white rot of wood is. Therefore, in practical application, fungal pigments can be extracted by organic solvents such as DCM and applied directly to wood dyeing, or dissolved in flaxseed oil after extraction by organic solvents and then dyeing wood, which can solve the problem of white rot of wood and shorten the whole dyeing time (Robinson et al. 2014a, b; Robinson et al. 2014a, b; Hinsch et al. 2015; Weber et al. 2015; Agurto et al. 2017; Robinson et al. 2017; Hinsch and Robinson 2018). In terms of zone lines, the researchers found that *Bjerkandera adusta/Trametes versicolor* and *Polyporus brumalis/Trametes versicolor* combinations, as well as *X. polymorphism, Coriolus versicolor*, etc. could produce stable zone lines (Robinson et al. 2010; Robinson et al. 2011). There are many reasons for the formation of zone lines, but generally they are all caused by changes in the growth environment, which are "Guard" and "Death" of hyphae (Mallett and Hiratsuka 1986; Cease et al. 1989).

In addition, fungi have different preferences for wood because of different functional groups and nutrients received from wood. Studies have shown that the spalted fungi are preferring alder, maple, poplar, phoebe, birch and beech (Chapela 1994; Robinson et al. 2011; Hai-Shan et al. 2013).

Due to the increase in value associated with spalted wood, efforts had recently been undertaken to utilize the process on low value woods. Of particular interest in southeastern China is the potential for *PT* (*Paulownia*) and *CL* (*Cunninghamia lanceolate*). Both species are native to China, although they are also commonly cultivated in other ethnic areas. These two species of tree from China has wide range of uses and has fixed value due to the relatively fast growth cycle. But *CL* is a soft, fine, aromatic, and straight grain wood that is easy to process and suitable for furniture, architecture and other applications. *PT* has a straight texture, a uniform structure and easy processing. *PT* is suitable for use in construction, furniture, wood-based panels and other applications. These two species of wood are easy to buy, but are currently underutilized in China, making them ideal for value-added processes.

A substantial body of literature exists on the controlled spalting of hardwoods and their use in woodcraft and furniture. There is currently few known record of controlled spalting being attempted with fast-growing wood. For example, using some of the poorly-formed wood, the wood is subjected to fungal infestation without destroying the board to achieve wood dyeing, and ultimately increase the vassal value of these fast-growing woods.

AP (*Arthrinium phaeospermum*), *VA* (*Vibrio anguillarum*), and *AS* (*Aspergillacea*) were used to inoculate two fast-growing wood (*CL* and *PT*). The purpose of this research was to investigate which unknown spalting fungi might be suitable for inoculation on the fast-growing wood, with the intended result of producing extra pigments that would increase the marketability of the lumber. And screened new spotted fungi and study the ability of fast-growing wood in fungal staining. Whether fungi that produce large amounts of extracellular pigments can also treat cork under controlled conditions. Successful pigmentation of the fast-growing wood by any of the tested fungi would offer an opportunity for sawmills to generate additional income, and then market lumber to interested consumers.

Materials and methods

Materials

PT and *CL* were selected to improve the economic value of fast-growing wood and explore the possibility of producing spalted in fast-growing wood, and the average moisture contents of the tested wood species were 19.04% for *CL* and 18.00% for *PT*, respectively. The specifications of the experimental block were 20 mm × 20 mm × 20 mm.

We recorded whether the pigments of the three fungi changed significantly on both woods. These three wood fungus, which were isolated and screened from spalted *PT*. The strains fungi were shown in Table 1.

Fungi isolation and screening and culturing

The portion with the spalted was cut into small pieces of length and width less than 10 mm. Alcohol lamps, ultrapure

Table 1 Wood and fungus

Wood species	Fungi species	Collection code
Cunninghamia lanceolata	*Arthrinium phaeospermum*	*AP*
	Vibrio anguillarum	*VA*
	Aspergillacea	*AS*
Paulownia	*Arthrinium phaeospermum*	*AP*
	Vibrio anguillarum	*VA*
	Aspergillacea	*AS*

water, 0.001 g/ml mercury chloride solution, scalpels etc., that were placed in a clean bench and sterilized by UV lamp for 1 h. The small pieces were washed by three times with 0.1% mercury chloride solution, soaked for 1 min each time, then washed with sterile water for 3–5 min, and finally the wooden blocks were cut with a sterile scalpel on a sterile workbench. The block was placed on the surface of the medium, and the sealed medium was cultured in a dark environment of 27 °C ± 2 °C for about one week. After the obvious mycelium grows around the small wooden block in the medium, the purification was repeated by 2–3 times according to different morphological colors until obtaining a purer fungus.

Inoculation

The moisture content test was performed using a modified decay jar test with vermiculite instead of soil, as outlined in Robinson et al. (2009), to avoid eventual influence of soil substrates on pigment formation. Jars with plastic lids (250 mL) were prepared with 15 g vermiculite and 30 mL water. Before incubating the fungi, blocks were oven dried at 60 °C for 48 h and weighed, and then a piece of wood was put into the jar.

Culture jars with vermiculite, water and wood samples were autoclaved for 40 min at 121 °C. The entire sterile bench was UV sterilized for 30 min before inoculated. After sterilizing, the inoculum strip (approximately 20 mm × 20 mm agar with actively growing mycelium) was placed on both sides of the block (longitudinal section), and the block was covered with perlite, and the jar was sealed. Inoculum consisted of mycelium and agar approximately 20 mm × 20 mm cut from an actively growing culture in a Petri dish. Each fungus was repeated in 20 jars for one wood, and a total of 120 jars were used. After inoculating, jars were placed in an incubation room (27 °C ± 2 °C, 80% ± 5% RH) and incubated in the dark. There were 20 replicate jars of each pairing made (Table 1). After meeting the incubation time, mycelium was scraped off and blocks were evaluated externally for bleaching, pigmentation, and zone lines. Blocks were then cut in half perpendicular to the grain on the transverse plane and one internal side was also evaluated. All internal spalting, pigmentation, and zone lines were noted for the internal face being evaluated. At 4, 6, 8, and 10 weeks, five jars of each combination (a fungus combines a wood) were removed, and those blocks were cleaned, oven dried (60 °C for 48 h), evaluated for color change, and then weighed again (to determine percent weight loss).

Pigment evaluation

Pigment evaluation involved RGB distribution, amount of spalting and dyeing depth. Firstly, the wood samples inoculated fungi were dried and scanned with Epson Expression 10000XL Scanner at 2400 dpi to obtain stain images, by which external spalting evaluation were carried out with Scion Image software, following the protocol described in Robinson et al. (2009). Secondly, RGB of these images were analyzed to explore color change three fungal-forming spalted wood by MATLAB. Finally, the sample after being scanned was cut in half to expose the inner plane and the inner surface was also scanned to analyze the internal staining. Meanwhile, the depth of internal spalted was measured by vernier caliper. And five points were selected on both sides and the average value was taken as the dyeing depth of the wood.

Weight loss analysis

Before inoculating the fungus in the wood block, the wood block was dried for 48 h, and the M was weighed; after completing the culture, the surface impurities of the wood block were taken out, dried, and weighed m_0. Where, M is oven dry weight of wood sample prior to exposure and m_0 is the oven dry weight following exposure to fungus. The calculation for weight loss rate (R) is as Eq. 1:

$$R = \frac{M - m_0}{M} \times 100\% \quad (1)$$

Results and discussion

Multivariate analysis

Multivariate analysis was performed by using fungi, wood species, and culture time as independent variables to determine which fungus produced the greatest amount of spalting in the experiment, and which wood was more suitable for spalted fungal inoculation and optimal incubation time.

RGB distribution

The obtained RGB values (Tables 2 and 3) were compared with the RGB color query comparison table to obtain their expression color. Three fungi were inoculated on *CL*, and the color changes after different incubation time were shown in Fig. 1.

The three columns of Fig. 1 were the average RGB color block of the corresponding *CL* block at week 4, 6, 8, and 10, inoculated by *AP*, *VA* and *AS*. And the specific code of corresponding color blocks (RGB color comparison table) of each color were shown on the right side of the column. From Fig. 1, we could find that when *AP* was inoculated on *CL*, the color of the wood changed from orange to dark red as the

Table 2 RGB value of *CL* (*Cunninghamia lanceolate*)

Fungus	Week											
	4			6			8			10		
	R	G	B	R	G	B	R	G	B	R	G	B
AP	200	134	85	203	108	55	152	85	49	147	79	54
VA	218	170	104	227	172	93	177	147	105	167	134	92
AS	215	165	103	227	164	93	165	134	94	162	131	92

Table 3 RGB value of *PT* (*Paulownia*)

Fungus	Week											
	4			6			8			10		
	R	G	B	R	G	B	R	G	B	R	G	B
AP	239	208	168	228	193	144	133	124	112	194	185	166
VA	240	219	162	245	224	159	203	202	177	206	199	165
AS	240	227	182	246	230	175	205	199	189	201	203	185

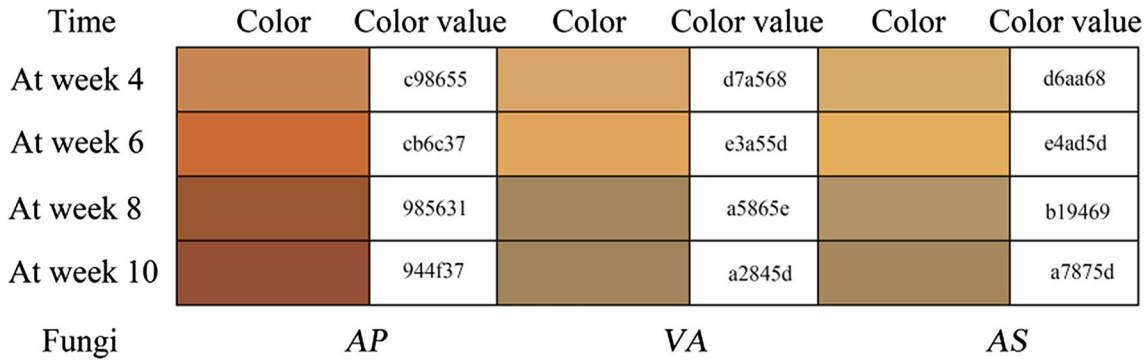

Fig. 1 Color expressed by RGB value of *CL* (*Cunninghamia lanceolate*)

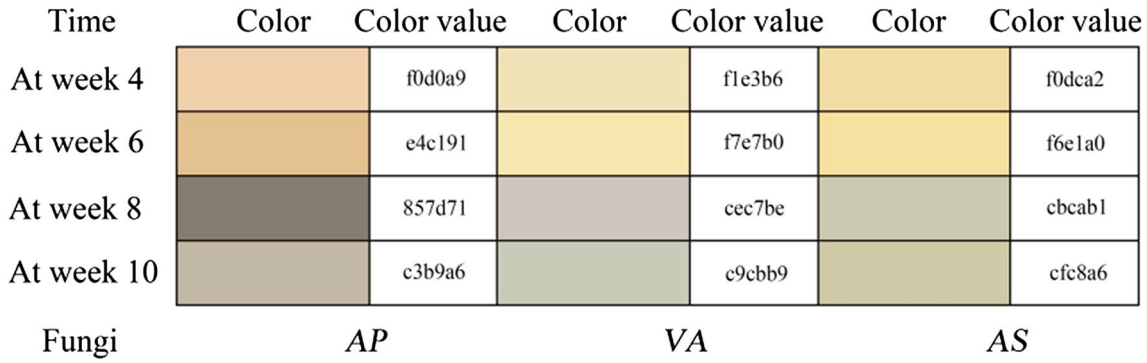

Fig. 2 Color expressed by RGB value of *PT* (Paulownia)

incubation time prolonged, but from light yellow to taupe for *VA* and *AS*. Moreover, during the first three incubation times, the color changed from orange to red; and the longer the incubation time was, the deeper the wood color was. During the last period, there was almost not color change except *AP*. The main reason was the pigment secreted by the fungus. It also could be seen that the third inoculated period, that is week 8, was the best time for dyeing *CL* for the three fungi, which agreed with the result of Robinson et al. (2007a, b).

From Fig. 1, it could be found that the maximum change of RGB was R, followed by B and G. With the extension of incubation time, the R and G values of wood decreased significantly for the three fungi. But the B value was influenced significantly by *AP*, and there is no influence for the other

two fungi. This indicated that the wood color change was more biased towards red and green gray scales.

Figure 2 showed the color change of *PT*. With the extension of the incubation period, the R and G values of *PT* have been significantly reduced. On *PT*, *AP* caused the most obvious change in wood color (The largest decrease at week 8), while *VA* and *AS* plants had not significant change. When *VA* and *AS* were inoculated into the *PT*, the B value was increased slightly at week 4, 6 and 8, but it almost did not change at week 10. On the contrary, *AP* was a special case, the B value was decreased significantly, and rebounded significantly at week 10, the color approached wood of the firstly incubation time. On account of, *AP* produced white rot at week 10, which causes wood color to gray.

From the Fig. 2 we could find that when *AP* was inoculated into *PT*, the color of the wood gradually changed from light yellow to gray as the incubation time prolonged, and eventually became grayish white; then for *VA*, the color of the wood gradually changed from light yellow to gray as the culture period prolonged, and eventually became pale grayish green at week 10; finally for *AS*, the color of the wood gradually changed from light yellow to gray as the incubation time was extended, and finally became pale grayish brown in the fourth incubation time. From this, we knew that *VA* and *AS* were not produce effective pigments to cause pronounced color difference in the wood. It could be seen that the color change of wood was not obvious at week 4 with 6, but significant changes occurred at week 8. At week 10, there were significantly external white rot on wood blocks of inoculated with *AP*, but other two fungi were not produced. This suggests that *AP* might be a white rot fungus.

In conclusion, on the one hand, *AP* could produce obvious red matter on *CL* and *PT*, among them the staining effect was not stable on *PT*, but *CL* was perfect. After the sample was placed for one year, the dyeing effect still existed on *CL*. It indicated the potential of *AP* for large-scale dyeing of wood. On the other hand, the incubation time has a close relationship with the color change of wood. The longer the incubation time, the more the amount of spalting produced by fungus, resulted in a more pronounced change in the color of the wood. The third incubation time might be the best period of pigmented material produced by the fungus during this period (Not sure the most pigmented period).

Amount of spalting

Figure 3 was the percentage average of external spalting of the wood formed by the three fungi on *CL* (Fig. 3a) and *PT* (Fig. 3b). From Fig. 3a, there was significantly more spalting on *CL* at week 10 than those at week 4, 6 and 8 ($P<0.05$). And the maximum spalting percentage was 48% for *AP*, 46% for *VA*, and 43% for *AS* at week 10, and the minimum spalting was 4%, 11% and 25%, respectively, for *AP*, *VA* and *AS* at week 4. With the extension of inoculated time, the percent of spalting gradually increased. For *CL*, there was more spalting for *AP* than those of the two other fungi. From Fig. 3b, the maximum spalting on *PT* was at week 10 for *VA* and *AS*, but at week 8 for *AP*. The highest value was significant by SPSS one-way ANOVA ($P<0.05$). The spalting caused by *AP* and *AS* was climbed steadily at week 4, 6, 8 and 10, and there was similar trend for *VA* expect at week 10, at which it was dropped down. It might be an unexpected result of the experiment.

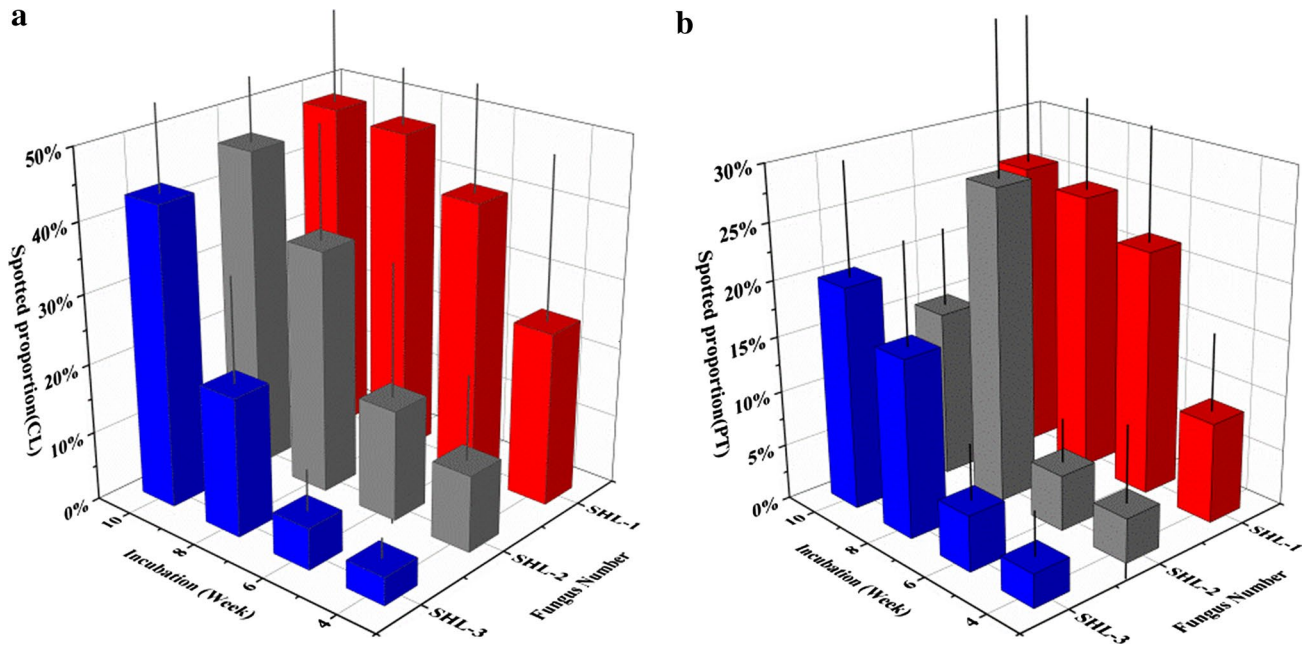

Fig. 3 **a** The percentage of spalting on *CL*; **b**The percentage of spalting on *PT*

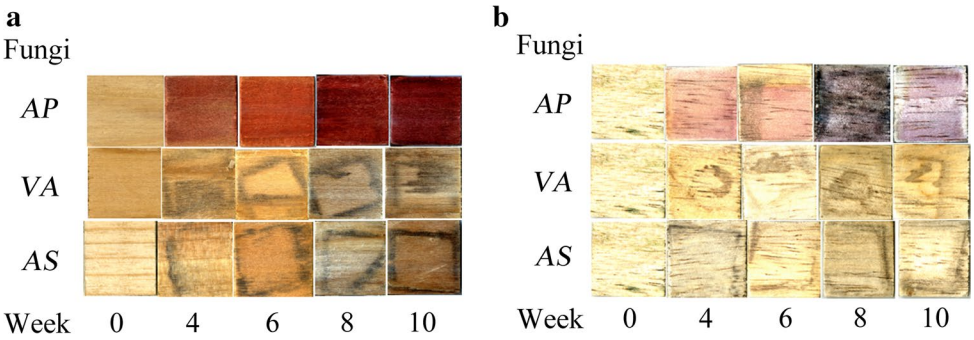

Fig. 4 **a** The part effect of three fungus dyeing on *CL*; **b** The part effect of three fungus dyeing on *PT*

Comparison of three fungus inoculated in *CL* and *PT* (Fig. 4). The external spalting on the *CL* were more than that in the *PT* at same incubation time and fungus ($P < 0.05$). Among the three fungi, *AP* external spalting was significantly higher than the other fungus at same incubation time and wood species ($P < 0.05$). Fungi had different preferences for wood, for example, *AP* preferring to *CL*. It was possible that there were some special matter in *CL*, and it could be beneficial to colonization of fungi and promote the growth of fungi or the production of pigment. In addition, *PT* might had some substances that inhibit the growth of fungi. Therefore, there had more pigment on *CT* that *PT*.

Dyeing depth analysis

The combination of *AP* and *CL* was the only one that could produce internal spalting. Therefore, the depths of these wooden blocks were measured, and the average of the depths on both sides of the spalted wood block was taken as the dyeing depth of the wood block. The average value of five test samples per batch was taken as the dyeing depth, and the curve of dyeing depth and percentage of internal spalting as the incubation time. Figure 5 shows the percentage average of internal spalting at different incubation time, and dyeing depth. The mean number of internally spalting and dyeing depth varied significantly depending on time. Internal spalting and dyeing depth were steadily increased for weeks 4, 6, 8 and 10. Later weeks (8 and 10) showed significantly more internally spalting than earlier weeks. It could be seen from Fig. 5 that the minimum average depth was 1.8 mm at week 4, and the maximum average depth was 5.06 mm at week 10. In addition, the dyeing depth of a single wood block reached 7 mm at week 10. Summarizing the internal spalting of *AP*, it could be found that the extension of the incubation time has a significant influence and regularity on the generation of internal spalting.

In the early stages, *AP* could observe significant pigment diffusion on the solid medium, so the pigment might be an extracellular pigment with good diffusibility. So, as the incubation time, the more pigments were produced by the fungus, the deeper the pigment penetrates the wood and

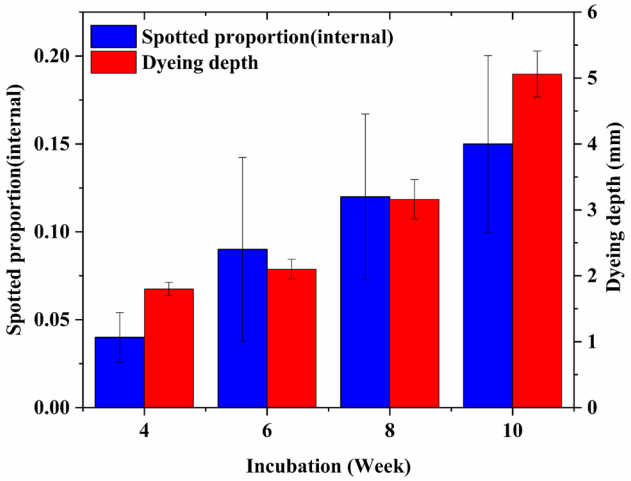

Fig. 5 The depth of internal spalting formed by fungi *AP* on *CL* blocks, and the percentage of internal spalting by fungi *AP* on *CL* blocks

the larger the proportion of internal spalting. However, it was not prove that the depth of the pigment was caused by physical infiltration or colonization of hyphae in the experiment. Again, results indicate that *AP* was a better fungus for spalting due to the higher levels of dyeing depth and internally spalting.

Weight loss

Figure 6 showed the weight loss of the spalted wood block, when the three fungi were inoculated on two fast-growing wood.

Significant difference was analyzed by SPSS one-way ANOVA, and it was found that the influence of incubation time on wood weight loss was not significant ($P > 0.05$). When the three fungus were inoculated into *PT*, the maximum weight loss rate was 16.27% (*AP*, at week 10), and the minimum value was 0.40% (*AS*, at week 4). On *CL*, the maximum weight loss rate was 1.92% (*AP*, at week 6), and the minimum value was 0.90% (*AS*, at week 4). When *AP* was inoculated, the weight loss of *PT* and *CL* was significantly

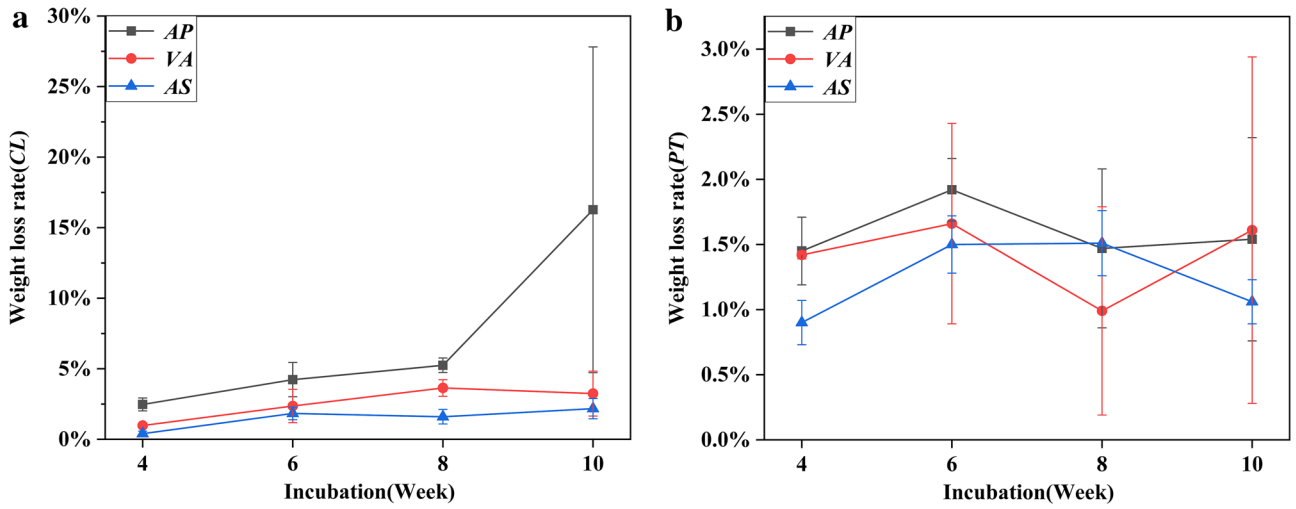

Fig. 6 **a** Weight loss rate of *CL* block; **b**) Weight loss rate of *PT* block

different. Surprisingly, *PT* had lower external spalting (pigment), but significantly higher weight loss than *CL*. Especially, the weight loss of *PT* was significantly higher than that of *CL* at week 10, and the other two fungi were not exception. Among the three fungi, only *PT* inoculated with *AP* showed a regular increase in weight loss with the extension of incubation time, but other fungus and wood combination was not obvious change. Combined with the weight loss rates of the wood, the results showed that there was no significant relationship between the weight loss of *CL* blocks with fungi and incubation time.

Conclusion

In this work, *AP* (*Arthrinium phaeospermum*), *VA* (*Vibrio anguillarum*) and *AS* (*Aspergillacea*) were inoculated on *CL* (*Cunninghamia lanceolate*) and *PT* (*Paulownia*), respectively, and then wood samples were evaluated. The results showed that *AP* could produce the most percentage average of external and internal spalting on *CL*, which were 48% and 15%, respectively (The average staining depth was 5.06 mm). And the weight loss of the combination was low (0.46 g), which had little influence on wood strength. The results showed that *AP* was an ideal wood staining fungus, which could produce pigment stably and had a broad application prospect. *CL* was an ideal application in wood fungus dyeing.

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