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FUNGICIDAL ACTIVITY AND BAMBOO PRESERVATION OF *PINUS ELLIOTTII* NEEDLES EXTRACTS

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ABSTRACT

China is rich in bamboo resources. But there has been a huge gap in timber supply. The most effective solution to eliminate the existing timber supply gap is to carry out the industrialization of bamboo so that the bamboo resources can be fully used. Bamboo is full of nutriments, but it is perishable and difficult to store. Usually bamboo is vulnerable to mildew and insects. Therefore, it is of great significance to research bamboo mold corrosion protection technology for the high-efficient development of bamboo resources. The antifungal activity of different solvent extracting tests were conducted from *Pinus elliottii* needles (hexane extract(W1), ethyl acetate extract (W2),

anhydrous ethanol extract (W3) and water extract (W4)) in white-rot fungus *Coriolus versicolor* and brown-rot fungi *Gloeophyllum trabeum*, *Polyporus vaporaria* Fr. The tests concequence verified that *Pinus elliottii* needles extracts W1 and W2 have better inhibitory effect on white-rot fungus *Coriolus versicolor* and brown-rot fungi *Gloeophyllum trabeum*, *Polyporus vaporaria* Fr, which indicated that W1 and W2 had possibility to develop as natural fungicide. Further analysis of indoor anti-corrosion of bamboo indicated that W1 and W2 played the best role in bamboo antiseptic effect. The latest research manifested that W1 and W2 have potential to be developed as natural bamboo preservative.

KEYWORDS: Fungicidal activity, bamboo preservation, *Pinus elliottii* needles extracts, white-rot fungi, brown-rot fungi, *Coriolus versicolor*, *Gloeophyllum trabeum*, *Polyporus vaporaria* Fr.

INTRODUCTION

China is rich in bamboo resources, but has a shortage of timber supply. The most effective solution to ease this timber shortage situation is to utilize bamboo as substitutes for timbers (Erfen 2017). Bamboo is vulnerable to mildew and insects which hinder its potential applications in indoor application such as furniture and flooring. There is an urgency of carrying out fundamental research to fully understand bamboo mold corrosion process and develop bamboo protection technologies.

White-rot and brown-rot funguses are responsible for bamboo fungal degradation. Preservatives chemically synthesized from organic compounds are toxic to human bodies (Bolin and Smith 2011, de Medeiros et al. 2016, Kartal et al. 2015, Lin et al. 2009, Wang et al. 2016), and not applicable for indoor applications. Therefore, seeking for environmentally friendly wood preservatives is future research direction (de Medeiros et al. 2016, De Vetter et al. 2006, Hill et al. 2004, Kiran and Raveesha 2005, Li et al. 2013, Mai and Militz 2004, Qi and Jellison 2004, Salem et al. 2016, Wang et al. 2005). Using natural plants and marine extracts as preservatives for bamboo materials is one of the alternatives (Jeloková and Šindler 1997, Kirker et al. 2013, Li et al. 2013, Mansour et al. 2015, Mburu et al. 2007, Mohammed et al. 2016, Nakayama et al. 2001, Philp et al. 1995, Qi and Jellison 2004, Salem et al. 2016, Syofuna et al. 2012, Tascioglu et al. 2013, Wang et al. 2005).

Studies showed that good antimicrobial activities were found in natural extracts from plants and marine life (Chen et al. 2013, Cheng et al. 2006, Hammer et al. 2002, Hassiotis and Dina, 2011, Jing et al. 2014, Juárez et al. 2015, Kedia et al. 2014, Pawar and Thaker 2006, Regnier et al. 2014, Salem et al. 2016, Vieira et al. 2014, Wang et al. 2005, Yang and Clausen 2007, Zabka et al. 2009, Pánek et al. 2014). Aromatic leaf alcohols, citral, and eugenol are essential ingredients of natural compounds restraining rot fungi in wood (Chang et al. 1999, Cheng et al. 2004, Kondo and Imamura 1986, Morita et al. 1997, Wang et al. 2005, Wu et al. 2005, Yen and Chang 2008). Humar and Lesar (2013) indicated that wax emulsion and grease compounds had a significant inhibition effect on rot fungi in wood. Obviously, most natural plants and marine organisms will have better antifungal activities because of their aliphatic, phenolic, and terpene compounds.

This paper investigated antifungal activities of different extracts from *Pinus elliottii* needles to white-rot fungus *Coriolus versicolor* and brown-rot fungi *Gloeophyllum trabeum*, *Polyporus vaporaria* Fr in bamboo.

MATERIALS AND METHODS

Materials

Pinus elliottii needles were gathered from Miluo, Yueyang, Hunan Province, in China, and processed through the following steps: washing, being dried in an oven at 50 to 60°C, grinding, and extracting (Basarian and Tahir 2017, Yang et al. 2017). Extracted samples were kept in airtight polythene bags stored at 4°C condition.

Bamboo (*Phyllostachys pubescens*) materials were from Taojiang, Yiyang, Hunan Province, China. Bamboo samples were selected using the following steps: (selecting a test material from the ground base 1.3 m above the interception of 1 m), shaving off bamboo green and outer, cut shaven pieces into 20 (long) ×20 (wide) ×10 mm (thick) (length in texture direction).

Three fungus strains, white-rot fungus *Coriolus versicolor* CFCC-5336, brown-rot fungi *Gloeophyllum trabeum* CFCC-86019 and *Polyporus vaporaria* Fr. CFCC-5608 were obtained from China Forestry Culture Collection Center (Beijing, China). The applied solvents and chemicals were all reached the analytical level from Hunan Chemical Reagent Factory (Changsha, China) and the receiving and drying procedures are all based on the standard principle, unless otherwise mentioned.

Obtaining the Eucommia ulmoides leaf extractive solutions

Weighing out 200 g crushed *Pinus elliottii* needles, in turn, petroleum ether, ethyl Acetate, anhydrous ethanol and water as solvent, the liquid ratio is 1:10 (M/V) ultrasound 10 min, the back of the extraction of the back -2 h, first sieve mesh coarse filter, then decompression pumping, combined with filtrate, filtrate decompression concentrated, recovery of organic solvents, extract 60°C vacuum drying paste (hexane extract (W1), ethyl acetate extract (W2), anhydrous ethanol extract (W3) and water extract (W4)). And then keep them in the refrigeration of the temperature of 4°C. In the end, repeat three times the above same extracting steps and merge the three extracts (Yasin et al. 2017).

Antifungal evaluation

The antifungal ability of different extracts from *Pinus elliottii* needles was evaluated through following steps. Each of 32, 20, 16, 8, 4 and 2 $mg \cdot m^{-1}$ *Pinus elliottii* needles extracts was first added to the sterilization of potato dextrose agar (PDA) placed in a Petri dish (dia. 9 cm), respectively, followed by transferring the mycelium of the strains to the Petri dish cultivated at 26±2°C and 70% RH. Each sample was repeated 3 times. After 10-14 days of the blank to the same fungal mycelium reaching the control edge, antifungal index was calculated using the formula:

$$\text{Antifungal index} = (1-D_a/D_b) \times 100 \quad (\%)$$

where: D_a - growth zone diameter in experimental plate (cm),
D_b - growth zone diameter in control plate (cm).

Wood treatment

Five gradient concentrations of solution standby were prepared for each preservative specimen (GB/T 13942.1, 1992). Bamboo specimens measured 20 × 20 ×10 mm (LY/T 1283, 1998) (texture direction). Numbered specimens were put into a blast dryer at 40±2°C, dried constant weight. Dried specimens were taken out and weighted with the precise to 0.01 g. Preservative solution was

loaded in the vacuum dryer. Specimens were immersed for 30 min, and then taken out. Treated specimens were weighted right after removing leftover liquid on their surface using sterilized absorbent paper. The retention representing the preservative absorption capacity in tested samples ($kg \cdot m^{-3}$) was calculated using the equation:

$$\text{Retention} = [(M_2-M_1) \times c/V] \times 10$$

where: M_1 - the weight of a specimen before preservative treatment (g),
M_2 - the weight of a specimen after preservative treatment (g),
c - the preservative solution concentration (%),
V - the sample volume (cm^3).

Decay resistance evaluation

In accordance with the method of LY/T 1283-1998, when the specimen lost and volatile after treatment, weigh out M_3 (keeping the precise to 0.01g). The same set of doses of the specimen with multi-layer gauze is wrapped in a steam sterilizer for treatment under atmospheric pressure 105±2°C for use after cooling. As per the method of LY/T 1283-1998, *Pinus massoniana* sapwood is adopted and the cross-section size can be kept in same with the specimen or slightly larger than the specimen and the thickness is 3-5 cm. After preparation of agar medium and river sand sawdust medium, inoculation, put into feed wood, to feed the surface of the wood is fully covered by mycelium, put in the processing of the bamboo specimen and maintain the temperature at 28±2°C and keep the relative moisture content at 75%-85%. The specimen can be removed after 12 weeks. Gently scrape the mycelium and impurities surface and then put them in a 40°C air drying oven to bake to a constant weight, finally weigh out M_4 (keep the precise to 0.01 g). And work out the mass loss rate of bamboo after the decay of the sample, which can be expressed as a percentage: $ML=((M_3-M_4)/M_3) \times 100\%$, ML is the sample mass loss rate (%).

RESULTS AND DISCUSSION

Antifungal activity analysis

Make a comparison and assessment of the weight loss and the values shown in Tab. 1.

Tab. 1: Durability rating scale according to GB/T 13942.1-92.

Durability class	Description	Mass loss (%)
1	Very durable	≤10
2	Durable	>10 to ≤25
3	Slightly durable	>25 to ≤45
4	Not durable	>45

Fig. 1 shows the results of antifungal activity (antifungal index?) of the extracts at the concentration of 32 $mg \cdot ml^{-1}$ from *Pinus elliottii* needles against white-rot fungus *Coriolus versicolor* and brown-rot fungi *Gloeophyllum trabeum*, *Polyporus vaporaria* Fr. W1 is the best activity against white-rot fungus *Coriolus versicolor* and the antifungal index was higher than 75.57%. In contrast, the other extracts from *Pinus elliottii* needles (W2, W3, W4) were the relative lower activity and the antifungal index were lower than 50%. In the same way, extracts of *Pinus elliottii* needles W1, W2 and W3 at the concentration of 32 $mg \cdot ml^{-1}$ have strong antifungal activity and the

antifungal index is higher than 68.49% against brown-rot fungi *Gloeophyllum trabeum*, but W4 did not express antifungal activities at the same concentration with the antifungal index lower than 40%. Besides, extracts from *Pinus elliottii* needles W1, W2 and W3 at the concentration of 32 mg·ml^{-1} shows fairly strong antifungal activity and the antifungal index is higher than 70% against brown-rot fungi *Polyporus vaporaria* Fr., while W4 did not express antifungal activities at the same concentration with antifungal index lower than 30%. From the above we can come into the conclusion that the extracts from *Pinus elliottii* needles W1 expressed the best antifungal activity against white-rot fungus *Coriolus versicolor* and brown-rot fungi *Gloeophyllum trabeum*, *Polyporus vaporaria* Fr.

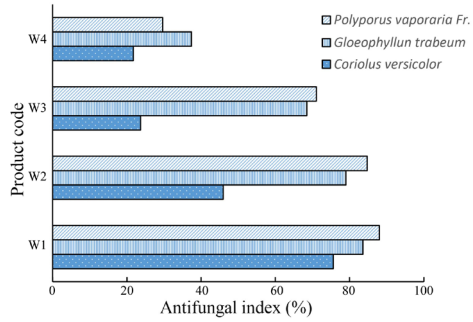

Fig. 1: Antifungal index (%) of different extracts from Pinus elliottii needles (W1,W2,W3,W4) against white-rot fungus Coriolus versicolor and brown-rot fungi Gloeophyllum trabeum, Polyporus vaporaria Fr. at the concentration 32 mg·ml^{-1}.

Tab. 2 summarizes the IC50 values of 4 extracts from *Pinus elliottii* needles (W1, W2, W3, W4). The antifungal activity IC50 values against white-rot fungus *Coriolus versicolor* are ranked as follows: W1 (8.44 mg·ml^{-1})> W2 (24.17 mg·ml^{-1}). Similarly, antifungal activity IC50 values of W1, W2, W3 and W4 against brown-rot fungi *Gloeophyllum trabeum* keep the order: W1 (9.29 mg·ml^{-1})> W2 (15.86 mg·ml^{-1}) > W3 (19.12 mg·ml^{-1}) > W4 (29.14 mg·ml^{-1}). Additionally, the IC50 values order against brown-rot fungi *Polyporus vaporaria* Fr. are W2 (8.88 mg·ml^{-1})> W1 (10.51 mg·ml^{-1}) > W3 (13.93 mg·ml^{-1}).

Tab. 2: IC50 values (mg·ml^{-1}) of different extracts from Pinus elliottii needles (W1,W2,W3,W4) against white-rot fungus Coriolus versicolor and brown-rot fungi Gloeophyllum trabeum, Polyporus vaporaria Fr.

Product code	Fungi		
	Coriolus versicolor	*Gloeophyllum trabeum*	*Polyporus vaporaria* Fr.
W1	8.44	9.29	10.51
W2	24.17	15.86	8.88
W3	>32	19.12	13.93
W4	>32	29.14	>32

These results indicated that W1 and W2 was the strongest antifungal activity against the 3 tested fungi. The corresponding IC_{50} values against white-rot fungus *Coriolus versicolor* and brown-rot fungi *Gloeophyllum trabeum*, *Polyporus vaporaria* Fr. were 8.44 mg·ml^{-1}, 9.29 mg·ml^{-1} and 10.51 mg·ml^{-1} respectively. The corresponding IC50 values of W2 against white-rot fungus

Coriolus versicolor and brown-rot fungi *Gloeophyllum trabeum*, *Polyporus vaporaria* Fr. were 24.17, 15.86, and 8.88 mg·ml^{-1} respectively.

Previous studies (Abdelgaleil 2010, Cheng et al. 2008, Regnault-Roger and Hamraoui, 1995, Xie et al. 2014, Yen and Chang 2008) indicated that the main antifungal substances of water extracts are organic acids and alcohol extracts contain abundant antifungal flavonoids. There are more hydrocarbon compounds (olefins, alkanes) and phenolic alcohols in the extracts of hexane and ethyl acetate (Cheng et al. 2006, Pandey et al. 2012, Wang et al. 2005, Xie et al. 2014). Aromatic leaf alcohols, citral, eugenol, and other ingredients are found in the natural compounds which have effective inhibition effects on wood rot fungi. All these findings indicated that W1 and W2 can be potentially developed into natural bamboo preservatives.

Preservative retention of preservative pre-treatment of bamboo

Tabs. 3, 4, and 5 summarize the retentions of different preservatives in treated bamboo samples. It is a linear relationship that the retention of four preservatives increases along with the increase of the preservative concentration.

Tab. 3: Preservative retention (kg·m^{-3}), mass loss (ML, %) and durability class (DC) of untreated and preservative pre-treatment bamboo treated with white-rot fungus Coriolus versicolor.

Treatment	Concentration (%)	*Coriolus versicolor*		
		Preservative retention (kg·m^{-3})	ML (%)	DC
Untreated			24.56	2
W1	0.2	0.58	16.89	2
	0.4	1.19	13.91	2
	0.8	2.39	10.67	2
	1.6	2.78	8.54	1
	3.2	3.21	4.51	1
W2	0.2	0.48	22.49	2
	0.4	0.95	18.58	2
	0.8	1.87	15.81	2
	1.6	2.31	10.84	2
	3.2	2.43	8.56	1
W3	0.2	0.50	24.93	2
	0.4	0.88	19.09	2
	0.8	1.58	18.89	2
	1.6	1.99	13.98	2
	3.2	2.02	13.88	2
W4	0.2	0.46	24.41	2
	0.4	1.09	17.00	2
	0.8	2.57	17.51	2
	1.6	2.64	13.85	2
	3.2	2.89	13.64	2

Tab. 4: Preservative retention (kg·m^{-3}), mass loss (ML, %) and durability class (DC) of untreated and preservative pre-treatment bamboo treated with brown-rot fungi Gloeophyllum trabeum.

Treatment	Concentration (%)	*Gloeophyllum trabeum*		
		Preservative retention (kg·m^{-3})	ML (%)	DC
Untreated			16.99	2
W1	0.2	0.60	14.49	2
	0.4	1.23	10.81	2
	0.8	2.19	8.28	1
	1.6	2.32	6.07	1
	3.2	2.85	4.64	1
W2	0.2	0.59	16.15	2
	0.4	1.16	12.78	2
	0.8	2.09	11.28	2
	1.6	2.19	11.40	2
	3.2	2.54	11.25	2
W3	0.2	0.62	16.43	2
	0.4	1.07	14.23	2
	0.8	1.77	14.11	2
	1.6	1.88	12.22	2
	3.2	2.11	11.81	2
W4	0.2	0.57	16.54	2
	0.4	1.33	16.00	2
	0.8	2.37	16.30	2
	1.6	2.50	14.15	2
	3.2	3.02	13.49	2

Tab. 5: Preservative retention (kg·m^{-3}), mass loss (ML, %) and durability class (DC) of untreated and preservative pre-treatment bamboo treated with brown-rot fungi Polyporus vaporaria Fr.

Treatment	Concentration (%)	*Polyporus vaporaria* Fr.		
		Preservative retention (kg·m^{-3})	ML (%)	DC
Untreated			17.24	2
W1	0.2	0.62	13.78	2
	0.4	1.11	12.47	2
	0.8	2.23	8.22	1
	1.6	2.81	7.68	1
	3.2	3.96	6.14	1
W2	0.2	0.54	16.97	2
	0.4	0.86	13.35	2
	0.8	1.55	10.90	2
	1.6	2.61	9.40	1
	3.2	3.27	8.10	1

W3	0.2	0.55	16.43	2
	0.4	0.96	13.23	2
	0.8	1.94	13.11	2
	1.6	2.79	12.22	2
	3.2	3.43	11.81	2
W4	0.2	0.55	15.53	2
	0.4	1.08	13.00	2
	0.8	2.24	13.30	2
	1.6	3.24	13.15	2
	3.2	3.92	12.50	2

In short, the W2 and W3 loaded doses were lower than those of the W1 and W4 when the preservative concentrations are the same. The data indicate that the preservative retention of preservative pre-treatment of bamboo is much lower than that of wood. When the preservative concentration reaches 3.2%, the preservative retention of the W1 takes up the highest 3.96 and the preservative retention of the W4 is only 3.92 from the average of 3 decay fungi in bamboo preservative treatment.

Resistance to fungal decay of preservative pre-treatment of bamboo in relation to Retention

Figs. 2, 3 and 4 show the corresponding results of mass loss in terms of preservative retention of preservative pre-treatment bamboo against white-rot fungus *Coriolus versicolor* and brown-rot fungi *Gloeophyllum trabeum*, *Polyporus vaporaria* Fr.

Fig. 2 is a response curve between preservative retention and mass loss for preservative pre-treatment bamboo against white-rot fungus *Coriolus versicolor*. It shows such a relationship: when the preservative retention is low, the mass loss rate of the W2, W3, and W4 is less significant and the mass loss rate of the W1 treatment decreases. Along with the increase of preservative retention, the mass loss rate of all preservatives treated samples will decrease. When the mass loss rate of W1 and W2 processing samples keep the preservative retention decreasing trend, the decreasing trend of W3, W4 will be rather smooth. On the basis of China National standard GB/T 13942.1-92, the obtained mass losses are the results of a durability class as per the criteria given in Tab. 1. The preservative retention, the corresponding mass losses and durability classes obtained for unleached samples against white-rot fungus *Coriolus versicolor* are showed in Tab. 3. W1 and W2 will reach certain preservative retention when the protection of bamboo has the durability class 1 level, which means that products W1 and W2 can adequately protect the wood at preservative retention of approximately 2.7, and 2.4 $kg \cdot m^{-3}$, respectively. The weight loss rate of W1 processing samples will be less than 5% when preservative retention reaches 3.21 $kg \cdot m^{-3}$.

Fig. 3 is a response curve between preservative retention and mass loss for preservative pre-treatment bamboo against brown-rot fungi *Gloeophyllum trabeum*. When the low preservative retention is comparatively not treated, the mass loss rate of W2, W3, and W4 will be less significant, and the mass loss rate of W1 treatment will reduce. In pace with the increase of preservative retention, the mass loss rate of W1 treatment samples is plummeting. When preservative retention reaches 2 $kg \cdot m^{-3}$, the mass loss rate drops begin to become relaxed and the change becomes smaller. Relatively Speaking, the change of W3 and W4 are very small along with the changing trend of preservative retention. The preservative retention, the corresponding mass losses, and durability classes obtained for unleached samples against brown-rot fungi

Gloeophyllum trabeum are showed in Tab. 4. When the protection of bamboo has the durability class 1 level, W1 will reach certain preservative retention, which means that at preservative retention of approximately 1.5 kg·m^{-3}, products W1 can adequately protect the wood.

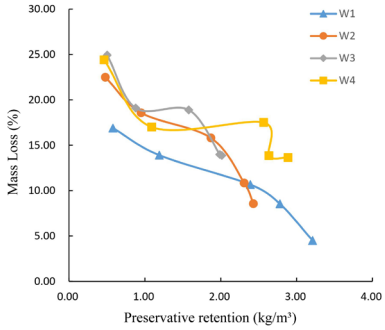

Fig. 2: Mass loss in relation to preservative retention of preservative pre-treatment bamboo treated with white-rot fungus Coriolus versicolor

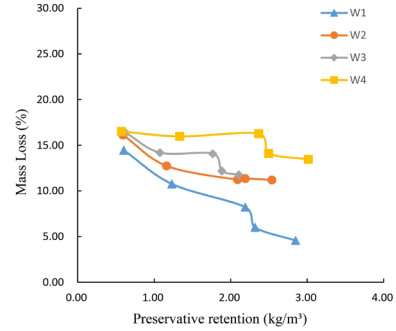

Fig. 3: Mass loss in relation to preservative retention of preservative pre-treatment bamboo treated with brown-rot fungi Gloeophyllum trabeum.

Fig. 4 is a response curve between preservative retention and mass loss for preservative pre-treatment bamboo against brown-rot fungi *Polyporus vaporaria* Fr. When the low preservative retention is relatively not treated, the mass loss rate of W2, W3, W4 is less significant, and the mass loss rate of W1 treatment will decrease.

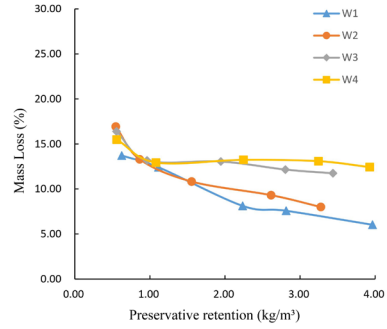

Fig. 4: Mass loss (%) in relation to preservative retention of preservative pre-treatment bamboo treated with brown-rot fungi Polyporus vaporaria Fr.

The mass loss rate of 4 kinds of preservatives treated samples will decrease along with the increase of preservative retention. The mass loss rate of W1, W2 treatment samples decreased apparently with the decreasing of preservative retention, and the W3 and W4 start to decrease drastically when the preservative retention changes. When the preservative retention reached 1%, the sagging tendency become stable and fewer changes occur (Cheung et al. 2017). The preservative retention, the corresponding mass losses, and durability classes obtained for unleached samples against brown-rot fungi *Polyporus vaporaria* Fr. are presented in Tab. 5. When the protection of bamboo has the durability class 1 level, W1 and W2 reaches certain preservative

retention, which means that at preservative retention of approximately 2.2 and 2.6 $kg \cdot m^{-3}$, products W1 and W2 can adequately protect the wood (Hamad et al. 2017).

CONCLUSIONS

Judging from the existing research results, the *Pinus elliottii* needles extract W1 (hexane extract) and W2 (ethyl acetate extract) own the best and strongest fungal activities treated with the three tested fungi. Further experiments demonstrated that it is practicable that they can be utilized for bamboo preservation as a preservative. As thus, the *Pinus elliottii* needles extract W1 and W2 have the potential to develop as a natural fungicide and it will be a suitable substitute for synthetic preservatives in bamboo preservatives. Nevertheless, the relative studies have not stopped here. The ingredients of inhibiting decaying fungi in W1 (hexane extract) and W2 (ethyl acetate extract) and their mechanism of inhibition of bamboo decay are still in suspense and remain to be studied. Moreover, we can take advantage of these results to choose plants to inhibit the active ingredients of bamboo rot fungi and to discover the natural plant extracts to be used in new fungicides.

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