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ORIGINAL ARTICLE

Mould-resistance of bamboo treated with the compound of chitosan-copper complex and organic fungicides

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Abstract Bamboo has received increasing attention as an alternative raw material for wood in the late twentieth century for its fast growing nature and good mechanical properties. But bamboo is readily discolored by mould fungi, which greatly limits the applications of bamboo. In this paper, mould-resistance of moso bamboo treated with chitosan-copper complex (CCC®), propiconazole (PPA), tebuconazole (TBA), the compound of CCC® and PPA or TBA was reported. Results showed that CCC® or PPA used alone as bamboo-mould inhibiter could defer or restrain the growth of *Penicillium citrinum* Thom (*P. citrinum*), while not being effective against *Trichoderma viride* Pers. ex Fr (*T. viride*) and *Aspergillus niger* V. Tiegh (*A. niger*). However, the compound of CCC® and PPA could inhibit all mould fungi in the test, showing a good synergetic effect. Additionally, TBA had better resisting effect against *P. citrinum* and *T. viride* than against *A. niger*, but showed no synergetic effect with CCC®.

Keywords Bamboo · Chitosan-copper complex · Mould-resistant effect · Organic fungicides · Synergistic effect

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Introduction

The ecological crisis facing the world brings sustainable development ideas to the people. A lot of countries imposed restrictions on felling natural forests, which caused the diminishing of wood resources [1]. Therefore, the world's attention is focused on the need to identify substitute materials. In view of its rapid growth (exceeding most fast growing trees) and wide availability, bamboo emerges as a very suitable alternative [2, 3]. Bamboo products have found more and more applications in daily necessities, furniture, crafts, indoor decoration, constructions, and so on [4–6]. With the development of adhesive bonding and processing technology, strand woven outdoor flooring has drawn more and more companies' attentions in China, and the products were loved in the United States, Europe, Southeast Asia, and so on [7, 8]. But as a natural material, bamboo is readily deteriorated by mould fungi, decay fungi, and insects, especially mould fungi, which greatly limits the application of bamboo, leading to much more economic losses and wastage of bamboo resources.

To protect bamboo from mould fungi, sodium pentachlorophenate (PCP-Na) was widely used for its high effectiveness and relative low cost, but faced rejection due to the public concerns on the environment. The implementation of the Stockholm Convention on Persistent Organic Pollutants in 2004 further limited the production and application of PCP-Na [9]. The PCP-Na alternatives used widely are mainly from the successful wood preservatives, such as chromated copper arsenate (CCA), amine copper quat (ACQ) or copper azole (CA). But concerns on the leaching of copper from treated wood to the environment and their bad effects against mould fungi limited the application of these preservatives [10–12]. Non-metallic and organic preservatives such as borax, 2-decyl dimethyl ammonium chloride (DDAC),

propiconazole, and tebuconazole 3-iodo-2-propyl-butyl carbamate (IPBC), and so on are developed or modified as mould-resistant chemicals. However, considerable research is still under way regarding the effectiveness against mould fungi and higher cost compared with PCP-Na.

Chitosan (deacetylated chitin) has a broad spectrum of unique biological activities [13–15]. Chitosan-copper complex (CCC®), produced from the chemical reactions between chitosan and copper salts, was shown to be a low-toxic preservative for wood and bamboo [16–21]. Bamboo treated with CCC® had better resistance against *Penicillium citrinum* Thom and *Trichoderma viride* Pers. ex Fr, but poor against *Aspergillus niger* V. Tiegh [20]. In order to improve the resistance of CCC® against a wide variety of the mould fungi, a compound of CCC® and organic fungicides was prepared and tested under laboratory conditions.

Experimental

Materials

Moso bamboo (*Phyllostachys pubescens* Mazel ex H. de Lehaie) was selected due to its popularity, abundance, and value in the world. Bamboo samples, 4 years old, were collected from Sankou town, Lin'an city, Zhejiang, China, on May 17th, 2009. A segment of 2–4 m of bamboo from bottom, with green and yellow faces planed off, were chosen and machined into specimens without wormhole and mildew. The dimension was 50 mm (length) by 20 mm (width) by 5 mm (thickness). The specimens were then weighed, measured, and marked.

Three kinds of mould fungi including *Trichoderma viride* Pers. ex Fr (*T. viride*), *P. citrinum* Thom (*P. citrinum*) and *Aspergillus niger* V. Tiegh (*A. niger*), separated from natural mildew bamboo by microorganism group of Zhejiang A&F University, were applied in mould-resistance test. These fungi are common species on bamboo [22]. Fungal resistant test was conducted referring to "Standard Method for Testing Fungicides for Controlling Sapstain and Mould on Unseasoned Lumber" [23] and "Testing method for anti-mould chemicals in controlling mould and blue stain fungi on wood" [24].

Chitosan-copper complex (CCC®, weight ratio of copper ion 11.8%) was prepared by reacting copper chloride with chitosan at the ratio of 1.2:1.0, 50°C for 2 h. CCC®–propiconazole or CCC®–tebuconazole complexes were prepared by compounding CCC® with propiconazole or tebuconazole at the ratio of CCC® to organic fungicide being 1:0.5. Propiconazole and tebuconazole were prepared in water solution with the help of nonionic surfactants.

Potato dextrose agar (PDA) substrate was used as culture media in the cultivation of mould fungi. For the preparation of PDA substrate, 200 g peeled potato was cut into small pieces, and put into 1000 ml boiling water. After 30 min, the solution was filtrated quickly into 1000 ml beaker. Then water was complemented to the filtrate until 1000 ml, and 20 g glucose, 25 g agar was put into the solution. The solution was heated until the agar was melted. Thus the PDA substrate was prepared. PDA substrate was then poured evenly into petri dishes while it was hot.

Steam sterilizer was used to sterilize the specimens and petri dishes filled with PDA substrate at 121°C, 0.1 MPa for 30 min. Incubation cabinet set at a temperature of 28 ± 2°C, and a relative humidity between 90 ± 5%, was used to incubate fungi. Petri dishes with the size of 100 mm in diameter and 20 mm in height were used in mould-resistance tests.

Treatment of specimens

Six specimens per concentration of a fungicide were used for each fungus tested. Also, 6 untreated control specimens were used for each fungus tested.

The specimens to be treated with the same concentration of the same fungicide were put into 500 ml beaker, and a piece of glass was placed between the specimens to avoid clinging. After 30 min dipping in fungicide, the specimens were taken out, wiping out the superficial fungicide. The treated specimens were autoclaved at 121°C, 0.1 MPa for 30 min before inoculation.

Inoculation and incubation

Test fungi were activated by inoculating the test fungi onto test tubes filled with PDA substrate, and cultivating for several days at the temperature of 28 ± 2°C and relative humidity of 90 ± 5%. Purified water was prepared by infusing 10 ml water into test tube, stuffing with cotton plug, and wrapping in kraft, enlacing with cotton string, then sterilizing at 121°C, 0.1 MPa for 30 min.

The mycelium and spore were picked and put carefully into purified water with inoculation loop. The spore suspension was stirred frequently during inoculation. 0.25 ml spore suspension was streaked into the petri dishes filled with PDA substrate, and was scratched evenly on the substrate. The petri dishes were then placed into incubation cabinet, maintaining at a temperature of 28 ± 2°C, and relative humidity of 90 ± 5%. After 4–7 days, the fungi in petri dishes grew, and prepared to inoculate the specimens.

Before inoculation, a sterilized U-shape glass rod (3 mm in diameter) was placed on the PDA substrate which was covered with mycelium, and two specimens were put separately on the glass rod (Fig. 1). After inoculation, the dishes were moved into incubation cabinet, maintaining at a temperature of 28 ± 2°C, and relative humidity of 90 ± 5% and lasted for 30 days.

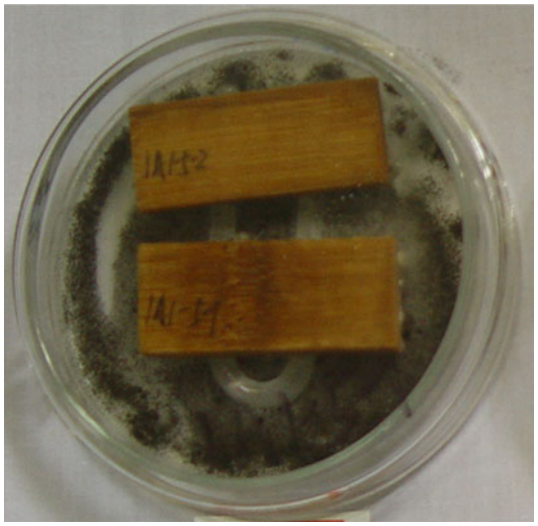

Fig. 1 Method for the inoculation

Evaluation of the test

The amount of chemicals absorbed in bamboo was calculated as follows:

$$R = (m_2 - m_1) \times 10^6/2(L \times W + W \times T + L \times T) \quad (1)$$

where R is the amount of chemicals absorbed, g/m^2; m_1 is the weight of specimens before impregnation, g; m_2 is the weight of specimens after impregnation, g; L is the length of specimens, mm; W is the width of specimens, mm; T is the thickness of specimens, mm.

Mould growth on each bamboo sample was visually rated using a scale of 0–4 (Table 1) from the next day after inoculation. The lower the infection value, the better the efficiency of chemicals.

The overall appraisal of each fungicide was defined as resisting effectiveness (RE) and obtained from the following formula:

$$\mathrm{RE} = (1 - D_t/D_0) \times 100 \quad (2)$$

where RE is the resisting effectiveness of fungicide against the three species of test fungi (including *T. viride*, *P. citrinum*, and *A. niger*); D_1 is the average infection value of the test specimens, and D_0 is the average infection value of the controls.

Results and discussion

The amount of chemicals absorbed in bamboo blocks

Different from wood, bamboo lacks radial transport systems, which has a significant influence on the efficacy of treatments. Therefore, the fungicides are mainly distributed on the surface of bamboo, and the absorption is closely related to the concentration and molecular weight of the fungicides [25].

Table 1 Standard method for rating the infection value

The infection value	Mould coverage on specimens
0	The surface of specimens have no mycelium
1	The area of mould infection <25%
2	The area of mould infection 25–50%
3	The area of mould infection 50–75%
4	The area of mould infection >75%

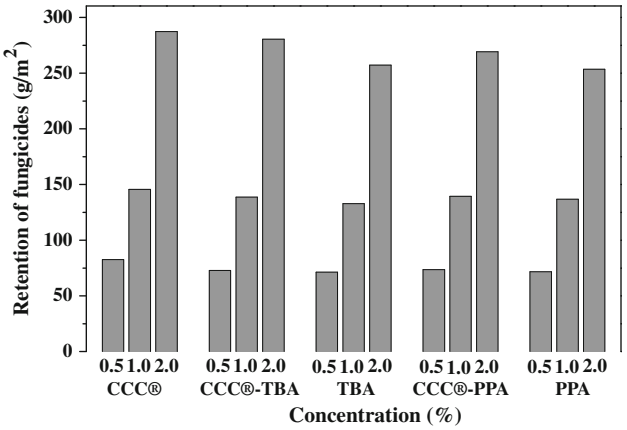

Fig. 2 Retention of fungicides in bamboo blocks

Figure 2 shows retentions of fungicides in bamboo blocks employing the same treating method. The amount of fungicides in bamboo blocks was nearly equivalent at the same concentrations among different fungicides, and with the increase of the concentration from 0.5 to 2.0%, the amount of fungicides absorbed in bamboo blocks increased from 70 to 280 g/m^2 except for CCC®. The amount of CCC absorbed in bamboo blocks was higher than organic fungicides and the compound of CCC®/organic fungicides because of the large viscosity owing to the high molecular weight ($M_w = 1.8 \times 10^5$, determined by gel permeation chromatography) of CCC®.

Resistances of different fungicides *against mould fungi*

Mould-resistant effects of different fungicides against P. citrinum

The mould-resistance of bamboo blocks treated with the test fungicides during one month's cultivation are presented in Fig. 3. Untreated bamboo blocks were seriously infected by *P. citrinum* and the face infection value reached 4 on the second day. When treated with CCC® at the concentration of 1.0% (w/v, similarly hereinafter) alone, mould infection appeared after 12 days, and grew slower than the controls. Our previous studies also revealed that CCC had certain resisting effect against *P. citrinum* at higher concentrations [20]. However, the infection value of

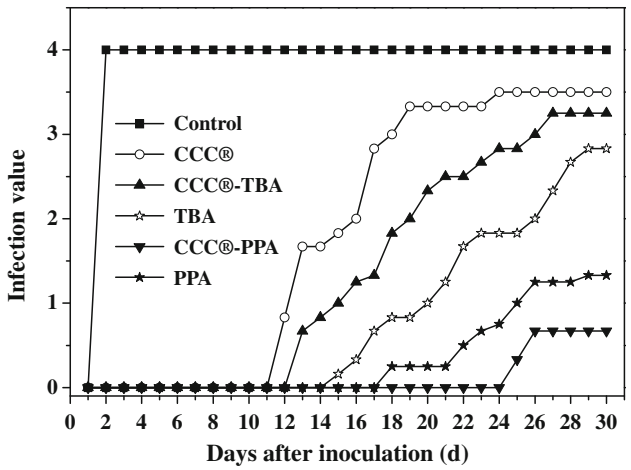

Fig. 3 Mould-resistant effect of different fungicides (1.0%, w/v) against *Penicillium citrinum* during one month's cultivation

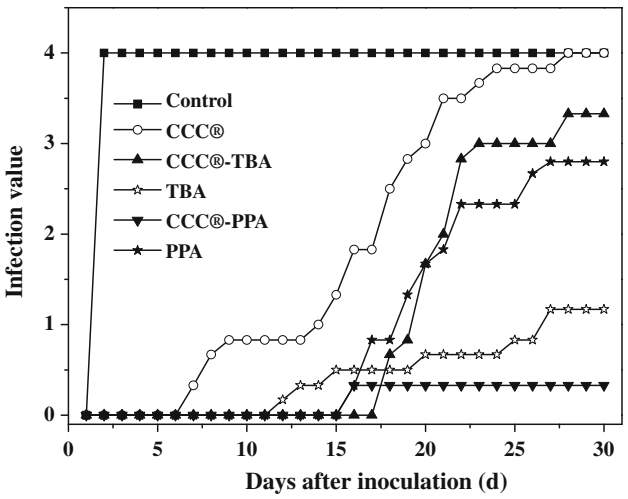

Fig. 4 Mould-resistant effect of different fungicides (1.0%, w/v) against *Trichoderma viride* during one month's cultivation

CCC®-treated bamboo reached 3.5 after a month of testing. The selected organic fungicides including TBA and PPA resisted *P. citrinum* more effectively than CCC® at the same concentrations. But neither could perfectly protect bamboo from *P. citrinum*.

Comparing the resistance of CCC® and PPA alone with that of the compound of CCC® and PPA (CCC®–PPA), one can find that the resistance of CCC®–PPA against *P. citrinum* improved significantly (Fig. 3). The initial infection of *P. citrinum* appeared after 25 days for CCC®–PPA treated bamboo, postponed 7–14 days comparing with CCC® or PPA-treated ones, and the final infection value was only 0.67. This phenomenon might result from a synergistic effect of the two fungicides. The resistance of CCC®–TBA was between CCC® and TBA, mainly from TBA, not the synergistic effect of both. The synergistic effect of organic fungicides with antioxidant or metal chelator has been reported in a number of literature [26–29]. CCC®, prepared by the reaction of chitosan and copper salts, may act as metal chelator which enhances the resistance of PPA against mould fungi. This inference can also be proved by the follows.

Mould-resistant effects of different fungicides against T. viride

Mould-resistant activities of each fungicide against *T. viride* were shown in Fig. 4. Like the activities of *P. citrinum* on the controls, mycelium of *T. viride* quickly covered all surfaces of the controls on the second day. The resistance of CCC® against *T. viride* was poorer than against *P. citrinum*, but the initial infection of mycelium on CCC®-treated blocks were also postponed and the growth rate lowered with the increased concentration. Comparing with CCC®, the two organic fungicides had better resisting

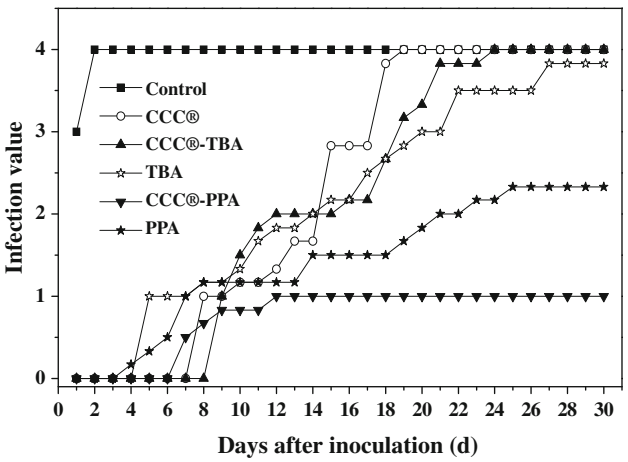

Fig. 5 Mould-resistant effect of different fungicides (1.0%, w/v) against *Aspergillus niger* during one month's cultivation

effects against *T. viride*, and between them, TBA behaved the better. Both CCC® and PPA used alone could not inhibit *T. viride* effectively, but the compound of them could resist *T. viride* much more effectively, again showing synergistic effect. No conclusions could be inferred on the synergistic effect of CCC® and TBA, because TBA alone behaved better against *T. viride*, and the infection value only reached 1.2.

Mould-resistant effect of different fungicides against A. niger

Aspergillus niger grew more vigorous than the two mould fungi mentioned above, and was very difficult to be controlled. Mycelium climbed onto the controls on the second day, and immediately spores were produced and covered all the surfaces, then the surface turned black. Comparing Figs. 3 and 4 with Fig. 5, one could find that the resistances

Table 2 Overall appraisal of the fungicides against the test fungi

Fungicides	Concentration (%)[a]	Infection value of treated bamboo[b]			Resisting effectiveness (%)
		P. citrinum	*T. viride*	*A. niger*	
Control		4.0	4.0	4.0	–
CCC®	0.5	4.0	4.0	4.0	0.0
	1.0	3.5	4.0	4.0	4.2
	2.0	2.8	3.3	4.0	15.8
PPA	0.5	3.0	3.3	3.8	15.8
	1.0	1.3	2.8	2.3	46.7
	2.0	0.8	2.3	1.7	60.0
CCC®–PPA	0.5	1.0	1.0	2.5	62.5
	1.0	0.7	0.3	1.0	83.3
	2.0	0.0	0.3	0.8	90.8
TBA	0.5	3.8	3.7	4.0	4.2
	1.0	2.8	1.2	3.8	35.0
	2.0	1.7	2.3	3.0	41.7
CCC®–TBA	0.5	4.0	4.0	4.0	0.0
	1.0	3.3	3.3	4.0	11.7
	2.0	2.7	2.8	3.3	26.7

[a] Weight percent

[b] Rated according to Table 1

[c] Calculated according to formula 2

of test fungicides were poorer against *A. niger* than against *P. citrinum* and *T. viride*. At the concentration of 1%, CCC®, CCC®–TBA, and TBA can only lower the growth of *A. niger*, and the treated bamboo was nearly completely covered with mycelium of *A. niger*. PPA had better resistance against *A. niger* than fungicides mentioned above, especially when compounding with CCC®, with infection values of 1 after a month of testing, again showing synergistic effect.

Results analyzed above showed that the organic fungicides (including PPA and TBA) resisted mould fungi more effectively than CCC®. Among the test mould fungi, *A. niger* was the most serious one, only the compound of CCC® and PPA or higher concentrations of PPA could restrain or inhibit it. The significant resistance of CCC®–PPA indicated that the two fungicides had good synergistic effect. However, CCC®–TBA, at the concentration of 1%, only had certain resistance against *P. citrinum* and *T. viride*, and the effects were mainly attributed to TBA, not to the synergistic effect of CCC® and TBA.

Overall appraisal of the test fungicides

Bamboo in natural conditions is readily attacked by a number of mould fungi [22]. In order to develop universally effective fungicides for bamboo, overall appraisal of the test fungicides is needed, which is defined as resisting effectiveness (RE), calculated according to formulae 2 [24], and the results were listed in Table 2.

As could be seen from Table 2, CCC®, TBA, and CCC®–TBA had lower RE, even at the concentration of 2%, CCC®–TBA showed no synergistic effect against the test fungi. RE of PPA was higher than TBA, CCC® and CCC®–TBA, but when used alone, could not resist all of the three test fungi effectively. The compound of CCC® and PPA had the highest resistance against all of the test fungi, even at low concentrations, showing synergistic effect.

Conclusion

Mould-resistant effect of CCC®, PPA, TBA, CCC®–PPA, and CCC®–TBA against *P. citrinum*, *T. viride*, and *A. niger* indicated that CCC® or PPA used alone could not resist the test fungi effectively, but the compound of CCC® and PPA behaved the best against all of the test fungi, even at low concentrations, showing a good synergistic effect. However, no synergistic effect could be inferred from the resisting results of CCC®–TBA against the test fungi. Further tests on field are recommended for the outdoor applications of bamboo treated with these fungicides.

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