INFLUENCE OF SOLVENT TREATMENT ON MOULD RESISTANCE OF BAMBOO

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Bamboo timber is very vulnerable to mould fungi, a characteristic that is attributed to the rich sugar, starch, and protein present in bamboo. Solvents including cold water, hot water, benzene/ethanol, ethanol/ether, 1% NaOH, and 1% HCl can dissolve corresponding components from bamboo, which might be helpful to the resistance of bamboo against mould fungi. In order to study the relationship between surface nutrition and mildew of bamboo, mould resistances of bamboo blocks treated with different solvents were tested in the laboratory and field. Results showed that bamboo treated with cold water, hot water, benzene/ethanol or ethanol/ether had almost the same resistances with the controls against mould fungi, and the surfaces became covered with mycelium within 10 days in laboratory tests, and 5 weeks in field tests. 1% NaOH and 1% HCl were helpful to the mould resistance of bamboo, of which, 1% HCl treatments behaved the best, especially in the field tests.

Keywords: Bamboo timber; Solvents treatment; Mould resistance; Laboratory test; Field test

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INTRODUCTION

Moso bamboo accounts for about 70% of the total bamboo forest in the world, and it is one of the most popular and important economic species in the world (Pérez et al. 1999; Zhang 2003). Without preservation, it becomes susceptible to attack by mould fungi, especially *Aspergillus* sp., *Penicillium* sp., and *Trichoderma* sp. (Nielsen et al. 2004; Pasanen et al. 2000), resulting in degraded performances, shortened service life, and reduced value. Thus, it is necessary to protect bamboo from mould fungi (Liese and Hamburg 1987; Zhang 2003). In contrast to timber, bamboo culm consists of about 45 to 55% parenchyma cells, which are filled with nutritious starch (2 to 6%), sugar (2%), protein (1.5 to 6%), fat (2 to 4%), and so on, even in older culms. These nutrients, especially the sugar and starch, are believed to be the main factors leading to the decay and mould of bamboo (Wu 1992; Zhang 1995; Liese 2003). Okahisa et al. (2006) have reported on the relationships between the free glucose or starch contents on the survival of decay fungi. Results showed that the fungal attack was independent of starch contents, but was influenced by the free glucose. Immersing the freshly felled bamboo into flowing water for some time has been frequently used by a number of companies to improve the mould resistance (Duan 2005; Wu 1992).

In this study, mould resistances of bamboo treated with cold water, hot water, 1% HCl, 1% NaOH, benzene/ethanol (1:1), and ethanol/ether (1:1) were investigated. Cold water extracts account for about 6.54 % of the dry weight of bamboo, mainly containing monosaccharides, oligosaccharides, and small amounts of tannin, amino acid, soluble pigment, and inorganic salts, etc. Hot water extracts account for about 8.24%, containing starch, gum, and other polysaccharides, apart from cold water extracts. 1% NaOH extracts mainly include tannin, pigment, alkaloid, soluble mineral composition, saccharide, starch, pectic, protein, amino acid, a part of hemicellulose and lignin, a few fats and volatile oils, etc. 1% HCl extracts are similar to 1% NaOH extracts except for the additional presence of wax, fat, and resin. Benzene/ethanol and ethanol/ether extracts mainly contain small amounts of fat, wax, resin, volatile oil, tannin, pigment, and fatty acid (Zhang 1995; Zhao and Yu 2002). Mould fungi mainly deteriorate the surface of bamboo, seldom intruding into the inner part. Therefore, surface treatment is of importance for the protection of bamboo from mould fungi. When bamboo samples are immersed into solvents mentioned above, the corresponding chemicals on the surface would be extracted, leading to the changed resistance against mould fungi. The following experiments were carried out in view of the above-mentioned considerations.

EXPERIMENTAL

Materials

Moso bamboo (*Phyllostachys pubescens Mazel* ex H.de lenaie), four years old, was collected from Sankou town, Lin'an city, Zhejiang, China, on Mar 25th, 2007. Bamboo blocks, 50 mm (length) × 20 mm (width) × 5 mm (thickness) for laboratory tests, and 120 mm (length) × 20 mm (width) × 5 mm (thickness) for field tests, with green and yellow faces planed off, were chosen and machined into specimens. 12 specimens per concentration of a solvent were chosen for laboratory tests and 20 for field tests, of which, half were with knots and half were without knots.

Three kinds of mould fungi including *Trichoderma viride* pers.ex Fr (*T. viride*), *Penicillium 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 the laboratory mould-resistance tests.

Commercially available products including HCl, NaOH, ethanol, ether, and benzene were purchased from Huadong Medicine Group Co. Ltd, and used without further purification.

Treatment of Specimens

Bamboo specimens were treated with cold water (25 ^{o}C), hot water (60 ^{o}C), 1% HCl, 1% NaOH, benzene/ethanol (1:1), and ethanol/ether (1:1), respectively. The treatment was carried out in a 1000-mL beaker with magnetic stirring for 36 h. The treating solvent was replaced with a fresh portion at every 12-h interval. As for 1% HCl and 1% NaOH treatment, two supplementary tests were set by immersing the treated specimens into water for another 12 h, changing the water twice during the treatment.

Both the treated and untreated blocks were placed into the fume hood for about two weeks before mould resistance test.

Mould Resistance Test

For laboratory tests, the treated and untreated blocks were placed on a 3-day culture of the test fungus malt agar (2 % malt extract and 2 % agar) in petri dishes (Fig. 1) and incubated for 30 days at 25 ^{o}C ± 2 under controlled humidity between 85 and 90 % (ASTM: D4445-03).

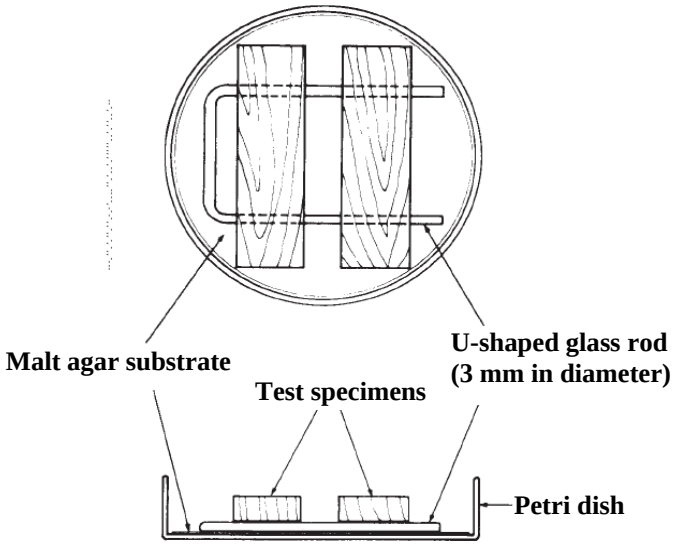

Fig.1. Method in the laboratory test

The field test was done in Lin'an city, Zhejiang, China. A shelf standing on the grass was employed to do the test (Fig. 2). The blocks with face up were placed on the mouldy bamboo strips (Chinese Standard: GB/T 18261-2000). In order to keep the same environment, all of the test blocks were put on the same horizontal plane, 20 cm off the ground, lasting for five months (from Apr 1st to Sept 1st).

Fig. 2. Shelf employed in the field test

Evaluation of the Test

The growth of fungi in the laboratory test was visually estimated and scored every day using a scale of 0 to 4, with 4 being the maximum infection (Table 1). In the field test, the temperature and humidity was measured with a hygrothermograph three times each day, and from which the weekly average temperature and humidity could be obtained. When the samples began to mildew, vernier caliper was applied to measure the length and width of the moldy part. Mould resistance of samples in the field test was evaluated by the infection area (*IA* for short), which was calculated as follows,

$$IA = \frac{\sum_{i=1}^{12} IA_i}{6200 \times 12} \times 100\% \quad (1)$$

where *IA* is the percentage of infection area; IA_i is the infection area of sample i (mm^2); 6200 = 120 × 20 × 2 + 120 × 5 × 2 + 20 × 5 × 2, is the area of each sample (mm^2), and 12 is the number of replicate samples.

Table 1. Standard Method for Scoring the Infection Value

Infection value	Percent of mould
0	No mycelium on the block
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%

RESULTS AND DISCUSSION

Mould Resistance in the Laboratory Test

Resistances of the samples against *T. viride, P. citrinum* and *A. niger* are shown in Figs. 3 through 5. Bamboo treated with cold water, hot water, benzene/ethanol, or ethanol/ether had similar resistances against the three test fungi, with only slightly improved in mould resistance. Mycelia spreaded rapidly once they climbed onto the blocks, and all surfaces became covered with mycelia after 5 days at least and 11 days at most.

1% NaOH treated blocks without supplementary water treatment resisted against *T. viride* and *P. citrinum* more efficiently than against *A. niger*. Spore germination of *T. viride* and *P. citrinum* was completely inhibited during the experiment, but the infection value increased to 3.5 after one month of testing against *A. niger*. *A. niger* is a vigorous fungus, and the hyphae that can stretch deeper than *T. viride* and *P. citrinum* into bamboo (Wu and Weng 2000), which might be the main reason for the poor efficiency of most treatments. In previous studies, we also found that most fungicides resisted *T. viride* and *P. citrinum* more efficiently than against *A. niger* (Sun et al.2006). In order to obviate the pH effect on the resistance, 1% NaOH

treated blocks were followed by immersing into water for 12 h (changing the water every 6 h), and results showed that the resistances of treated blocks decreased a lot, especially against *T. viride* and *A. niger*.

Different from 1% NaOH treated blocks, those treated with 1% HCl resisted the three test fungi efficiently, no matter whether the blocks had been further treated with water or not. No mycelia existed on 1% HCl treated blocks, which was better than most fungicides under the similar experimental conditions (Sun et al. 2006).

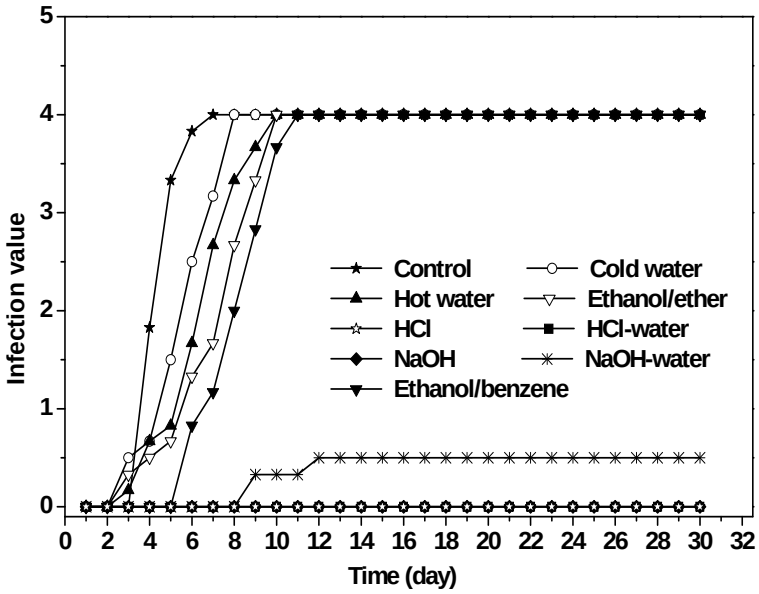

Fig. 3. Mould resistance of blocks against *Trichoderma viride* during one month of cultivation

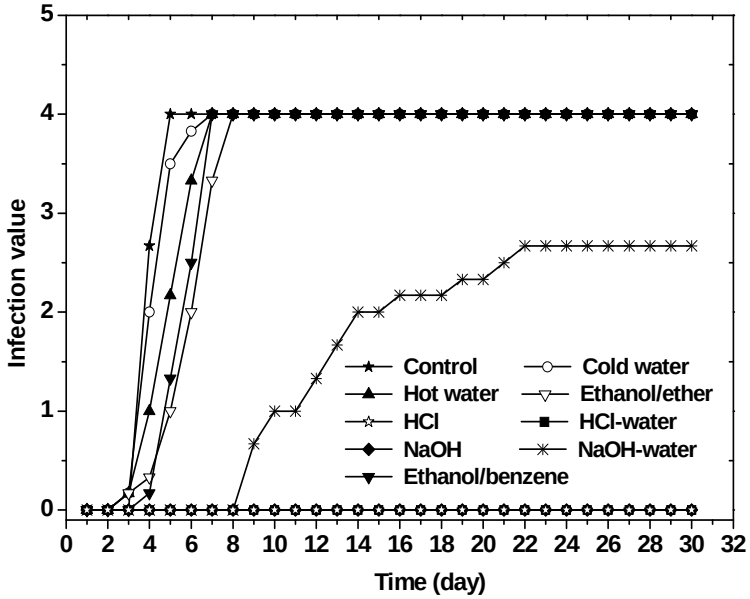

Fig. 4. Mould resistance of blocks against *Penicillium citrinum* during one month of cultivation

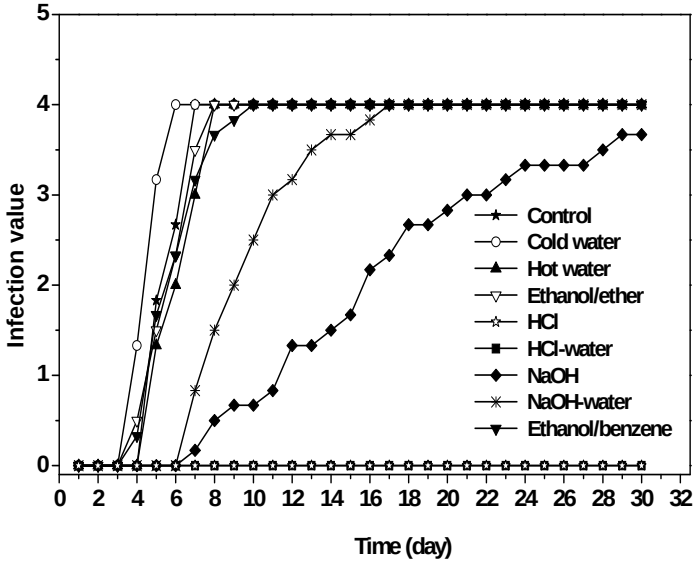

Fig. 5. Mould resistance of blocks against *Aspergillus niger* during one month of cultivation

Mould Resistance in the Field Test

Bamboo applied outdoors is deteriorated by the complex interplay of biology and environment, which always leads to a deviation between laboratory results and practical results. Therefore, field test should be carried out to make further evaluation. It is reported that the three most important growth conditions of temperature, humidity, and substrate must exist simultaneously over a certain period of time in order to enable fungal growth (Sedlbauer 2001; Isaksson et al 2010). Bamboo is a better substrate for mould fungi than wood, allowing them to adapt and survive over wider ranges of humidity (>63%) and temperature (5 to 30 ^{o}C) (Wu et al. 2000; Ran et al. 1997). In Zhejiang, China, bamboo is usually severely infected by mould fungi from April to September, especially in the rainy seasons. Therefore, the field tests were carried out from Apr 1st to Sept 1st. The weekly variation of temperature and humidity is presented in Fig. 6. In the first three weeks, the temperature and humidity increased gradually, but during the next 8 weeks, the humidity varied greatly from 62 to 89%, with a little fluctuation of temperature from 22.5 to 26 ^{o}C, then the humidity remained at 74 to 79%, and the temperature increased from 24 to 28 ^{o}C.

Once the temperature and humidity were appropriate, spores of mould fungi germinated and grew rapidly. As shown in Fig. 7, during the first three weeks no mycelia existed on the blocks, even on the fresh bamboo samples, indicating the unsuitability of the temperature and humidity. In week 4, spores began to germinate and grew rapidly. At the end of week 6, the moldy areas of the controls, samples treated with cold water, hot water, benzene/ethanol, and ethanol/ether exceeded 94 %. By contrast, those treated with 1% NaOH or 1% HCl resisted mould fungi more efficiently, especially the 1% HCl treatment. At the end of week 11, moldy areas of blocks treated with 1% HCl were 0%, and those treated with 1% HCl/water were less than 10%. But over time, the surfaces of treated blocks became coarse, which might arise from surface aging (Wang and Ren

2009). And at the same time, both the temperature and humidity reached higher levels at the beginning of rainy July, and 1 % HCl treated blocks began to mildew. At the end of week 16, all the surface were covered with mold fungi.

Both the laboratory test and field test demonstrates that bamboo treated with 1% NaOH or 1% HCl could resist mould fungi more efficiently than samples treated with cold water, hot water, benzene/ethanol or ethanol/ether. 1% HCl treated blocks were more resistant than 1 % NaOH treated ones.

As previously mentioned, sugar, starch, protein, and fat are the main nutrients for mould fungi. When bamboo samples were treated with hot water or cold water, sugar and starch would leach from the surface, then mould resistance could accordingly be increased. Unexpectedly, both laboratory and field tests did not show improved resistance of hot or cold water treated samples against mould fungi in comparison with the controls, some treatments even promoting the infection of mould. Similar results could be found in the samples treated with organic solvents such as benzene/ethanol or ethanol/ether. Further studies are required in order to explore the action mechanism of mould fungi on bamboo. The reason for the improved resistance of 1% HCl or 1% NaOH treated bamboo might involve synergistic results of multiple factors. 1% HCl (pH = 0.58) or 1% NaOH (pH = 12.33) treatments could not only dissolve nutrients required by mould fungi, but also change the pH value of bamboo samples, and there is a possibility of reaction with some components. The optimum pH for mould fungi is 5 to 8, and they can grow normally on PDA substrate (composed of potato and glucose) with a pH value from 4 to 11 (Ma et al. 2009). Accordingly, mould resistance of samples treated with 1% HCl or 1% NaOH might be improved as a result of the extreme pH value of the treating solution. But the significant resistance of 1% HCl and 1% HCl-water treated samples could not be simply attributed to the extreme pH value. Much more research is necessary to reveal the relationship between components of bamboo and the biological characteristics of mould fungi.

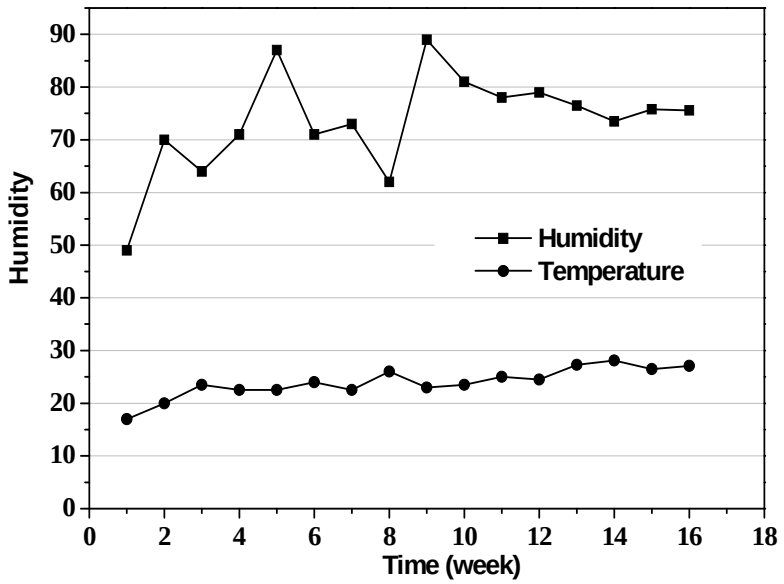

Fig. 6 . Weekly humidity and temperature in the field test

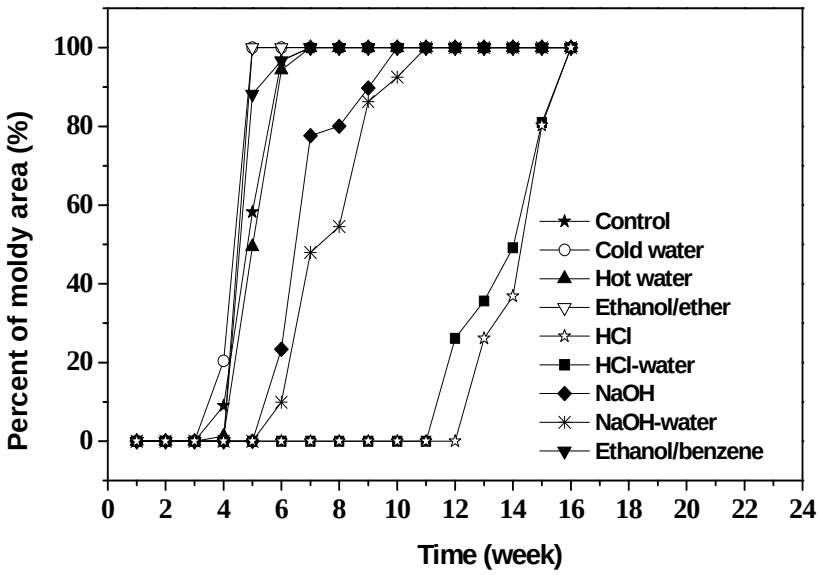

Fig. 7. Mould resistance of blocks in the field test

CONCLUSIONS

Mould resistance of bamboo blocks treated with different solvents has been illustrated in this paper. Results showed that treatments with cold water, hot water, benzene/ethanol, and ethanol/ether were not effective for improving the resistance of bamboo blocks against mould fungi, some treaments even promoting the germination and growth of mould fungi. 1% NaOH and 1% HCl treatments were more effective than the solutions mentioned above, of which 1% HCl treatment was prominent. 1% NaOH treament resisted *T. viride* and *P. citrinum* more efficiently than *A. niger* in the laboratory test, but not so efficiently in the field test. 1% HCl treatment not only showed remarkable resistance against the three test fungi in the laboratory test, but also behaved well in the field test.

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