Application of Wood Vinegars Prepared from Branches and Tree Tops of Cryptomeria japonica at Different Collection Temperatures to Evaluate their Fungi Resistance for Moso Bamboo Material

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Application of Wood Vinegars Prepared from Branches and Tree Tops of *Cryptomeria japonica* at Different Collection Temperatures to Evaluate their Fungi Resistance for Moso Bamboo Material

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In this study, wood vinegars were collected from the chimney outflow of earthen kiln at 6 different temperatures (80–159, 80–90, 91–100, 101–120, 121–140 and 141–159 °C) during the charcoal preparation from the branches and tree tops of *Cryptomeria japonica*. These vinegars were used to evaluate the fungi resistance of airborne fungi particles, *Aspergillus niger*, *Aspergillus flavus* and *Trichoderma viride* after they as well as acetic acid and tap water (as the controls) were used to impregnate Moso bamboo specimens for 240 min using the vacuum method. The basic properties of the wood vinegars obtained were: pH value of 2.54 to 3.45, specific gravity ranging from 1.006 to 1.008, contents of organic acid ranged between 1.75 and 3.03%, soluble tar content was from 0.186 to 0.629%, and the color difference (ΔE^*) ranged from 8.37 to 14.36. The absorption by the bamboo specimens after having been processed with the various vinegars using the vacuum method ranged from 10.85 to 19.35 mg/cm^3. The results of the macro–observation showed that after 70 days the growth rate of airborne fungi particle on the surface of bamboo specimens impregnated with vinegar at 80 to 159 °C range between 10% and 15%, but it was close to 100% for either of the controls after only about 30 days. The FTIR analysis showed that the specimens treated with vinegar at 80 to 159 °C did not contain the fungal chemical compounds, at 1654 cm^{-1} (C = O) and 1552 cm^{-1} (N–H), while the acetic acid and the tap water both showed absorbance peaks. The C/N ratio of the airborne fungi particles of the specimen treated with vinegar at 80 to 159 °C was 131.4%, but it was only about 90.5 to 102.8% for the controls. These results suggest that the compositions of the wood vinegars collected at different temperatures affect the fungi resistance of the bamboo material.

Keywords: Wood Vinegar, Branches and Tree Tops Wood of *Cryptomeria japonica*, Different Collection Temperatures, Fungi Resistance, Moso Bamboo Material

INTRODUCTION

The Taiwan Forestry Bureau reported that in 2005 forests occupied 2,102,400 hectares (ha), or 59.0% of the total area (3,591,500 ha) of the main island of Taiwan. The natural forest portion measures 1,527,500 ha or 73.0% of the forestland. Artificial forests cover another 20.0%, and the others 7% make up the rest. The majority (60.0%) of the natural forests are broad–leaved trees. The majority (51%) of the artificial forests are coniferous trees. The total forest volume of the island amounts to about 358,744,000 cubic meters.

Among these forests, *Cryptomeria japonica* D. Don (Japanese cedar), one of the main plantation species covers 62,500 ha, or about 10% of the artificial forests. The Japanese cedars are over 30 years old, occupy about 2,300 ha, and have a timber volume of approximately 4 million cubic meters. Plantations need to be thinned, and this is usually done by mechanical means. The results are small–diameter logs (SDLs) and branches and tree tops (BTW)". It is obvious why SDLs and BTW must be removed, such as reducing the forest fire hazard, managing the stand species, obtain a more desirable mix, provide a better wildlife habitat, protect the watersheds, and others (Levan–Green and Livingston, 2001). The SDLs, even low density and high knots material, is utilized for nonstructural lumber, pulp, chips, firewood, paneling (Tsai *et al.*, 1995; Chen *et al.*, 2000; Kim H. G. and Kim K. T., 2000; Hwang, 2003; Lin *et al.*, 2005), biomass energy (Demirbas, 2005; Onay and Kockar, 2003; Stamatov *et al.*, 2006), composting, laminating and so on, but BTW is generally discarded and is under–utilized in Taiwan.

Wood charcoal, a wood product, plays an important role to reduce climate change because it signifies the reduction of carbon in the atmosphere. Therefore, using BTW to make wood charcoal as a valued–added product that accumulates CO_2 without spreading it into the atmosphere and at the same time provides jobs for the local

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population is a good deal (Okimori *et al*., 2003; Ogawa *et al*., 2006). Wood charcoal made with BTW is a simple way to reduce climate change, even if it no longer absorbs the CO_2 from the atmosphere. This is because it directly creates a new way to produce wood charcoal (Hwang *et al*., 2008), and indirectly plays an important role to increase the efficiency of the forests. However, wood vinegar, a by–product of the wood charcoal manufacturing process using BTW of *Cryptomeria japonica*, is under–utilized in Taiwan.

Previous studies (Lin *et al*., 2006a; Lin *et al*., 2006b; Lin *et al*., 2008) suggested that bamboo vinegar was useful to restrain molds on bamboo materials and prevented microbiological deterioration. The composition of wood vinegar includes more than 200 kinds of organic compounds, is much more complex than that of bamboo vinegar (Chen *et al*., 2006; Hwang *et al*., 2008; Lu and Shau, 2009), and can repel termites and disease germ of plants, improve the soil, promote crop or prevent worm growth, reduce the use of agricultural chemicals, and sterilization (Furuda, 1987; Nishimiya *et al*., 1998; Yataga, 2002; Ishihara, 2004; Kou, 2005). However, the effect of wood vinegar collected at different temperatures during the production process has never been investigated to determine its fungi resistance on bamboo materials. Therefore, the objective of this study was to evaluate if different collection temperatures of wood vinegars during the manufacturing process of charcoal from BTW of *Cryptomeria japonica* would influence its fungi resistance, in the hope that it could delete or at least decrease the need for chemical fungicides and their impact on the environment.

MATERIALS AND METHODS

Specimen preparations

Bamboo material

Moso bamboo (*Phyllostachys heterocycla* Milf) was provided by Pu Yuan Co. Ltd., Nantou, Taiwan. The specimens were pre–processed by heating at a temperature of 120 to 130 ˚C for 1.5 hours. After they were air–dried, the specimens, measuring 25 mm×25 mm×3 mm (Length × Weight × Thickness) were conditioned to equilibrium at 20 ˚C with 65 % relative humidity (RH) for about four weeks. The average moisture content and the density of the specimens was 8.9 to 9.7% and 0.67 (0.01) g/cm^3, respectively.

Wood vinegar

These vinegars, collected at different temperatures during the manufacturing process of charcoal from BTW of *Cryptomeria japonica*, were provided by the Division of Forest Utilization, Taiwan Forestry Research Institute (TFRI), Taipei, Taiwan (Hwang *et al*., 2008). The vinegars were collected at temperatures ranging from 80 to 159 ˚C (Furuda, 1987; Nishimiya *et al*., 1998). The temperature was measured by a thermocouple at the chimney outflow of the earthen kiln during the charcoal manufacturing process (pyrolysis) using BTW of *Cryptomeria japonica*. The different groups of bamboo vinegar were collected at 80–159, 80–90, 91–100, 101–120, 121–140 and 141–159 ˚C.

Nutrient media

Water agar (WA) was used as a nutrient media to maintain/grow the fungi to be used in the tests with either of the specimens in the petri dishes (TAPPI, 1993). WA was purchased from Merck Co, and was prepared using 8 g of Agar–Agar with 400 mL of tap water. The preparations were then mixed thoroughly prior to sterilization.

Inoculation

The 3 strains of fungi used at an average concentration of suspending liquid of 10^{3-4} spore/mL were *Aspergillus flavus*, *Aspergillus niger*, and *Trichoderma viride*. In addition, the fungi from air borne spores (air-borne fungi particles) were also tested in this study. To obtain the air borne spores the bamboo specimens were exposed to the air in an indoor atmosphere for a period of 10 min.

Experimental methods

Basic properties

The experimental tests to determine the pH value, specific gravity, contents of organic acid and soluble tar content for the various vinegars were the same as in previous studies (Lin *et al*., 2006b; Lin *et al*., 2008; Tsai *et al*., 2009). The results of the basic properties were statistically analyzed based on Duncan's multiple range tests at a 5% significance level using the Statistical Package for Social Science (SPSS) software.

Vacuum treatments

The heated specimens were treated using the vacuum method. The vinegars were kept under a constant pressure of over 600 mm/Hg. The specimens were then impregnated with each type of wood vinegar, tap water and acetic acid for 60 min of vacuum time and 240 min of impregnation time, respectively. The specimens were then individually removed to a drainage pad, tipped on end to allow for drainage and wiped with a paper towel to remove the surface vinegar. They were then weighed to determine the amount of absorption. The absorption formula was the same as in previous studies (Lin *et al*., 2006b; Lin *et al*., 2008). The specimens were air dried for 24 h, and then placed into a convection oven at 30 ˚C for over 30 min after which they were cooled and stored in plastic bags for at least 3 days to allow the bamboo vinegar fixation reaction to proceed. They were then reconditioned at 20 ˚C with 65% RH for 2 weeks. Six replicates of the specimen were prepared for each type of fungus, respectively.

Color value

After the specimens were heat treated and impregnated with the various vinegars, they were each checked using a colorimeter (Nippon Denshoku NR–3000) at 3 randomly selected spots.

Fungi inoculation and fungal resistance

Each fungus was inoculated by distributing 3 drops (about 3 mL) onto the surface of the specimen in a petri dish, and then incubating the petri dishes containing the inoculated test–specimens at a temperature of 25 ˚C in a humid atmosphere till the growth of each fungus being tested was visible. The fungal resistance experiments were determined by visual (macro) examination.

Observation by Microscope

In order to determine the growth of the tested fungi, an inoculated section measuring 5 mm×5 mm was taken from each specimen, and then observed using a scanning electron microscope (SEM, HITACH S–2400 types) at 300 X magnification.

C/N ratio measurement

The oven dried samples weighed about 2–4 mg. Each sample was inoculated individually with one of the various fungi. Each specimen was analyzed after 70 days using an Elemental Vario CHNS/O analyzer (EA, Germany). The C and N elements were determined and the C/N ratio was calculated.

FTIR analysis

Seventy days after having been inoculated with the fungi the specimens, each treated with a specific vinegar, were analyzed using the reflectance mode of Fourier Transform Infrared Spectroscopy (FTIR, Perkin Elmer Spectrum RX 1) at a resolution of 4 cm^{-1}. Thirty–two scans were co–added for each spectrum. Infrared transmittance spectra were measured over a range of 650 – 4000 cm^{-1}. The peak assignments were performed using the Win FIRST software program.

RESULTS AND DISCUSSION

Basic properties

Table 1 shows the basic properties of wood vinegars collected at different temperatures. The pH value of the vinegars ranged from 2.54 to 3.45, the organic acid content ranged from 1.75 to 3.03%, specific gravity was between 1.006 and 1.008 (between 29.0 and 30 ˚C), and the soluble tar content ranged from 0.186 to 0.629%. The results of the colorimetric measurements of the specimens treated with various wood vinegars are based on the CIE L* a* b* system, and are shown in Table 1. The color difference (ΔE*) of these specimens was from 8.37 to 14.36. Their color ranged from light yellow to yellowish–brown, and the ΔE* of the treated specimens increased with the increase of the collecting temperature. It is suggested that the color difference is influenced by the soluble tar content and the composition of the various vinegars. The absorption by the heated specimens of wood vinegars from different collection temperatures using the vacuum method ranged from 10.85 to 19.35 mg/cm^3. The absorption of tap water was 24.72 mg/cm^3 and 13.02 mg/cm^3 for acetic acid.

Effect of vinegar collected at different temperatures on fungi resistance

The results of the specimens treated with vinegars collected at different temperatures are shown in Fig. 1. The fungi resistance of the controls, no treatment and treated with acetic acid, was ineffective. It resulted in 100% fungal colony growth after having been inoculated by various fungi for 5 to 10 days (only the results for specimens inoculated with airborne fungi particles are shown in the top of Fig. 1). The specimens treated with tap water had a fungal colony growth of 82% after have been inoculated for about 20 days (see the top of Fig. 1). However, after having been treated with a vinegar collected at the respective temperatures, the specimens were fungi resistant to various degrees, as shown in Fig. 1. For the vinegar collected at 80–159 ˚C, the growth rate of *Aspergillus niger* was about 15% 70 days after inoculation. The growth rate was about 8–10% for airborne fungal particles and for *Aspergillus flavus*, but 0% for *Trichoderma viride*. The growth rate of the specimens treated with vinegar collected at 80–90 and 91–100 ˚C 70 days after inoculation was about 15–90% for both *Aspergillus flavus* and *Aspergillus niger*, but it was only 0–22% for both *Trichoderma viride* and the airborne fungi particles (the left side of Fig. 1). For the specimens treated with vinegar collected at 101–120, 121–140 and 141–159 ˚C, the growth rate of both *Aspergillus flavus* and *Aspergillus niger* was about 20–80%, but it was only 0 to 15% for *Trichoderma viride* and the airborne fungal particles (the right side of Fig. 1) 70 days after inoculation. Moreover, the growth rate of the specimens treated with vinegar collected at 80–159, 91–100, 101–120 and 121–140 ˚C was 0 % for both *Trichoderma viride* and the airborne fungi particles, 70 days after inoculation. These findings indicate

Table 1. Basic properties of wood vinegars prepared from branches and tree tops wood of *Cryptomeria japonica* with the different collected temperatures

Bamboo vinegar (˚C)	pH value	Organic acid content (%)	Specific gravity tests		Soluble tar content (%)	ΔE*[2)]
			Temperature (˚C)	SG[1)]		
80–159	2.59	2.81 (0.06) [a 3)]	29.5	1.007	0.389 (0.01) [a]	11.31 (0.12) [a]
80–90	2.54	1.90 (0.09) [c]	29.5	1.006	0.186 (0.00) [d]	8.37 (0.31) [d]
91–100	2.55	3.03 (0.16) [a]	29.0	1.008	0.566 (0.02) [b]	11.97 (0.06) [a]
101–120	2.80	1.75 (0.08) [c]	29.5	1.007	0.409 (0.05) [a]	10.04 (0.44) [b]
121–140	2.79	2.65 (0.06) [a]	29.0	1.007	0.629 (0.04) [c]	14.36 (0.30) [c]
141–159	3.45	2.03 (0.12) [b]	30.0	1.008	0.615 (0.04) [c]	13.25 (0.39) [c]

[1)] SG: Specific Gravity.

[2)] $\Delta E^* = (\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2})^{1/2}$: Color difference.

[3)] Mean (standard deviation) separation within columns by Duncan's multiple range tests at 5% significant level.

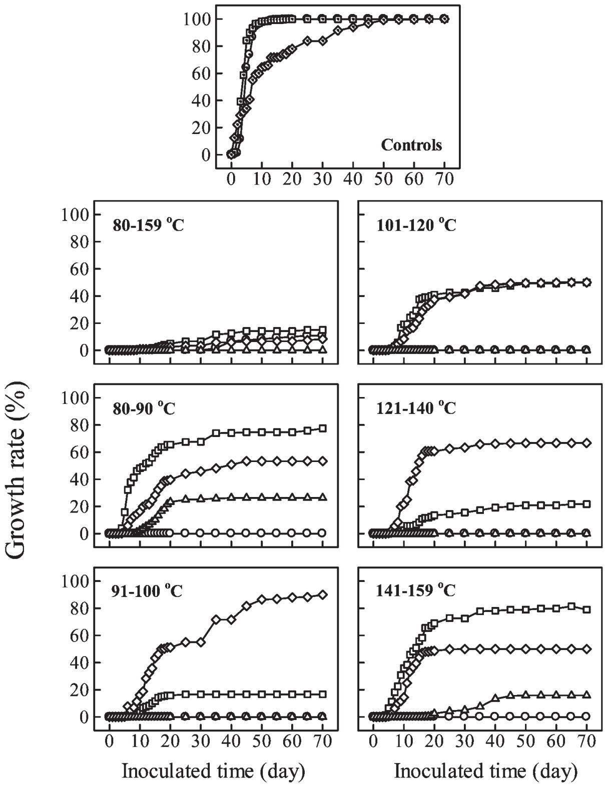

Fig. 1. Fungi resistance of Moso bamboo specimen with wood vinegars from different collection temperatures.
Symbols: Controls (Inoculatied with airborne fungi particles):
–⊙–: Blank; –⊡–: Acetic acid; –◈–: Tap water.
Experimentals:
–○–: Airborne fungi particles; –□–: *Aspergillus niger*;
–◇–: *Aspergillus flavus*; –△– : *Trichoderma viride*.

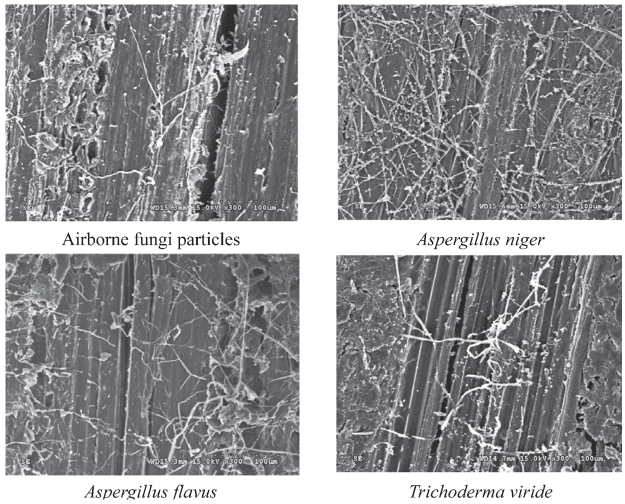

Fig. 2. SEM photographs (×300) of various fungi at inoculated side for Moso bamboo specimen treating with 80–159 °C of wood vinegar by using vacuum treatment after 70 days.

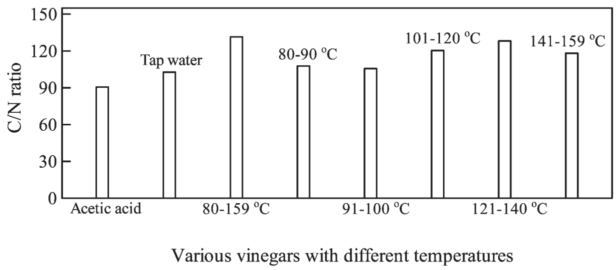

Fig. 3. C/N ratio for bamboo specimen treating with different collected temperatures of wood vinegars after inoculated airborne fungi particles for 70 days.

that wood vinegars collected at different temperatures can restrain molds and decrease the microbiological deterioration of products made with bamboo. It also indicates that the temperature of the wood vinegar at the time of collection influences the effectiveness of the fungi resistance of the treated bamboo materials. This study suggests that it is critical for future research to investigate which of the organic compounds in the wood vinegars prepared from the BTW of *Cryptomeria japonica* are the main fungicides, and which are the most effective collection temperatures.

A micrograph by scanning electron microscope (SEM) for the resistance to fungi of specimens treated with vinegar collected at a temperature of 80–159 °C is shown in Fig. 2. The 300 times SEM micrograph of the side view of the specimens 70 days after having been inoculated with airborne fungi particles, indicates that there was only a small fungal colony growth for both the airborne fungi particles (top figure – left side of Fig. 2) and for *Trichoderma viride* (bottom figure – right side of Fig. 2). However, for the specimens with both *Aspergillus flavus* and *Aspergillus niger*, it showed a substantial amount of fungal strains on the side of the specimens.

The C/N ratios of the control specimens, treated with both tap water and acetic acid, were lower than those of the specimens treated with wood vinegars at different temperatures after having been inoculated by airborne fungal particles for 70 days (Fig. 3). This is because the amino acids of the inoculated fungi increased as a result of their metabolism of fungal microorganism (Fujida, 1993; Rayner and Boddy, 1988; Fukuda, 2000; Lin, 2003). In other words, the N element increased. It is confirmed that the lower the C/N ratio, the greater the rate of biodegradation (Lin *et al.*, 2006a; Lin *et al.*, 2006b; Lin *et al.*, 2008).

FTIR analysis

To understand the effectiveness of the resistance to fungus of the specimens treated with vinegars collected at various temperatures and the controls (no treatment, acetic acid and tap water), the specimens inoculated with various fungi were examined using FTIR analysis. One of the results for the specimens inoculated with *Aspergillus niger* are shown in Fig. 4.

The results of the FTIR spectra for these specimens after 70 days showed that for the controls, two absorbance peaks located at 1654 cm^{-1} and 1552 cm^{-1}, were assigned to C = O (carbonyl group) stretching and N–H deformation vibration, respectively. And the weaker peaks for both functional groups also showed on the specimens treated with vinegars collected at 80–90, 91–100,

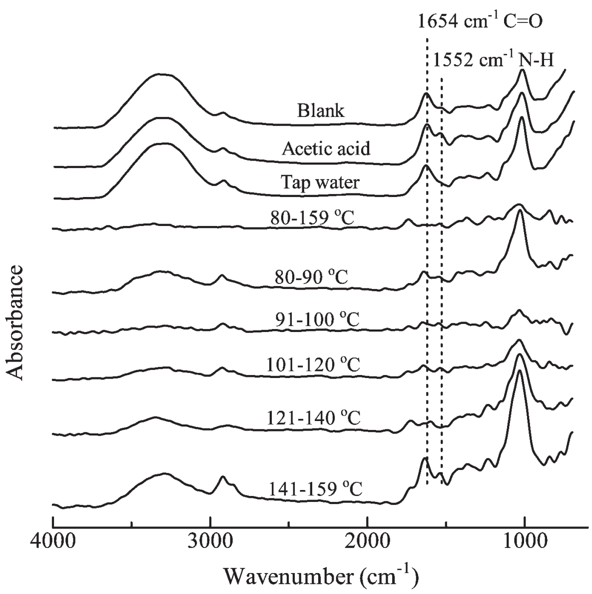

Fig. 4. FTIR spectra of bamboo specimens treating with different collected temperatures of wood vinegars by using vacuum treatment after inoculated *Aspergillus niger* for 70 days.
Note: The controls are included Blank, Acetic acid and Tap water.

101–120 and 141–159 ˚C. However, neither peak was present for the specimens with vinegars at 80–159 and 121–140 ˚C. These results are similar to the results of the resistance of bamboo specimen to *Aspergillus niger* when treated with wood vinegars collected at different temperatures (see Fig.1). In other words, it seems that both peaks, 1654 cm^{-1} (C = O) and 1552 cm^{-1} (N–H), represented the chemical compounds of the representative function group, as indicated by the fungi on the bamboo surface. This is because the main compounds of the cell wall of the fungi are made up of chitin, cellulose, aminosaccharides, protein, etc. As such C = O can be a representative for their function group (Chang, 1990). For the function group N–H, it could be the amino acid products that were increased through the metabolization of fungal microorganisms (Lin, 2003; Lin *et al.*, 2006). Therefore, it can be concluded that wood vinegars collected at different temperatures are useful for restraining molds by treating the bamboo product by the vacuum method, applying 60 min of vacuum and 240 min of impregnation.

CONCLUSIONS

This study used wood vinegar prepared from BTW of *Cryptomeria japonica* and collected at different temperatures to evaluate the fungi resistance of Moso bamboo material when treated with these wood vinegars. The results obtained are summarized as follows. The pH value, specific gravity, contents of organic acid, soluble tar content and color difference of wood vinegars were 2.54 to 3.45, 1.006 to 1.008, 1.75 to 3.03%, 0.186 to 0.629% and 8.37 to 14.36, respectively. The absorption with each wood vinegar by the bamboo specimens using the vacuum method was about 10.85 to 19.35 mg/cm^3. Seventy days after the surface of the bamboo specimens were inoculated with vinegars at 80 to 159 ˚C the growth rate of the airborne fungi particles was only about 10 to 15% of that of the other specimens. At the same time, the growth rate for both controls was close to 100% after only 30 days. It is evident that wood vinegars collected at different temperatures can restrain bamboo molds and reduce the microbiological deterioration of bamboo products. The C/N ratio of the specimen inoculated with airborne fungus particles and treated with vinegar collected at 80 to 159 ˚C was 131.4%, and about 90.5 to 102.8 % for the controls. The FTIR analysis showed that the specimens treated with vinegars collected at temperatures from 80 to 159 ˚C were found not to be the functional groups of the fungal chemical compounds, at 1654 cm^{-1} (C = O) and 1552 cm^{-1} (N–H). It is evident from the results of the C/N ratio and the FTIR analysis, that wood vinegar can reduce the microbiological deterioration in bamboo materials.

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