Laboratory Evaluation of the Anti-stain Efficacy of Crude Wood Vinegar for *Pinus densiflora*

Sabiha Salim, Syaidatul Shahomlail, Yong-Seok Choi, Min-Ji Kim, and Gyu-Hyeok Kim*

In Korea, *Pinus densiflora* is one of the most important indigenous tree species in terms of making high-value wood products. Therefore, Korean sawmills exercise extreme caution to prevent fungal discoloration such as that caused by sapstains and molds on the timber. In this study, the effectiveness of using natural crude wood vinegar to inhibit sapstains and molds, especially on *Pinus densiflora* (Japanese red pine) was examined. *Pinus densiflora* wood samples were dipped in absolute and diluted wood vinegar at different concentrations (1:1, 2:1, and 3:1; deionized water to wood vinegar dilution ratio) for 3 minutes and immediately air-dried. In addition, volatile wood vinegar was also used in this study to imitate the condition of wood vinegar when exposed to open air. The degree of discoloration was examined and evaluated every 2, 4, 6, and 8 weeks according to the ASTM D4445-91 Standard Method for laboratory test. Crude wood vinegar inhibited sapstains more efficiently than it inhibited molds. Wood vinegar at a 1:1 concentration was found to be the most optimum treatment for inhibiting sapstains for at least 8 weeks.

Keywords: Discoloration; Sapstain; Mold; Wood vinegar; Inhibitors

Contact information: a: Division of Environmental Science and Ecological Engineering, Korea University, 136-713, Seoul, South Korea; Corresponding author:lovewood@korea.ac.kr.

INTRODUCTION

Wood discoloration by sapstain and mold fungi considerably reduces the market values of wood products. In Korea, most of the lumber is air-dried during storage before manufacture; however, for effective protection against fungal staining, this wood must be kiln-dried. Nevertheless, most manufacturers are hesitant to incur additional production costs for this purpose, and thus chemical treatment is the most preferred option due to economic viability and effectiveness. Although sawmills take precautionary measures to prevent fungal discoloration on lumber and timber using anti-stain chemicals, this can result in environmental pollution due to the toxicity of these chemicals. As alternatives, various natural products, such as plant essential oils (*e.g.*, cedar leaf) and wood vinegar (Dawson-Andoh *et al.* 2000), are being investigated, although currently these are not being produced commercially. Previous studies (Sulaiman *et al.* 2005; Velmurugan *et al.* 2009) have demonstrated that the presence of acids (mainly acetic acid) and phenols in wood vinegar made from bamboo and broad-leaved trees have antifungal properties useful to treat wood. Although Ikergami *et al.* (1992) showed that wood vinegar, which contains guaiacol, cresol, 4-ethyl-2-methoxyphenol, and 2, 6-dimethoxyphenol, has good antifungal activity, there is, in general, very little scientific evidence regarding the antifungal properties of wood vinegar.

Therefore, the objective of this study was to determine whether wood vinegar treatment has a significant effect on fungal discoloration of wood when the wood is exposed. Volatile wood vinegar was used in this study to simulate conditions similar to treated wood exposed to the atmosphere, where wood vinegar is readily volatilized with the potential loss of antifungal components. To the best of our knowledge, this is the first attempt to evaluate the effectiveness of wood vinegar in preventing discoloration of timber when exposed to the atmosphere.

EXPERIMENTAL

Material Preparation

The efficacy of wood vinegar in controlling fungal discoloration was evaluated according to ASTM D4445-91 (ASTM 1996). Wood strips with a dimension of 5 x 10 x 50 mm were prepared from sapwood of Japanese red pine (*Pinus densiflora*) with approximately 30% moisture content.

Fungi preparation

The following 12 sapstain and 8 mold fungi used in this study were obtained from the Korea University Culture Collection (KUC): (sapstain fungi) *Hyalorhinocladiella* sp. KUC2518, *Leptographium koreanum* KUC2078, *Ophiostoma floccosum* KUC2014, *O. huntii*-like KUC2026, *O. ips* KUC2089, *O. minutum*-like KUC2126, *O. nigrocarpum* KUC2761, *O. piceae* KUC2015, *O. piliferum* KUC2325, *O. pluriannulatum*-like KUC2763, *O. quercus* KUC2034, and *Pesotum fragrans* KUC2080; (mold fungi), *Aureobasidium pullulans* KUC1632, *Alternaria alternata* KUC5005, *Aspergillus niger* KUC1361, *Cladosporium cladosporioides* KUC1701, *C. perangustum* KUC1767, *Penicillium decaturense* KUC1522, *Trichoderma harzianum* KUC1459, and *T. viride* KUC5062.

Wood vinegar treatment

Crude wood vinegar, which had been prepared in a block-type kiln for carbonization of mixed chips of *Quercus* spp., was obtained from the charcoal manufacturer at Hongcheon, Korea. The crude vinegar was diluted in 3:1, 2:1, and 1:1 dilutions (deionized water : wood vinegar, on a volume basis), along with undiluted solution. For preparation of volatile wood vinegar (VWV), 3 L of crude wood vinegar in an open flask was exposed to the air at a room temperature and a relative humidity in a fume hood for 2 weeks until approximately 1.5 L of wood vinegar remained. VWV was diluted at the same concentrations used for the non-volatile wood vinegar (NVWV).

Wood Vinegar Treatment and Evaluation of Wood Discoloration

The wood samples were dipped into the solutions of NVWV and VWV for 3 min and then allowed to drain for 3 min. Samples dipped in deionized water served as a control. A 1% solution of the commercial anti-stain chemical, Hylite Extra, was also used for comparison. After treatment, the samples were placed on glass rods over moistened filter papers in glass plates, and then sprayed with a cocktail of sapstain and mold fungi, prepared earlier in accordance with ASTM D4445-91. The samples were then incubated in sealed plates at 25 °C for 8 weeks. Finally, fungal discoloration was visually examined after 2, 4, 6, and 8 weeks. The percentage of discoloration was evaluated from the percentage area covered by hyphae and sporulation.

RESULTS AND DISCUSSION

After 2 weeks, untreated wood samples were completely discolored by the sapstain and mold fungi (Table 1), whereas those treated with 1% Hylite Extra did not discolor even after 8 weeks. All dilutions of NVWV inhibited sapstain, whereas 2:1 and 3:1 diluted VWV inhibited sapstain for 6 and 2 weeks, respectively. After 8 weeks, wood treated with VWV at 2:1 and 3:1 dilutions showed 39% and 100% discoloration, but NVWV at the same concentrations provided complete protection against sapstain. VWV was less effective than NVWV in protecting against sapstain. It was reported that wood vinegar from *Quercus* spp. contains mainly 3.5 to 4.0% acetic acid and 80 to 90% water with some strong antifungal compounds such as guaiacol, cresol, 4-ethyl-2-methoxyphenol, and 2, 6-dimethoxyphenol (Yatagai and Unrinin 1989; Ikergami *et al*. 1992; Kim *et al.* 2001). The antifungal compounds such as carboxylic acids and phenols derived from cellulose and lignin, respectively, are considered as volatile organic compounds (Kartal *et al.* 2004; Nakai *et al.* 2007). Thus, it is considered that NVWV was more effective than VWV because the antifungal compounds were vaporized during the preparation process of VWV.

Discoloration by mold fungi was inhibited when using undiluted NVWV and a 1:1 dilution of NVWV. However, after 2 weeks, wood treated with 2:1 and 3:1 dilutions exhibited 5% and 44.3% discoloration by mold, respectively (Table 3). Undiluted VWV provided complete protection against mold after 8 weeks. At 1:1 dilution, NVWV inhibited mold for 8 weeks, but VWV could only inhibit mold for 2 weeks. This clearly indicates that VWV was less effective than NVWV against discoloration caused by either sapstain or mold. These results confirm that the volatile substances in wood vinegar consist of a strong antifungal compound. NVWV at a 1:1 dilution provided complete protection against sapstain and mold after 8 weeks. However, if it is used in conditions of outdoor exposure (simulated by VWV), concentrations of wood vinegar higher than 1:1 dilution might need to be applied in field. Undiluted VWV completely inhibited discoloration by sapstain and mold after 8 weeks; however, when storing timber for less than 2 weeks, a 1:1 dilution of wood vinegar can also be used.

As shown by results of this study, volatile compounds in wood vinegar played important roles as inhibitors to prevent the development of sapstains and molds. There is no evidence to support the identification of volatile compounds as polyphenolic compounds, though 2,6-dimethoxy phenol was described as the main component of wood vinegar; this is a volatile substance that could improve resistance of wood against termite and fungi attack (Sameshima *et al*. 2002; Yatagai *et al.* 2002 and Kartal *et al.* 2004; Nakai *et al.* 2007).

Results of this study also show that volatile compounds in wood vinegar influenced the inhibition of sapstain and mold fungi. This strongly suggests that polyphenolic compounds affect the antifungal activity in addition to the previously reported acidic nature of wood vinegar (Velmurugan *et al.* 2009). Nonetheless, mold can be removed easily by surfacing or planing, and does not discolor wood as severely as sapstain. However, discoloration might penetrate far enough into the wood to be visible in the finished wood product. Thus, the anti-stain treatment should be considered according to the period of storage and end products.

Further studies should be conducted to assess the application of wood vinegar as anti-stain in the field. Moreover, as the antifungal efficiency of wood vinegars was reported to be strongly dependent on the phenolic compound contents (Baimark and

Niamsaa 2009), it is recommended that further studies should be conducted on the chemistry and reactive sites of wood vinegar volatile compounds.

Table 1. Degree of Discoloration of Japanese Red Pine Exposed to Pure Cultures of Sapstain Fungi

Treatment solution	Dilution[a]	Incubation period (weeks)			
		2	4	6	8
Control		100.0 (0.0)[b]	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)
Hylite Extra		0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Non-volatile wood vinegar	undiluted	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	1:1	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	2:1	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	3:1	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Volatile wood vinegar	undiluted	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	1:1	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	2:1	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	39.0 (1.0)
	3:1	0.0 (0.0)	15.3 (0.7)	16.3 (0.0)	100.0 (0.0)

[a]Dilution indicates deionized water : wood vinegar on a volume basis
[b]Degree of discoloration was calculated based on a rating of 0 (no stain) to 100 (completely discolored).

Table 2. Degree of Discoloration of Japanese Red Pine Exposed to Pure Cultures of Mold Fungi

Treatment solution	Dilution[a]	Incubation period (weeks)			
		2	4	6	8
Control		100.0 (0.0)[b]	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)
Hylite Extra		0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Non-volatile wood vinegar	undiluted	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	1:1	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	2:1	5.0 (0.0)	16.3 (0.6)	20.0 (0.0)	23.0 (0.0)
	3:1	44.3 (1.3)	43.3 (1.0)	61.3 (0.6)	64.3 (0.2)
Volatile wood vinegar	undiluted	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	1:1	0.0 (0.0)	18.3 (0.5)	40.7 (0.6)	49.3 (0.6)
	2:1	28.3 (0.5)	43.3 (0.6)	68.7 (1.3)	75.3 (0.9)
	3:1	29.7 (1.0)	70.0 (1.2)	82.7 (1.0)	84.3 (0.6)

[a]Dilution indicates deionized water : wood vinegar on a volume basis
[b]Degree of discoloration was calculated based on a rating of 0 (no stain) to 100 (completely discolored).

CONCLUSIONS

1. Crude wood vinegar is a potential anti-stain agent against sapstain and mold fungi for Japanese red pine. Volatile wood vinegar (VWV) is less effective than non-volatile wood vinegar (NVWV).
2. We suggest using a 1:1 dilution of crude wood vinegar to protect Japanese red pine wood from sapstain for at least 8 weeks. In the case of shorter storage periods, a 3:1 dilution could be used.
3. For protection against mold, undiluted wood vinegar provides protection for at least 8 weeks, whereas a 1:1 dilution could protect against mold for short-term storage of 2 weeks.

REFERENCES CITED

American Society for Testing and Materials (ASTM) (1996). ASTM D4445-91. "Standard test methods for testing fungicides for controlling sapstain and mold on unseasoned lumber (Laboratory Method)."

Baimark, Y., and Niamsaa, N. (2009). "Study on wood vinegars for use as coagulating and antifungal agents on the production of natural rubber sheets," *Biomass Bioenergy* 33, 994-998.

Dawson-Andoh, B. E., Lovell, R., and Kamdem, D. P. (2000). "Inhibitory and compatibility effects of essential oils on sapstain and biological control fungi," *J. Essent. Oil Res*. 12, 509-515.

Ikergami, F., Sekin T., and Fuji, Y. (1992). "Antidemaptophyte activity of phenolic compounds in *mokusaki-eki*," *Yakugaku Zasshi* 118, 27-30.

Kartal, S. N., Imamura, Y, Tsuchiya, F., and Ohsato, K. (2004). "Preliminary evaluation of fungicidal and termiticidal activities of filtrates from biomass slurry fuel production," *Bioresource Technology* 95, 41-47.

Kim, Y. H., Kim, S. K., Kim, K. S., and Lee, Y. H. (2001). "Compounds identification of commercial wood vinegar liquors," *Journal of the Korean Society of Agricultural Chemistry and Biotechnology* 44, 262-268.

Nakai, T., Kartal, S. N., Hata, T., and Imamura, Y. (2007). "Chemical characterization of pyrolysis liquids of wood-based composites and evaluation of their bio-efficiency," *Building and Environment* 42, 1236-1241.

Sameshima, K., Sasaki, M., and Sameshima, I. (2002). "Fundamental evaluation on termiticidal activity of various vinegar liquids from charcoal making," in: Proceedings of the Fourth International Wood Science Symposium, Sept. 2-5, 2002, Serpong, Indonesia, 134-138.

Sulaiman, O., Murphy, R. J., Hashim, R., and Gritsch, C. S. (2005). "The inhibition of microbial growth by bamboo vinegar," *J. Bamboo Rattan* 4(1), 71-80.

Velmurugan, N., Han, S. S., and Lee, Y. S. (2009). "Characterization of *chikusaku-eki* and *mokusaku-eki* and its inhibitory effect on sapstaining fungal growth in laboratory scale," *Int. J. Env. Res.* 3(2), 167-176.

Yatagai, M, and Unrinin, G. (1989). "Byproducts of wood carbonization: Germination and growth regulation effects of wood vinegar components and their homologues on plant seeds: Acids and neutrals," *Mokuzal Gakkai Shi* 35, 564-571.

Yatagai, M., Nishimoto, M., Hori, K., Ohira, T., and Shibata, A. (2002). "Termiticidal activity of wood vinegar, its components," *J. Wood Sci.* 48, 338-342.

Article submitted: September 18, 2013; Peer review completed: November 2, 2013; Revised version received and accepted: December 2, 2013; Published: December 11, 2013.