

# Application of Plant Essential Oils in Controlling Wood Mold and Stain Fungi

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The antifungal activities of 19 plant essential oils against six wood mold and stain fungi (*Aspergillus niger*, *Penicillium citrinum*, *Trichoderma viride*, *Botryodiplodia theobromae*, *Fusarium moniliforme*, and *Alternaria alternata*) were investigated with the *in vitro* medium method. The chemical compositions and volatilization rates of the essential oils were analyzed by gas chromatography–mass spectrometry and oven heating, respectively. Antifungal effects of the essential oils on fresh *Pinus massoniana* wood were evaluated by dipping treatment. The average antifungal efficacies of the essential oils varied from 0.1 to 1.0, and oils of *Cinnamomum cassia*, *Syzygium aromaticum*, and *Thymus mongolicus* showed the greatest antifungal activities and completely inhibited the growth of all six fungi. The essential oils presented great differences in their main chemical components, and a significant negative linear correlation (Pearson correlation coefficient = -0.627,  $p < 0.01$ ) was found between antifungal efficacy and volatilization rate, indicating that both chemical composition and volatilization rate are important factors influencing the antifungal activities of essential oils. Eight essential oils effectively inhibited the growth of fungi in *Pinus massoniana* wood with an absorption of  $65.51 \text{ g/m}^2 \pm 13.78 \text{ g/m}^2$ , and they have the potential to be environmentally friendly anti-mildew agents.

*Keywords:* Plant essential oils; Antifungal activity; Mold and stain fungi; Chemical composition; Volatilization rate

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## INTRODUCTION

Fresh timbers from some important plantations (such as those of *Pinus massoniana* and *Hevea brasiliensis*) in southern China are prone to suffering from infection by mold and stain fungi. Although fungal colonization and disfigurement of wood usually have no substantial effect on its density and mechanical properties, they are detrimental to its aesthetic value and cause economic losses (Salem *et al.* 2016; Chang *et al.* 2018). Therefore, effective prevention of fungal growth is beneficial to resource saving and the value-added utilization of plantation wood.

Environmentally friendly natural products have attracted increasing attention in protecting wood and wood-based materials against deteriorating organisms such as fungi, bacteria, and termites (Singh and Singh 2012). Some extracts from plants (Mohareb *et al.* 2013), heartwood (Belt *et al.* 2018), and seeds (Chang *et al.* 2018) inhibit wood decay fungi and mold fungi. Among these extracts, plant essential oils are secondary metabolites of natural plants and have a complex chemical composition, mainly including terpenoid derivatives, aromatic compounds, and aliphatic compounds (Pandey *et al.* 2017). They

exhibit antioxidative and antibacterial characteristics, health benefits, and mosquito repellency owing to the variety of functional groups in their molecules, and they are thus widely used in the food, pharmaceutical, agricultural, and textile industries (Jugreet *et al.* 2020). Considering their wide variety of sources and good antifungal properties, plant essential oils show great potential for controlling wood mold and stain fungi. They can be used to treat wood by impregnating, brushing or spraying, to prevent fresh felled logs from fungi infection, or to prevent air-dried or kiln-dried timbers from fungi infection especially in hot and humid weather during storage. For the wood applied in furniture or buildings, plant essential oils can be used to protect the products from mold and stain fungi, or to provide the products with special fragrance. Nevertheless, the effect of the essential oil treatment on wood bonding and finishing performance and the persistence of fragrance remain to be investigated.

Recently, many studies have been done on applying plant essential oils to wood protection. For *in vitro* antifungal tests, essential oils from *Cinnamomum osmophloeum* (Wang *et al.* 2005), *Eucalyptus* (Varshney *et al.* 2012), Egyptian plants (Mohareb *et al.* 2013), *Lippia origanoides* (Medeiros *et al.* 2016), and *Origanum vulgare* (Xie *et al.* 2017) were reported to have strong inhibitory effects on typical wood decay fungi, while essential oils of geranium, cinnamaldehyde, and eugenol inhibit the growth of four sapstain fungi at a concentration of 1% w/v (Chittenden and Singh 2011). In *in vivo* antifungal tests on wood samples, cinnamon oil completely inhibited the growth of *Aspergillus niger* on rubber wood (Ma-in *et al.* 2014), and essential oils from *Pinus rigida* wood completely inhibited the growth of four common mold fungi (*Alternaria alternata*, *Fusarium subglutinans*, *Chaetomium globosum*, and *Aspergillus niger*) on wood surfaces of *Pinus sylvestris*, *Pinus rigida*, and *Fagus sylvatica* (Salem *et al.* 2016). Additionally, oils from myrtlewood, orange, lime, and Leyland cypress needles significantly improved the anti-fungal activity of *Bacillus subtilis* B26 (Wang *et al.* 2012). However, as far as the authors know, few studies have focused on the antifungal activities of plant essential oils against wood mold and stain fungi in common occurrence in China's forestry.

In this study, the antifungal efficacies of nineteen plant essential oils against six common deteriorating mold and stain fungi on plantation timbers in China were investigated. The chemical compositions and volatilization rates of the essential oils were further analyzed to illustrate their antifungal activities. The actual antifungal effects of essential oils on *Pinus massoniana* wood blocks were also evaluated.

## EXPERIMENTAL

### Materials

Logs with a length of 1 m were obtained from a freshly felled 25-year-old *Pinus massoniana* and immediately sawn to timbers. The timbers from the sapwood were then processed to 50 mm (longitudinal) × 20 mm × 5 mm blocks and stored frozen until use.

Nineteen plant essential oils (Table 1) were purchased from Guangzhou Bosilin Co., Ltd. (Guangzhou, China). Three wood mold fungi (*Aspergillus niger*, *Penicillium citrinum*, and *Trichoderma viride*) and three wood stain fungi (*Botryodiplodia theobromae*, *Fusarium moniliforme*, and *Alternaria alternata*) were provided by the China Forestry Culture Collection Center (Beijing, China). Potato dextrose agar (PDA, BR grade) nutrient medium was purchased from Qingdao Hope Bio-Technology Co., Ltd. (Qingdao, China). Sterile water was made in the laboratory.

**Table 1.** Basic Information about Plant Essential Oils

| No. | Plant Species                 | Source                | Extraction Method | Color         |
|-----|-------------------------------|-----------------------|-------------------|---------------|
| 1   | <i>Laurus nobilis</i>         | Leaves                | Distillation      | Brownish red  |
| 2   | <i>Cedrus deodara</i>         | Leaves, wood          | Distillation      | Light yellow  |
| 3   | <i>Cinnamomum cassia</i>      | Buds, bark and leaves | Distillation      | Light yellow  |
| 4   | <i>Cupressus sempervirens</i> | Leaves, cones         | Distillation      | Colorless     |
| 5   | <i>Eucalyptus robusta</i>     | Leaves                | Distillation      | Colorless     |
| 6   | <i>Syzygium aromaticum</i>    | Leaves, buds          | Distillation      | Orange-yellow |
| 7   | <i>Pelargonium x hortorum</i> | Leaves                | Distillation      | Light green   |
| 8   | <i>Lavandula angustifolia</i> | Flowers               | Distillation      | Colorless     |
| 9   | <i>Cymbopogon citratus</i>    | Stem, leaves          | Distillation      | Light yellow  |
| 10  | <i>Myristica fragrans</i>     | Fruits                | Distillation      | Light yellow  |
| 11  | <i>Citrus sinensis</i>        | Fruits                | Squeeze           | Light yellow  |
| 12  | <i>Mentha haplocalyx</i>      | Flowers, leaves       | Distillation      | Colorless     |
| 13  | <i>Pinus</i>                  | Leaves, cones         | Distillation      | Light green   |
| 14  | <i>Rosmarinus officinalis</i> | Flowers, leaves       | Distillation      | Colorless     |
| 15  | <i>Ormosia henryi</i>         | Wood                  | Distillation      | Colorless     |
| 16  | <i>Thymus mongolicus</i>      | Leaves, flowers       | Distillation      | Orange-yellow |
| 17  | <i>Camellia sinensis</i>      | Leaves                | Distillation      | Colorless     |
| 18  | Styracaceae                   | Resin                 | Distillation      | Brown         |
| 19  | <i>Ilex chinensis</i>         | Leaves                | Distillation      | Colorless     |

## Methods

### *In vitro antifungal test*

The test was conducted according to the method used in Chittenden and Singh (2011) and Mohareb *et al.* (2013). A solution of the nutrient medium was prepared by steaming 46 g of the PDA and 1000 mL of sterile water at 115 °C for 30 min in a pressure steam sterilizer (LDZM-80KCS, Shanghai Medical Instruments Co., Ltd., Shanghai, China). The solution was poured into sterilized 9-mm Petri dishes to obtain solid plate mediums in a clean bench (SW-CJ-IFD, Suzhou Purification Equipment Co., Ltd., Suzhou, China). The strains of the test fungi were transferred with inoculating needles to the centers of the mediums and cultivated in a climate chamber (temperature 27 °C, relative humidity 65%) for 1 week until the colonies matured.

A nutrient medium solution was prepared following the above method and stored in a water bath at 65 °C. The medium solution was mixed evenly with plant essential oils (0.05 vol%) in a 50-mL centrifuge tube by a vortex mixer and poured into sterilized Petri dishes to obtain an amended solid plate medium. The discs of mature fungi colonies (5-mm diameter) were transferred to the center of the plate medium and placed in the climate chamber (temperature 27 °C, relative humidity 65%) to cultivate. Three replicates were set for each treatment, and mediums without plant essential oils were used as the control group. The growth of the fungi was observed daily, and the test was ended when the control colony filled the whole medium. The final diameter of the colony was measured by the cross-over method. The antifungal efficacy was calculated as  $(D_1 - D_2) / D_1$ , where  $D_1$  and  $D_2$  are the colony diameters of the control and amended samples, respectively.

### *Measurement of chemical compositions and volatilization rates of plant essential oils*

The chemical compositions of the plant essential oils were analyzed by a gas chromatography–triple quadrupole mass spectrometer (SCION SQ-TQ, Bruker, Billerica, MA, USA) equipped with a flame ionization detector (FID) and a DB-5 capillary column (30 m × 0.32 mm × 0.25 μm). Helium was used as the carrier gas, and the oven temperature program was as follows: 55 °C (3 min), 70 °C to 250 °C (3 °C/min), and 250 °C (5 min). Samples (1 μL) were injected with a split ratio of 50:1, and the temperatures of the vaporization chamber and detector were 250 °C. The electron bombardment source was electron ionization (EI) with an electron energy of 70 eV, and the ion source temperature was 250 °C. The interface temperature was 250 °C, and the solvent delay time was 3 min.

The essential oil sample (2 mL) was transferred into a 5-mL centrifuge tube by a pipette and heated in an oven (60 °C) for 3 h. The initial and final weights of the essential oil were recorded. The volatilization rate was calculated as  $(m_1 - m_2) / (S \cdot t)$ , where  $m_1$  and  $m_2$  are the initial and final masses of the essential oil, and  $S$  and  $t$  are the liquid surface area and volatilization time, respectively. Ultrapure water was taken as the control group, and three replicates were used for each essential oil.

### *Antifungal effect of plant essential oils on Pinus massoniana wood*

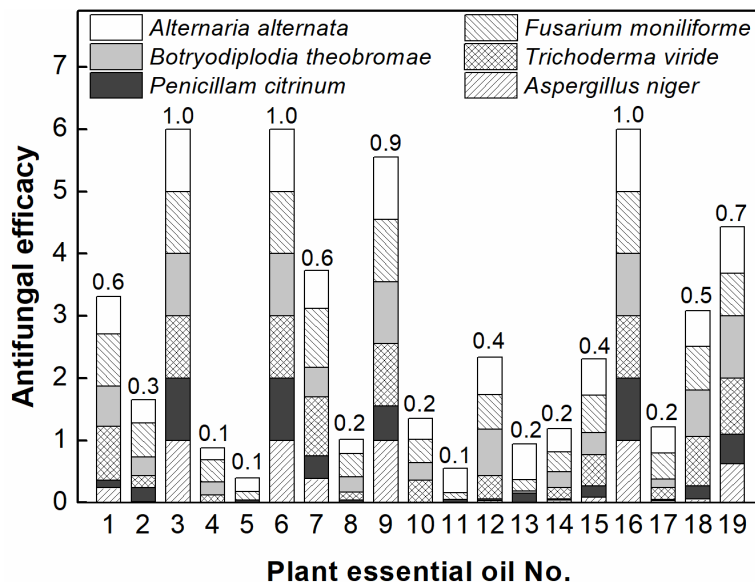
This test was conducted referring to the Chinese standard GB/T 18261 (2013) with some modifications. Frozen wood blocks were taken out, thawed for 30 min, and dipped into beakers with plant essential oils for 10 min. The weights of the blocks before and after treatment were recorded to determine the absorption of the oils. The PDA solid plate mediums were prepared following the above method, and two sterilized glass rods were parallelly placed on the medium. Two treated blocks were then put on the glass rods, and the Petri dishes were transferred into the climate chamber (temperature 28 °C, relative humidity 85%) for 4 weeks. Six replicated blocks were used for each essential oil treatment, and untreated blocks were set as the control group. The number of blocks with growing fungi was recorded weekly. The inhibitory efficacy of a plant essential oil was simply measured by the number of blocks without fungi divided by the total number of blocks used. Pearson correlation analysis and least squares regression analysis were used to investigate the relationship between antifungal efficacy and volatilization rate with SPSS Statistics.

## RESULTS AND DISCUSSION

### Antifungal Efficacies of Plant Essential Oils

The antifungal efficacies of the selected plant essential oils against the six fungi are presented in Fig. 1. The average antifungal efficacy varied from 0.1 to 1.0, indicating substantial differences in antifungal activity among these essential oils. The essential oils of *C. cassia* (#3), *S. aromaticum* (#6), and *T. mongolicus* (#16) showed the greatest antifungal activities and completely inhibited the growth of all wood mold and stain fungi. The essential oil of *C. citratus* (#9) also had high antifungal activity, effectively inhibiting the growth of five fungi (all except *Penicillium citrinum*). The essential oils of *L. nobilis* (#1), *P. × hortorum* (#7), Styracaceae (#18), and *I. chinensis* (#19) showed moderate antifungal activities, with average antifungal efficacies of 0.5 to 0.7. The remaining ten categories of essential oils presented relatively poor antifungal activities, having average antifungal efficacies of 0.1 to 0.4. Additionally, the average antifungal efficacies of the

nineteen essential oils against *A. niger*, *P. citrinum*, *T. viride*, *B. theobromae*, *F. moniliforme*, and *A. alternata* were 0.3, 0.3, 0.5, 0.5, 0.6, and 0.6, respectively. The variation in antifungal efficacy of plant essential oils with different sources has also been demonstrated in other reports (Ma-in *et al.* 2014; Xie *et al.* 2017).



**Fig. 1.** Antifungal efficacies of plant essential oils against wood mold and stain fungi. Numbers above the columns represent the average antifungal efficacies of the plant essential oils against the six fungi. The height of each different section in a bar represents the antifungal efficacy of the essential oil against the corresponding fungal.

### Chemical Compositions of Plant Essential Oils

The main chemical compositions (constituents with concentrations greater than 5%) of the nineteen plant essential oils are shown in Table 2. Thirty-seven chemicals were determined, and 1 to 5 constituents were found for each essential oil. Although a few compounds such as d-limonene,  $\alpha$ -pinene, eugenol, and linalool were present in several essential oils, the chemical components of the samples presented notable differences overall. These differences should be an important reason for their variation in antifungal activity. The most abundant components in the essential oils of *C. cassia* (#3), *S. aromaticum* (#6), and *C. citratus* (#9) were consistent with previous reports (Deng *et al.* 2014; Ma-in *et al.* 2014; Xie *et al.* 2017), whereas obvious differences were found in some other constituents due to the variations in raw materials and the extraction process.

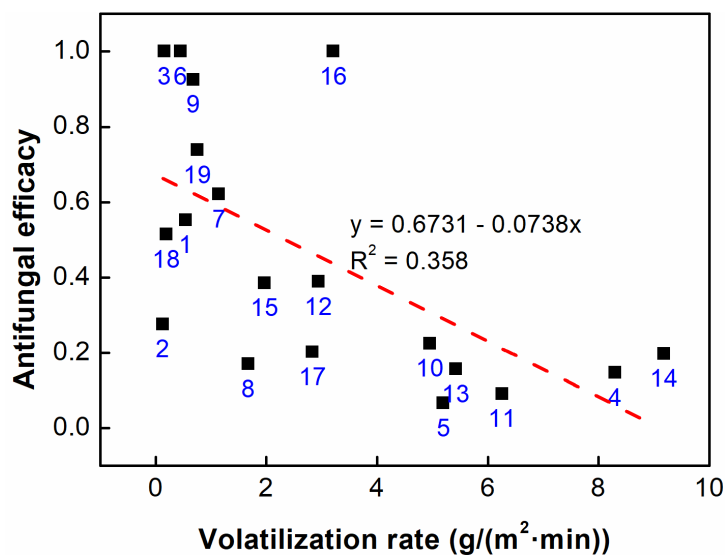
The strong fungicidal activities of cinnamaldehyde, eugenol, and thymol (Xie *et al.* 2017) accounted for the high antifungal efficacies of the essential oils of *C. cassia* (#3), *S. aromaticum* (#6), and *T. mongolicus* (#16). In contrast, the main constituents such as  $\alpha$ -pinene, 1,8-cineole, and d-limonene from essential oils with low antifungal efficacies should have poor antifungal activities. Moreover, the antifungal activities of the essential oils were also influenced by the concentrations of the constituents (Salem *et al.* 2016; Xie *et al.* 2017). For example, plant essential oils of *S. aromaticum* (#6), *Laurus nobilis* (#1), and *Myristica fragrans* (#10), with eugenol concentrations of 87.19%, 24.78%, and 6.50%, had average antifungal efficacies of 1.0, 0.6, and 0.2, respectively. It is suggested that the types and concentrations of chemical components of plant essential oils are decisive factors for their antifungal activities.

**Table 2.** Main Chemical Constituents of Plant Essential Oils

| No. | Constituents and Their Concentrations (%)   |
|-----|---|
| 1   | $\beta$ -caryophyllene (51.77); eugenol (24.78)   |
| 2   | (+)- $\alpha$ -longipinene (44.72); (-)- $\alpha$ -himachalene (15.83); ethanol, 2-(3,3-dimethylbicyclo[2.2.1] hept-2-ylidene)- (10.97); (+)-g-gurjunene (8.98) |
| 3   | Cinnamaldehyde (84.46); methyl benzoate (5.03)  |
| 4   | $\alpha$ -pinene (64.61); 3-thujene (6.71); bornyl acetate (6.48)   |
| 5   | 1,8-cineole (80.44); d-limonene (6.62)  |
| 6   | Eugenol (87.19); $\beta$ -caryophyllene (10.24)   |
| 7   | Citronellol (29.66); neryl alcohol (25.01); 3,7-dimethyl-1-octene (13.76); linalool (11.29)   |
| 8   | Linalyl anthranilate (69.64); linalool (19.16)  |
| 9   | Geranial (49.30); neral (34.70); neryl alcohol (7.86)   |
| 10  | d-limonene (34.01); $\alpha$ -terpineol (19.25); $\alpha$ -pinene (18.97); eugenol (6.50); gamma-terpineol (5.82)   |
| 11  | d-limonene (98.26)  |
| 12  | (+/-)-p-menthan-3-ol (44.65); isomenthone (17.40); d-limonene (9.49); (-)-menthone (6.22)   |
| 13  | Bornyl acetate (30.45); d-limonene (20.64); camphene (19.62); $\alpha$ -pinene (15.28)  |
| 14  | $\alpha$ -pinene (29.00); 1,8-cineole (27.00); L-camphor (25.37); isoborneol (7.25)   |
| 15  | Linalool (83.68)  |
| 16  | g-terpinene (53.98); thymol (22.92); 1-isopropyl-3-methylbenzene (21.74)  |
| 17  | 4-carvomenthenol (56.41); g-terpinene (18.88); a-terpinene (7.41)   |
| 18  | Benzyl benzoate (73.11); cinnamyl alcohol (12.32)   |
| 19  | Methyl hydroxybenzoate (99.80)  |

### Volatilization Rate and Its Correlation with Antifungal Efficacy

The volatilization rates of the plant essential oils ranged from 0.13 g/(m<sup>2</sup>·min) to 9.18 g/(m<sup>2</sup>·min) (Fig. 2), lower than that of the ultrapure water (14.98 g/(m<sup>2</sup>·min)).

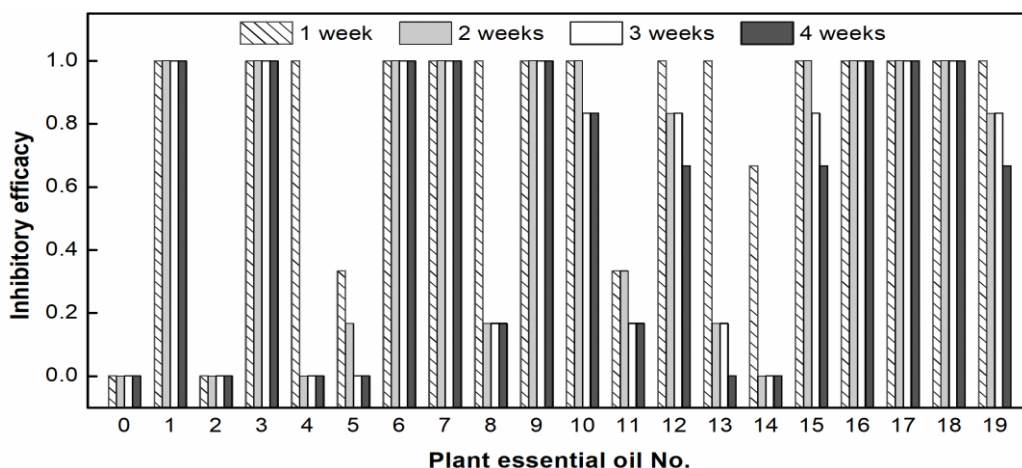


**Fig. 2.** Correlation between antifungal efficacy and volatilization rate in plant essential oils. Blue numbers below squares represent the identifying numbers of the plant essential oils.

Interestingly, a significant negative linear correlation (Pearson correlation coefficient = minus 0.627,  $p < 0.01$ ) was found between antifungal efficacy and volatilization rate. Plant essential oils with high antifungal efficacies ( $> 0.4$ ) showed low volatilization rates ( $< 4 \text{ g}/(\text{m}^2 \cdot \text{min})$ ), while plant essential oils with high volatilization rates ( $> 4 \text{ g}/(\text{m}^2 \cdot \text{min})$ ) had low antifungal efficacies ( $< 0.3$ ). However, a few plant essential oils (such as #2 and #8) with low volatilization rates ( $< 2 \text{ g}/(\text{m}^2 \cdot \text{min})$ ) also possessed low antifungal efficacies ( $< 0.3$ ). Overall, it seems that plant essential oils with high volatilization rates would rarely have high antifungal activities, though research on more types of plant essential oils is needed.

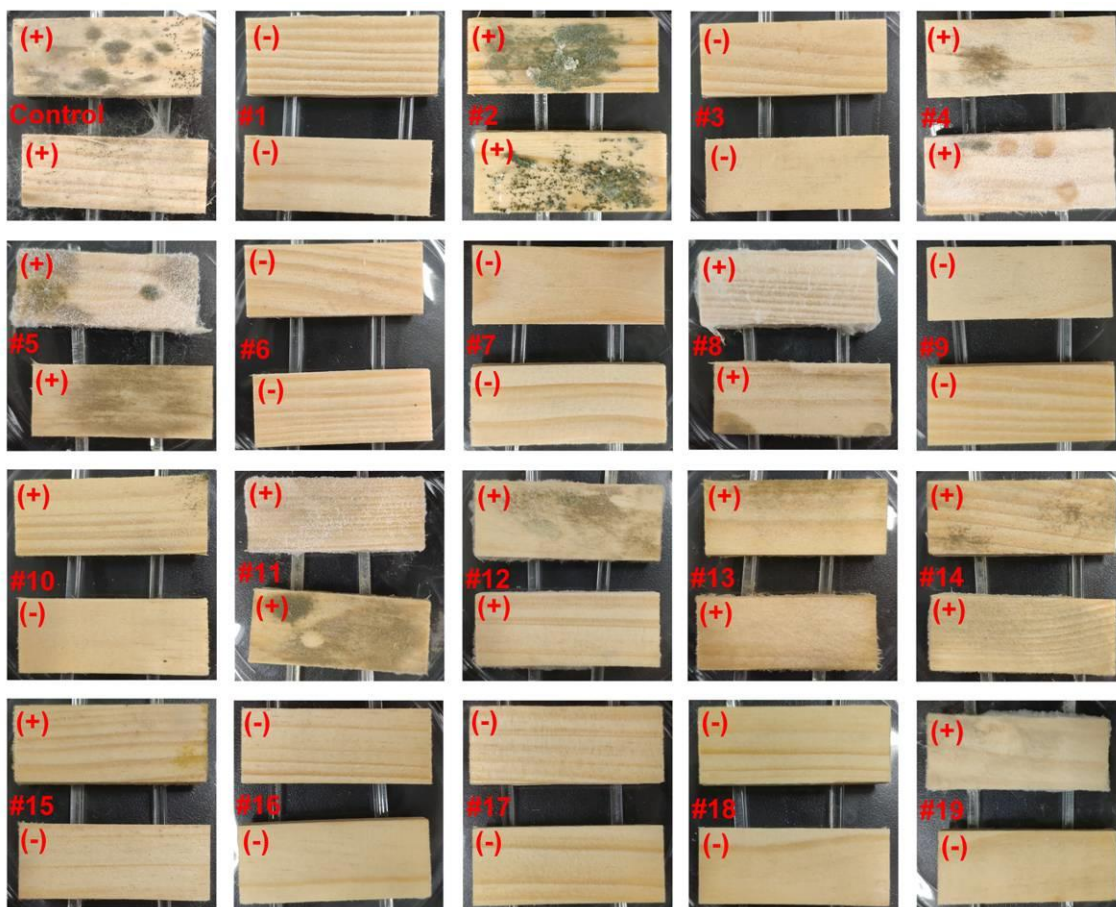
### Inhibitory Effects of Plant Essential Oils on the Growth of Fungi in Wood

The absorption of the plant essential oils in the block samples was  $65.51 \text{ g}/\text{m}^2 \pm 13.78 \text{ g}/\text{m}^2$ , and their inhibitory efficacies on fungi growth in the wood is presented in Fig. 3. After 4 weeks of testing, plant essential oils #1, #3, #6, #7, #9, #16, #17, and #18 completely inhibited the growth of fungi in *Pinus massoniana* wood, while the inhibitory efficacies of #2, #4, #5, #13, and #14 essential oils were 0. The *in vitro* antifungal efficacies of the plant essential oils were generally consistent with their inhibitory efficacies (Mohareb *et al.* 2013). However, the overall inhibitory effects of the essential oils were superior to the *in vitro* antifungal activities, probably due to their greater loadings (approximately 3.39 vol%) in the blocks test. Notably, the inhibitory efficacies of ten of the essential oils substantially decreased as the test time extended from 1 week to 4 weeks, indicating that the antifungal activities of some plant essential oils had validity periods.



**Fig. 3.** Inhibitory efficacies of plant essential oils on fungi growth in *Pinus massoniana* wood. No. 0 – control experimental group

The appearances of the *Pinus massoniana* wood blocks after the test are shown in Fig. 4. For the control group, mycelium growth was observed in all six samples after only 3 days, which confirmed that fresh *Pinus massoniana* wood is easily infected by mold and stain fungi. The mycelia on different block samples showed diversity in their colors and morphologies, possibly owing to the effects of the plant essential oils on fungi growth and the variation of fungi within the fresh wood. Some of the tested plant essential oils (such as #1, #3, #6, and #7) effectively prevented fungal colonization and disfigurement of the wood samples, and they have the potential to be developed as environmentally friendly wood protectants against mold and stain fungi.



**Fig. 4.** Appearances of the *Pinus massoniana* wood blocks after the antifungal effects test. Plus (+) and minus (-) signs represent growth and non-growth of fungi on the blocks, respectively.

## CONCLUSIONS

1. The average *in vitro* antifungal efficacies of the plant essential oils varied from 0.1 to 1.0, and *C. cassia*, *S. aromaticum*, and *T. mongolicus* showed the greatest antifungal activities and completely inhibited the growth of all six fungi.
2. Thirty-seven main constituents were determined in the plant essential oils. The essential oils presented great differences in their main chemical components, indicating that chemical composition was an important factor influencing the antifungal activities of the essential oils.
3. The volatilization rates of the plant essential oils ranged from 0.13 g/(m<sup>2</sup>·min) to 9.18 g/(m<sup>2</sup>·min), and a significant negative linear correlation (Pearson correlation coefficient = -0.627, *p* < 0.01) was found between antifungal efficacy and volatilization rate.
4. The plant essential oils of *L. nobilis*, *C. cassia*, *P. × hortorum*, *S. aromaticum*, *C. citratus*, *T. mongolicus*, *Styracaceae*, and *C. sinensis* completely inhibited the growth of fungi in the *Pinus massoniana* wood with an absorption of 65.51 g/m<sup>2</sup> ± 13.78 g/m<sup>2</sup>,



and they have the potential to be developed as environmentally friendly anti-mildew agents.

## ACKNOWLEDGMENTS

The authors are grateful for the support of the Science and Technology Major Project of Guangxi (Grant No. GUIKE AA17204087-16), the Guangxi Natural Science Foundation (Grant No. 2017GXNSFBA198064), and the Guangxi Science and Technology Base and Talent Special Project (Grant No. GUIKE AD19254004).

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Article submitted: September 9, 2020; Peer review completed: December 5, 2020;  
Revised version received and accepted: December 22, 2020; Published: December 23, 2020.

DOI: 10.15376/biores.16.1.1325-1334