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Short communication

Antifungal activity of monoterpenes against wood white-rot fungi

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ABSTRACT

Biodegradation of wood by fungi is a chronic problem for wood utilization. Natural products from plants have great potential as novel fungicide sources for controlling wood decay fungi as alternative to synthetic pesticides. Many plant extracts and essential oils contain monoterpenes, sesquiterpenes and aliphatic compounds. In the present study, 41 pure monoterpenes were tested for the toxicity against wood white-rot fungi, *Trametes hirsuta*, *Schizphylhls commune*, and *Pycnoporus sanguineus*. Results from antifungal tests revealed that β-citronellol, geraniol, carvacrol, thymol, eugenol and citral showed good antifungal activities against the three tested fungi. In addition, the carvacrol was the most toxic monoterpenes against the three tested fungi, the IC_{50} values against *T. hirsuta*, *S. commune* and *P. sanguineus* were 87.6, 53.6 and 71.7 μg ml^{-1}, respectively. The present results indicated that carvacrol have potential to be developed as natural fungicidal agents, suggesting that further study of wood preservation treatment be warranted.

1. Introduction

Biodegradation of wood by fungi is a serious problem in many tropical and some temperate region of the world, resulting in a significant economic and resource losses (Andrew et al., 2001). White-rot fungi are the only microorganisms that are known to degrade efficiently all the components of wood, including lignin (Kirk and Farrell, 1987; Shary et al., 2007; Bakar et al., 2013). Traditionally, preservative treatments to wood are used to be creosote, pentachlorophenol and inorganic arsenicals for addressing this durability problem (Cheng et al., 2008). However, these preservatives are used in long-term resulting to produce a significant problem on human health and environmental pollution. Therefore, the search for natural, safe and none polluting bioactive chemical compounds from plants as alternative to synthetic preservative becomes much essential (Loh et al., 2011; Hu et al., 2015).

Many plant materials are believed to have antifungal properties and fungicidal properties, and numerous essential oils have been extensively studied against various fungi species (Cheng et al., 2006, 2012; Yang and Clausen, 2007; Zabka et al., 2009; Hassiotis and Dina, 2011; Chen et al., 2013; Jing et al., 2014; Kedia et al., 2014; Regnier et al., 2014; Vieira et al., 2014; Juárez et al., 2015; Salem et al., 2016). Futhermore, it has been found that the natural compounds possess the fungicidal activities against wood decay fungi, among of these natural compounds, eugenol, α-cadinol, T-muurolol, T-cadinol, γ-cadinene, cryptomeridiol, chamaecynone, cinnamaldehyde and ferruginol were found to be more toxic (Rudman, 1965; Kondo and Imamura, 1986; Morita et al., 1997; Chang et al., 1999, 2000; Wang et al., 2005a,b; Wu et al., 2005; Cheng et al., 2004, 2008; Yen and Chang, 2008). Notoriously, many plant extracts and essential oils may be as the alternative sources of fungal control agents because of their rich sources of bioactive chemicals, including monoterpenes, sesquiterpenes and aliphatic compounds. Our previous study demonstrated that the 42 evaluated monoterpenes had excellent anti-termitic activity (Xie et al., 2014). Now we are interesting to know whether these monoterpenes possess the antifungal property against white-rot fungi. Therefore, this paper describes a laboratory study to examine the toxicity of 41 monoterpenes against the three white-rot fungi, *Trametes hirsuta*, *Schizphylhls commune*, and *Pycnoporus sanguineus*.

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2. Materials and methods

2.1. Chemicals

Forty-one monoterpenes, (−)-α-pinene (1), (−)-β-pinene (2), ρ-cymene (3), 3-carene (4), γ-terpinene (9), terpinolene (10), β-citronellene (11), eugenol (23), (−)-verbenone (29), (±)-citronellal (34) were purchased from Sigma–Aldrich chemical Co. (Shanghai, China). (±)-camphene (5), (±)-limonene (6), myrcene (7), α-terpinene (8), α-terpineol (12), (±)-terpinen-4-ol (13), (±)-menthol (14), isopulegol (15), (−)-borneol (16), nerol (17), linalool (18), dihydrolinalool (19), β-citronellol (20), geraniol (21), 3,7-dimethyl-1-octanol (22), thymol (24), carvacrol (25), isoeugenol (26), (+)-camphor (27), (−)-fenchone (28), (+)-pulegone (30), (−)-carvone (31), (−)-menthone (32), citral (33), cuminaldehyde (35), bornyl acetate (36), linalyl acetate (37), terpinyl acetate (38), neryl acetate (39), citronellyl acetate (40), geranyl acetate (41) were purchased from Tokyo chemical Industry Co., Ltd. (Shanghai, China).

2.2. Fungi

Three white-rot fungi (*T. hirsuta*, *S. commune*, and *P. sanguineus*) were obtained from the college of life science, Hubei Engineering University and used in antifungal experiments.

2.3. Antifungal assays

The method of Chang et al. (1999) was employed for antifungal evaluation of 41 monoterpenes. Briefly, 400, 200, 100 and 50 μg ml^{-1} of compounds were added to sterilize potato dextrose agar (PDA) in 9 cm Petri dishes. Fungicide stock solutions were prepared at a standard concentration of 100 mg ml^{-1} in analytical-grade acetone. For liquid monoterpenes compounds, directly took a certain amount for experimental study. Antifungal assays were performed three times. After fungal mycelia reached the edges of control plates (without adding compounds) by incubating at 26 ± 1 °C for ca. 7 d, the antifungal index was calculated as follows: Antifungal index (%) = $(1 - D_a/D_b) \times 100$ where, D_a = the diameter of growth zone in the experimental plate (cm), D_b = the diameter of growth zone in the control plate (cm).

2.4. Statistical analyses

All results were obtained from three independent experiments and expressed as mean ± SD ($n = 3$). Significant differences ($P < 0.05$) were determined by using the Scheffe's test.

3. Results and discussion

Fig. 1 presents the results of antifungal activity of these compounds against white-rot fungus *T. hirsuta*, *S. commune*, and *P. sanguineus* at the concentration of 400 μg ml^{-1}. Results from antifungal tests revealed that compounds 17, 20–21, 23–25 and 33 were the most active against *T. hirsuta* amongst the 41 tested compounds, with antifungal indices higher than 81.8%; while other compounds were the lower active, with antifungal indices lower than 53.7%. Similarly, compounds 17, 20–21, 23–26 and 33 also exhibited strong antifungal action with antifungal indices higher than 66.3% against *S. commune* at the concentration of 400 μg ml^{-1}, while other compounds did not express antifungal activities at the same concentration, with antifungal indices lower than 44.8%. In addition, compounds 17, 20, 22, 23–26 and 33 exhibited strong antifungal action with antifungal indices higher than 63.5% against *P. sanguineus* at the concentration of 400 μg ml^{-1}, while other compounds did not express antifungal activities at the same concentration, with antifungal indices lower than 53.3%. It was noticed that compounds 23–25 and 33 exhibited the strongest activity against *T. hirsuta*, *S. commune*, and *P. sanguineus*.

The IC_{50} values of the 9 compounds were determined in Table 1. In terms of the IC_{50} values, the antifungal activity of compounds 17, 20–21, 23–25 and 33 against *T. hirsuta* is ranked as eugenol (23) (85.1 μg ml^{-1}) > carvacrol (25) (87.6 μg ml^{-1}) > thymol (24) (139.0 μg ml^{-1}) > nerol (17) (166.3 μg ml^{-1}) > citral (33) (184.5 μg ml^{-1}) > geraniol (21) (189.3 μg ml^{-1}) > β-citronellol (20) (266.4 μg ml^{-1}). On the other hand, the order of IC_{50} values against *S. commune* are carvacrol (25) (53.6 μg ml^{-1}) > thymol (24) (67.1 μg ml^{-1}) > eugenol (23) (122.2 μg ml^{-1}) > isoeugenol (26) (132.0 μg ml^{-1}) > citral (33) (207.5 μg ml^{-1}) > β-citronellol (20) (219.5 μg ml^{-1}) > geraniol (21) (224.4 μg ml^{-1}) > nerol (17) (337.6 μg ml^{-1}). In addition, the IC_{50} values against *P. sanguineus* are carvacrol (25) (71.7 μg ml^{-1}) > thymol (24) (116.8 μg ml^{-1}) > eugenol (23) (137.7 μg ml^{-1}) > citral (33) (178.5 μg ml^{-1}) > β-citronellol (20) (190.2 μg ml^{-1}) > 3,7-dimethyl-1-octanol (22) (221.5 μg ml^{-1}) > nerol (17) (242.7 μg ml^{-1}) > isoeugenol (26) (247.1 μg ml^{-1}) > geraniol (21) (367.6 μg ml^{-1}).

In this study, we found carvacrol was the most toxic monoterpenes against the three tested fungi, the IC_{50} values of carvacrol against *T. hirsuta*, *S. commune* and *P. sanguineus* were 87.6, 53.6 and 71.7 μg ml^{-1}, respectively. As for IC_{50}, the similar results were found in earlier literature, such as that the IC_{50} values of T-muurolol against *Laetiporus sulphureus*, *Lenzites betulina*, *Trametes versicolor* and *Pcynoporus coccineus* were 93.3, 74.1, 81.0 and 57.3 μg ml^{-1}, respectively (Cheng et al., 2004). Cheng et al. (2008) had proven that cinnamaldehyde was a strong antifungal agent against *L. betulina* and *L. sulphureus* with the IC_{50} values of 58.9 and 35.3 μg ml^{-1}, respectively, and these observations were in accordance with the results reported previously (Wang et al., 2005a; Hsu et al., 2007; Yen and Chang, 2008). On the other hand, Cheng et al. (2004) reported that the IC_{50} values of α-cadinol against *L. sulphureus*, *L. betulina*, and *T. versicolor* were 9.9, 28.6, and 30.4 μg ml^{-1}, respectively. These results show that carvacrol is a potential compound for the development of fungicides in the near future.

In our study, eugenol exhibited good antifungal activities against the three tested fungi, the IC_{50} values of eugenol against *T. hirsuta*, *S. commune* and *P. sanguineus* were 85.1, 122.2 and 137.7 μg ml^{-1}, respectively. Comparing our results with those reported earlier in the literature, Cheng et al. (2008) stated that the IC_{50} values of eugenol against *L. betulina* and *L. sulphureus* were 36.9 μg ml^{-1} and 62.9 μg ml^{-1}, respectively. Similar results were also obtained by Wang et al. (2005a) and Yen and Chang (2008). These results suggested that the susceptibility of the same compounds to different fungi species varies considerably.

In the present study, we found that the antifungal activity of oxygenated monoterpenes was higher than that of monoterpene hydrocarbons against white-rot fungi. Relatively lower toxicity of monoterpene hydrocarbons can be attributed to their higher evaporation rate as compared with oxygenated monoterpenes. A similar observation was also noted in previous studies on the insecticidal property (Regnault-Roger and Hamraoui, 1995; Abdelgaleil, 2010; Xie et al., 2014). At the same time, a different finding was also observed in the other bioactivity assays. Kordali et al. (2006) showed that oxygenated monoterpenes were less toxic than monoterpene hydrocarbons against the larvae of *Leptinotarsa decemlineata*. In addition, phenolic compounds showed strongest antifungal activities against white-rot fungi, i.e. carvacrol, thymol and eugenol possesses much stronger antifungal activity, and carvacrol was more effective than thymol. Similarly, it has been found that carvacrol as fumigants were more effective than thymol