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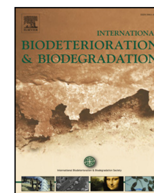

Resistance of fungal growth on Scots pine treated with caffeine

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ABSTRACT

Alkaloids like caffeine are well-known compounds of natural origin, economically suitable and commercial available, which could facilitate their future use for wood protection. The aim of our study was to determine the resistance of Scots pine (*Pinus sylvestris* L.) treated with various concentrations of caffeine water solutions (25–0.4 mg mL^{-1}) against common mold fungi and four wood-decaying fungi (*Coniophora puteana*, *Poria placenta*, *Gloeophyllum trabeum*, and *Trametes versicolor*). Half of the treated specimens before fungal exposition were subjected to leaching procedure. Mass loss from brown rotting fungi was significantly inhibited by all preparations. Moreover, caffeine imparted high resistance in Scots pine to the white-rot fungus *T. versicolor*. In this study we have shown that to effectively protect wood against all tested decay fungi the concentration of the caffeine solution should be 10 mg mL^{-1}. Complete inhibition of the growth of *Aspergillus niger*, *A. terreus*, *Chaetomium globosum, Cladosporium herbarum, Paecilomyces variotii, Penicillium cyclopium*, *P. funiculosum*, *Trichoderma viride*, except *A. versicolor* and *Phoma violacea* has been achieved by applying a higher concentration of 25 mg mL^{-1} of caffeine. Mold and decay resistance of wood treated caffeine is probably related to fact that caffeine has been shown to inhibit chitinases activity, which results in inhibition of fungal growth.

1. Introduction

The wide spectrum of biocides and certain commercial wood preservatives that contain heavy metals for wood protection purposes has recently been limited (Tascioglu and Tsunoda, 2010; Ashraf et al., 2014; Kartal et al., 2015). Increasingly stringent restrictions of their use and the developing general awareness of environmental protection and ecology have stimulated the search for new alternative wood preservatives (Kartal et al., 2012). The main goal is to reduce the quantity of conventional harmful biocides and enhance their efficiency by the addition of cheap and low toxicity compounds capable of wood protection. Recently, much interest has been paid to natural products due to their many advantages, such as availability, lower toxicity, as well as greater biodegradability (Singh and Singh, 2012). There are many reasons as to why natural products might be good sources of active molecules or molecular templates for pesticides. These compounds are a result of coevolution of the organisms producing them and its biotic environment (Boulogne et al., 2012). Nature is known to offer many different types of antifungal compounds, which play an important role in defence mechanisms protecting plants against pathogens (Wink, 1998). Compounds of natural origin, such as alkaloids often have a shorter environmental half-life than synthetic ones, thus their potential environmental impact is weaker (Rimando and Duke, 2006).

Caffeine (1,3,7-trimethylxanthine) is a naturally occurring alkaloid found primarily in tea, coffee, kola nuts, cocoa, chocolates, and pharmaceutical products (Smith, 2002). It is one of the major compounds generated by solid wastes in the coffee and tea industries, i.e. coffee pulp, husk, and tea waste. Coffee industry by-products, which are rich in proteins and carbohydrates, can be used as animal fodder if they are made free of caffeine due to their physiological effects (Gummadi et al., 2012). Such a practice is economical and reduces the ejection of waste produced by the coffee industry that is partly used for feeding cattle (Habtamu, 2014). Thus, with a wide array of applications, there is an even wider potential for future research pertaining to caffeine use in wood preservatives.

There are two hypotheses that concern the role of caffeine in plants, those are the “chemical defence” and “allelopathic function” theories (Ashihara and Suzuki, 2004). Caffeine is now documented to be insecticidal, larvicidal and inhibitory to mold, yeast and bacteria (Raut et al., 2013). Various *in vitro* and *in vivo* experiments have shown that caffeine itself proves to be antimicrobial, resulting in the inhibition of growth and death of bacterial strains (Kumar et al., 1995; Ibrahim et al., 2006; Pruthviraj et al., 2011). The antifungal potential of caffeine has not been widely described yet. There are only a few reports which are available on this subject. It has been shown that caffeine induces cell wall alteration in fungi (Park et al., 2005). At relatively low

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concentrations, this purine alkaloid has a significant effect on the inhibition of phosphodiesterase resulting in an increased effect on intracellular calcium levels and antagonism of adenosine receptors (Serafin, 1995). Buchanan and Lewis (1984) have found that the growth of *Aspergillus* spp. and *Penicillin* spp. was inhibited by caffeine. This purine derivative has been also tested for the inhibition of biosynthesis of mycotoxins, such as aflatoxins secreted by *A. parasiticus* and *A. flavus* (Maraqa et al., 2007). Rizvi et al. (1980) have determined an antifungal spectrum of caffeine isolated from *Coffea arabica* against 10 pathogenic fungi, i.a., *Alternaria solami*, *A. niger*, *A. flavus*, *Cladosporium herbarum*, *Helminthosporium oryzae* and *Syncephalastrum racemosum.* Moreover, the antifungal effect of pure caffeine has been tested at different concentrations against *Chaetomium globosum*, *Deadalea flavida, Duldiniu concentrica, Gloeophyllum trabeum, Phunerochaete chrysosporium, Phlebia radiate* and *Sporotrichum pulverulentum* where the majority of the fungi was totally inhibited by this compound (Arora and Ohlan, 1997). Furthermore, Lekounougou et al. (2007) have published results showing that caffeine has completely inhibited the growth of five species of wood decay fungi and the effects of caffeine on fungal growth are even higher in the presence of propiconazole (Lekounougou et al., 2008). On the basis of the above-mentioned works, it is assumed that caffeine as a natural biocide could be used in wood preservation formulations based on the association of different molecules acting together on the wood-rotting fungi.

Among the current results of studies on the fungistatic properties of pure caffeine against wood-based fungi, caffeine has never been applied directly to wood and has not been used to protect it effectively against fungal attack. Consequently, the objective of this research was to evaluate the resistance of Scots pine treated with caffeine solutions in different concentrations against various mold and wood decay fungi.

2. Materials and methods

2.1. Wood specimens

All test specimens were prepared from Scots pine sapwood (*Pinus sylvestris* L.). Wood samples came from several logs supplied by the Faculty of Wood Technology in Poznan. The samples were without knots, free of visible evidence of resins, and showed no infection by mold, stain and wood decay fungi. The specimens with the size of (40 ± 0.5) × (40 ± 0.5) × (5 ± 0.5) mm^3 (longitudinal × radial × tangential) for mold growth test were cut out, the specimens for the decay resistance test were prepared in the sizes of (50 ± 0.5) × (25 ± 0.5) × (15 ± 0.5) mm^3.

2.2. Bioassay solutions with caffeine

Caffeine was purchased from Sigma-Aldrich, Germany as a white powder ($C_8H_{10}N_4O_2$) (Fig. 1.) Deionized water only was used as a solvent for the preparation of working caffeine solutions. Caffeine water solutions were prepared at final concentrations of 4.0, 6.3, 10, 20 and

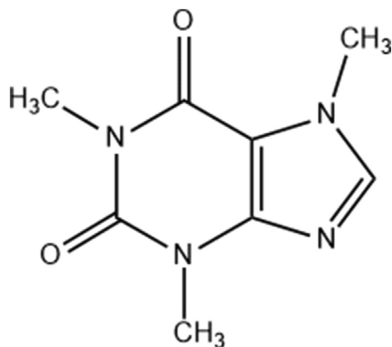

Fig. 1. Chemical structure of caffeine.

25 mg mL^{-1} chosen on the basis of the previous screening tests (Kwaśniewska et al., 2013). The prepared solutions were completely clear.

2.3. Treatment of wood samples

Before treatment, all samples were conditioned at 20 °C and 65% RH (relative humidity) to achieve a constant weight (m_{bi}) and an equilibrium moisture content (12%). Both types of samples for fungal resistance tests were first put in a vessel and treated by a vacuum for pressure impregnation (15 min under vacuum conditions of 0.08 MPa, and 2 h under atmospheric pressure) according to EN-113 with solutions of indicated concentrations of caffeine (c_{caf}). Immediately after impregnation, the mass of each sample was measured (m_{ai}) to determine the solution uptake. After treatment, all the samples were cured for four weeks in room conditions and then oven-dried. The retention of caffeine (CAF kg m^{-3}) based on solution absorption was calculated according to Eq. (1) for each specimen volume (v_s).

$$\text{CAF (kg/m}^3) = \frac{m_{ai} - m_{bi}}{V_s} \times c_{caf} \quad (1)$$

2.4. Leaching procedure

The leaching procedure was performed for decay and mold resistance tests based on the EN 84, 1997 test methods, as provided for outdoor applications. After the conditioning period, the samples were placed in a beaker and soaked in deionized water to then be subjected to vacuum-pressure impregnation via deionized water. The water was exchanged after 2 h, 1 day, 2 days and every 2nd day, thereafter for a total of 2 weeks. The retention of caffeine after the leaching procedure was calculated from the weight of the specimens. All leached and unleached samples were exposed to effects of fungi.

2.5. Decay resistance test

Treated and later leached wood samples were tested against basidiomycetous fungi according to the EN-113 standard method. Cultures were obtained from the BAM Federal Institute for Material Research and Testing. Four species of wood destroying fungi, the brown rot fungi *Coniophora puteana* (Schumacher ex Fries) Karsten BAM 112 (BAM Ebw.15), Rhodonia (*Poria) placenta* (Fries) Cooke sensu J. Eriksson BAM 113 (FPRL 280), *Gloeophyllum trabeum* (Persoon ex Fries) Murrill BAM 115 (BAM Ebw.109) and white rot fungus *Trametes versicolor* (Linnaeus) Quelet BAM 116 (CTB 863 A) were used in the test. The inoculum was transferred to a box with an agar medium containing 40 g malt extract powder, 20 g agar per litre of deionized water. After inoculation, the boxes were kept in conditions (22 ± 2 °C, 70 ± 5% RH) until the media surface was completely colonized by the fungi. The treated and untreated wood blocks were sterilized at 121 °C for 15 min after they were oven-dried. Subsequently to the conditioning period, the samples were exposed for 16 weeks in a conditioning room. After incubation, surface mycelium was removed from each sample. The decay resistance was determined according to average mass loss of the samples after the test (ML).

2.6. Mold resistance tests

The method described below was based on ISO EN 846, 1997 part B: 1997 Plastics- Evaluation of the action of microorganisms. The treated wood samples (5 replicates for each test variant) were evaluated for resistance to mold. First, 10 mold fungi *Aspergillus niger* ATCC 6275, *A. versicolor* ATCC 11730, *A. terreus* ATCC 10690*, Chaetomium globosum* ATCC 6205, *Cladosporium herbarum* ATCC 26362, *Paecilomyces variotii* ATCC 18502, *Penicillium cyclopium* IMI 229034, *P. funiculosum* BAM 22 (ATCC 11797), *Phoma violacea* BAM 27 (IMI 49948ii) and *Trichoderma*

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