Growth effectivity of molds in contact with methylxanthines

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Abstract. Molds on buildings can cause health- and aesthetic problems. Looking for suitable cheap and non-toxic substances to eliminate them is therefore an actual task. In the present study, effect of three selected methylxanthines (1,3,7-trimethylpurine-2,6-dione, 3,7-dihydro-3,7-dimethyl-1*H*-purine-2,6-dione and 1,3-dimethylxanthine) on growth of non-specific species of molds occurring on building materials were tested on agar medium under laboratory conditions. The chemical substances were dissolved in distilled water and applied into agar. Solid agar has been exposed to air molds for 24 hours. Agar plates were then covered with lids and placed for 5 days in a laboratory thermostat at stable test conditions (dark place, 25 °C). After that, the mycelium was observed. The results showed that 3,7-dihydro-3,7-dimethyl-1*H*-purine-2,6-dione and 1,3-dimethylxanthine were not effective against the molds growth. On the other hand, 1,3,7-trimethylpurine-2,6-dione was able to eliminate mold growth on agar medium at a concentration of 1 g·l⁻¹ or higher.

1 Introduction

Wood as a natural renewable resource plays an important role in the construction industry. Wood can be attacked by various fungi and molds. While fungi are able to decompose lignin or cellulose in wood in depth, molds can be found on a cover of wood and thus can cause unpleasant coloring of timber. Therefore, constructional timber has to be painted by some protecting mixtures. Presently, original chemical protective preparations containing metals or persistent organic substances are gradually banned for their toxicity, persistency and bioaccumulative potential [1-2]. Plant extracts or extraction of some biologically active substances seem to be therefore suitable substitutes. The most spread and effective natural biocides contain extracts from trees as bark sources of plant oils (either vegetal oils or essential oils), flax seeds [3], cinnamon [4-5], citrus peels [6], and tung seeds. They have been experimented with as potential wood protectors, showing diverse activities as antibacterial, antifungal and antiinsect agents [7-10].

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In the present study, methylxanthines were selected as model substances. They belong to a group of phytochemicals derived from the purine base xanthine obtained from plant metabolism [11-12]. The most known substances are 3,7-dihydro-3,7-dimethyl-1*H*-purine-2,6-dione (theophylline), 1,3-dimethylxanthine (theobromine) and 3,7-trimethylpurine-2,6-dione (caffeine). They were also selected for this research, being commonly found in coffee, tea and cocoa. All of the selected substances are white and crystalline with variable solubility in water. Some plants can produce them as natural metabolites for their own protection against insects [13]. Effectivity of caffeine was tested against fungi and molds in the past [14-15] but theobromine and theophylline have not been tested yet. Therefore, we tested the effectiveness of water concentrations of these three selected methylxanthines against aerial molds.

2 Materials and methods

The studied methylxanthines were theophylline, theobromine, and caffeine. They were purchased from Sigma Aldrich, Ltd., Czech Republic. The most soluble concentration of selected methylxanthines was used in the preliminary experiment. The basic properties of studied substances are described in Table 1.

Table 1. Formula, structural formula, molar weight (g.mol⁻¹) and solubility in water (g.l⁻¹) at 20 °C of studied substances.

Substance	Formula	Structural formula	Molar weight	Solubility in water
Caffeine	C ₈ H ₁₀ N ₄ O ₂		194.19	20
Theobromine	C ₇ H ₈ N ₄ O ₂		180.16	0.330
Theophylline	C ₇ H ₈ N ₄ O ₂		180.16	5.5

The appropriate amount of substance powder was dissolved in sterile 3% maltose agar (P-Lab, Ltd., Czech Republic) and filled into Petri glass (d = 9 cm). Three replicates were prepared for each substance and for control (clear agar without tested substances). One replicate containing each tested substance was also used as a control of substance- and agar purity and sterility during the test period. The Petri dishes with cool agar were placed for fall of mold spores from air for 2 hours. After that, the Petri dishes were covered by Petri lids, packaged into aluminum foil and stored in a biological incubator for 5 days under

controlled test conditions (in dark, temperature 25 °C). At the end of the incubation period, the growth of mold mycelium was observed visually or under stereomicroscope (Olympus) in all Petri dishes. The basic experiment was performed by the same methodology but only caffeine was tested, taking into account the results of the preliminary experiment. The concentration range was 0, 0.0625, 0.125, 0.25, 0.5, 1, 5, 10, 15, 20 g·l⁻¹, on the basis of results of the first experiment. The final experiment was designed according to the results of the basic experiment and the tested concentrations of caffeine were 0, 1, 2, 3, 4 and 5 g·l⁻¹.

3 Results and discussion

The results of the preliminary test indicated that theophylline and theobromine did not have any effect on the growth of mold mycelium (Fig. 1). Therefore, the basic experiment was performed with caffeine only. The results of basic experiment showed that caffeine had 100% inhibition effect for concentrations 1 g·l⁻¹ and higher. Concentrations in the range of 0.12 to 0.5 g·l⁻¹ caused certain growth of molds. Hence, the final experiment was realized using the concentration range of 1 to 5 g·l⁻¹. In this experiment, it was observed that caffeine caused 100% inhibition of mold mycelium at all tested concentrations (Fig. 2).

Fig. 1. Illustration of mold growth exposed to tested methyxanthines.

Fig. 2. The experiment with caffeine - growth of mold mycelium exposed to control agar without caffeine (= 0) and the concentration 1, 2, 3, 4, 5 g·l⁻¹.

The mold growth expressed as resistance (in %) against caffeine is summarized in Fig. 3. This calculation was modified for agar medium, contrary to the original use for wood materials [16]. The growth of molds had the same resistance for the individual concentrations. So, the Fig. 3 does not include error bars for individual columns.

Fig. 3. Average mold resistance rating for agar containing caffeine. Mold resistance rating scale: 0 – no visible growth under the stereomicroscope, 1 – growth invisible by eyes, but clearly visible under the stereomicroscope, 2 – coverage of up to 25% of the agar dish, 3 – coverage of up to 50% of the agar dish, 4 – coverage of more than 50% of the agar dish 5 – intensive growth covering the agar dish.

4 Conclusions

In the present study, agar tests with aerial unspecific molds exposed to three selected methylxanthines, namely caffeine, theobromine and theophylline, were performed. Both degradation products of caffeine (theobromine and theophylline) did not affect the growth ability of molds but caffeine totally inhibited mold mycelium on agar medium at the concentrations higher than 1 g.l⁻¹. Therefore, further work will be focused on the use of caffeine against selected mold species on various constructional timbers.

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