The *Aspergillus niger* growth on the treated concrete substrate using variable antifungals

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Abstract. The aim of this study was to evaluate the *Aspergillus niger* (*A. niger*) growth on substrates after incorporates with different compounds of antifungals which is normally used in food industry. The antifungals named as potassium sorbate (PS), calcium benzoate (CB) and zinc salicylate (ZS) were applied on concrete substrate covered with different wall finishing such as acrylic paint (AP), glycerol based paint (GBP), thin wallpaper (THIN) and thick wallpaper (THICK). The concrete substrate were inoculated with spore suspension, incubated at selected temperature (30°C) and relative humidity (90%) in plant growth chamber. The observations were done from the Day 3 until Day 27. The results showed that the growth of *A. niger* for concrete treated by PS for AP, GBP, THIN, and THICK were 64%, 32%, 11% and 100%, respectively. Meanwhile for CB, the growth of *A. niger* on AP, GBP, THIN, and THICK were 100%, 12%, 41%, and 13%, respectively. Similarly, treated concrete by ZS revealed that the growth of *A. niger* on the same substrate cover were 33%, 47%, 40%, and 39%, respectively. The results obtained in this study provide a valuable knowledge on the abilities of antifungals to remediate *A. niger* that inoculated on the concrete substrate. Consequently, this study proved that the PS covering with THIN more efficiency compares CB and ZS to prevent *A. niger* growth.

Introduction

Currently, indoor pollutants known as chemicals that are potentially harmful to people and easily found in the habitable portions of buildings, including homes, schools, offices, factories, and other public gathering places. Indoor pollutants are many and varied. Many exposures may be through indoor air. Some substances, like lead or ozone, are also ambient (outdoor) air pollutants. Others, like formaldehyde or asbestos, may be found either indoors or out, but are most often of concern

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when found at healthful concentrations indoors. Indoor pollutants may be natural (for example, carbon monoxide or radon) or synthetic (such as polychlorinated biphenyls [PCBs]), may originate indoors or outdoors, and may be deliberately produced, naturally occurring, or inadvertent by products of human activities [1].

On the other hand, various species of bacteria and fungi have been found growing on all building materials and surfaces. These organisms cause corrosion or degradation of materials and components of building materials and thereby load the indoor air environment with harmful spores and substances, thus leading to poor indoor air quality, sick building syndrome and building-related diseases [2]. Commonly the surface building construction materials such as plasterboard, concrete and wood can provide an environment favourable proliferation and growth of organisms. Various species of organisms have been found growing easily on building materials and surfaces. The most important factor that effect microbial growth on buildings materials are temperature connected with relative humidity, nutrients, moisture, nutrients and the times [3].

Besides, treatment of decay in buildings by synthetic antifungals has now been restricted due to their harmful effects to the environment, residual toxicity and carcinogenic nature. Moreover, the constant use of chemicals may induce the resistance in a target organism. This concern has encouraged researchers to search for efficient method to prevent and cure the harmful effects of microbes in a more eco-friendly manner. Antifungal are compound that are used to control the growth of microorganisms such as bacteria and fungi by killing and limiting their growth. Usually the antifungal used in building environments as disinfectants in cleaning agent to control the microbial growth. Instead being used in paint formulation, antifungal also used to protect substrate materials coated with paints. According to USEPA, antifungal have been used extensively to control the growth of fungi and bacteria during production and storage of interior, water-based, latex paints and to control fungal growth in exterior latex paints on a long-term basis. In order to protect dry films and products from fungal and microbial attack, several of antifungal usually added in the paints [4].

The antifungal that have been used in these studies are potassium sorbate normally known as potassium salt of corbate acid. This is normally presence in food preservative and other products and their safety has been established. In the US, PS suitable to avoid growth of fungi in fermented products by pH near 4-5 used from 0.01% to 0.02% [5]. In addition, Gregori et al [6] noted that the potassium sorbate known as antimicrobial agent for foods preservative. The PS was able to inhibit fungal growth but depends on a type of food and the conditions of storage and processing. As highlighted by Belloti et al [7] fungicide commonly used to control the fungal growth, more eco-friendly alternative compounds, non-toxic to humans and attractive for antimicrobial paints.

Additionally, the calcium benzoates are referred as calcium salt of benzoic acid. Currently, in food industry commonly used as a food preservative and approved for use as a food additive in the EU, USA, Australia and New Zealand. Calcium benzoate (CB) performed as a white crystalline powder. In chemistry, the terms salt refers to a particular set of compounds (ionic compounds) when an acid is neutralized by a base. Additionally, the sodium benzoate was used as antimicrobial next to selected food was spoiled to determine the effectiveness towards fungal growth. The resulted show when incorporate the CB and others antimicrobial more effectively to remediate the fungi on food [8]. However most of the studies in the open literature did not concurrently observe the effect of CB on the different materials in construction such as plasterboard, concrete and wood.

The other antifungals like zinc salicylate or salicylic acid performs as colourless crystalline organic acid used in organic synthesis and as a plant hormone, the formulae is C7H8O2. Another function of this ZS analgesic, antipyretic and anti-inflammatory agent. In addition, it has been used to reduce fever and pain because this salicylic acid is an originator to acetylsalicylic acid, known as aspirin [9-11]. The study by Belloti et al [12] prove that the effectiveness ZS as antifoulant as others applications. In food industry, ZS also used in food preservative as concern about the safety of chemical used. This is supported by Belloti et al., [5] study which reveals that the application of the ZS can inhibit the fungal growth in antimicrobial coating test. Furthermore, they concluded that in
painted coating, the ZS is more effective than PS and CB to reduce fungal growth. On the other hand no study has examined the coating bio resistance with different wall finishing in climate region.

Therefore, the objective of this study was to evaluate the \textit{A.niger} growth on concrete substrate after incorporate with different compounds antifungals, used in food industry as preservative. Three types of antifungals were tested against \textit{A.niger} isolated from interior painted wall. These antifungals were potassium sorbate (PS), calcium benzoate (CB) and zinc salicylate (ZS). The selected of these antifungals to explored the possible application of different compounds and eco-friendly used in food industry like preservatives.

\section*{Materials and method}
\subsection*{1.1 Antifungals compounds}
In the start of the test, sixteen samples of each supports were prepared; the materials are cutting into small size (50mmx50mm). First step support washed by ethanol 70% and left for some hours to avoid any contamination. The different of antifungal were prepared with 0.03% (w/v) was poured into two different paints (AP and GBP). Therefore, for two different wallpapers were glued onto substrates (THIN and THICK), the antifungals were sprayed on surfaces homogenously. Sample left to dry for 24-48 hours before inoculate with \textit{A. niger} spores [13- 15].

\subsection*{1.2 \textit{A. niger} isolates}
The colonies of indoor fungal are isolated based on their characteristic. Then the species was identified based on the colony morphology. Furthermore, the isolated of microorganisms sent for DNA sequencing to the commercial company for identification species level. Then the samples were assessed for enumeration of viable indoor fungal in the microbiology laboratory. The temperature was set 25\degree C to incubated agar plate. Observation for growth was done between 1-5 days after sampling. Thus, the result of fungal spore load per sample was expressed as colony forming units (CFU) per cubic meter of air (m\textsuperscript{3}). The colony counters are used to count the colonies manually. The main reason for choosing \textit{A. niger} was that it is often the most dominant mould genus found developing in humid conditions on a wide variability of materials.

\subsection*{1.3 \textit{A. niger} growth on concrete}
The efficacy of three types of antifungals was evaluated against \textit{A. niger} on concrete surfaces. The concrete were cut into blocks of 50 x 50 mm. The PS, CB and ZS antifungals were prepared. According to Belloti et al [5], the Malt Extract Agar (MEA) with 0.03% (w/v) antifungals was poured into two different paints acrylic paint (AP) and glycerol-based paint (GBP). Two different wallpapers (THIN and THICK) were glued onto concrete and antifungal was applied on the wall finishing. Samples of control (untreated) were also included for each experiment. Each test has done in triplicate at the same period. Based on study made by Belloti et al [5] to avoid any contamination, the sample irradiated with the UV lamp for 40 minute. The samples were placed in petri-dish contained MEA. Total 50 \textmu l of the spore suspension at concentration 0.34x106 spores/ml was inoculated on the each substrate. The observation of the sample conducted for every 3 days until 27 days; at optimum temperature (30\degree C) and relative humidity (90\%) in plant growth chamber (ThermoStable SWGC-450). According to ASTMD5590-00 standard method a visual assessment are needed to find the percentage growth of \textit{A.niger}. There are degrees of fungal growth according the scale according to ASTMD5590-00 standard which were 0 (scale 0-no growth), <10 \% (scale 1-trace growth), 10-30 \% (scale 2-light growth), 30-60 \% (scale 3-moderate growth) and 60-100 \% (scale 4-heavy growth).
1.4 Statistical analysis

All the experiments were conducted in triplicate for each sample including the control sample. The analysis of variance (one-way ANOVA)- SPSS 20 was conducted to evaluate the data analysis of the average, standard deviation, mean difference and significant difference level ($P<0.05$) for the growth of $A. niger$ on substrates.

Results and discussions

This finding highlights the potential of three different antifungals to remediate $A. niger$ on different wall finishing surfaces. The $A. niger$ growth on the concrete substrates is presented in Figure 1. The effect of the PS on the four different wall finishing is shown from Day 6 until Day 27 of observation period. The most effective wall finishing that inhibits the $A. niger$ growth on THIN at 11% of growth. Followed by GBP was achieved up to 32% of $A. niger$ growth. The percentages for AP and THICK were 64% and 100%, respectively. The scale rating for THICK and AP are at scale 4 (heavy growth), GBP is scale 3 (moderate growth), and THIN wallpaper is scale 2 (light growth). The scale ratings are referring to the ASTM D5590-00 standard specification. Thus, according to ANOVA analysis, the mean of samples are significant ($p<0.05$). In addition, the $A.niger$ growth on concrete wall finishing treated by CB. The CB prevents the $A. niger$ growth on GBP and THICK more than AP and THIN which the highest percentage are 100% for AP. Then next is THIN (41%), followed by THICK (13%) and GBP (12%). Based on ASTM standard specification the scale for AP are 4, THIN was scale 3 while for GBP and THICK were scale 2. The ANOVA analysis proved that the data result were significant where the mean percentage and diameter value were less than 0.05 ($P<0.05$). The effectiveness of the ZS to prevent the growth of $A. niger$ on the concrete surface to prevent the growth of $A.niger$ was ranged between 30% and 69%. Based on the result of ZS treatment, a slow growth of $A. niger$ was observed. The highest percentage growth was GBP while the lowest was AP. The scales for all substrates are at scale 3 (moderate growth). According to ANOVA analysis, all mean data are significant, $P<0.05$.

The addition of the antifungals does not significantly modify the physical properties of wall finishing. The concrete treated with PS and ZS (Figure 2 and Figure 4) have shown two different performances on wall finishing surfaces: on the GBP a surface, the growth of $A. niger$ was more affected associated to the THIN and AP. Under the same conditions, $A.niger$ has ability to grow rapidly on the THIN on the antifungals treated concrete by CB as present in Figure 3.

There are many factors that may promote the $A. niger$ growth on concrete surfaces. These factors include the biological, environmental and substrates factors. According to USEPA 2004 [10], the growth of $A. niger$ is related to biological exposure especially in the office, which impacts the environmental quality. The $A. niger$ do not have chlorophyll which enables them to provide their own food, thus they need to depend growing substrate and high abilities to degrade wall finishing. In addition, the environmental condition such as humidity, temperature and nutritive supplies encouraged the $A. niger$ growth on the concrete surfaces. Mostly, previous studies found that microbial growth on concrete materials is suitable at around 90-95% of relative humidity [11-13]. In this condition, the $A.niger$ favours to growth. Other factor such as the temperature also effects growth speed. Since the optimal growth temperature is about 30°C [16] ; therefore $A. niger$ survived from the nutritive supplied from material of wall finishing (wallpaper and paint).

The concrete substrate is defined as a composite material composed of water, aggregate and cement. According to substrates factor, $A. niger$ growth on porosity, roughness and mineral composition of the support.[17]. The surface mineral composition plays a role since it may either be used as nutriments by $A. niger$. In addition, surface roughness and porosity could favour the attachment of nutrient components carried by dust resulting from the activities in buildings. The findings of the previous study show that the presences of selected antifungals are might be other factor the growth of $A. niger$ [3], [14], [18]
Figure 1 Observation result of fungal growth percentage on concrete with different types of wall finishing and different antifungal.

Figure 2. Treated concrete with potassium sorbate on different covering after 27 days
a) AP, b) GBP, c) THIN and d) THICK

Figure 3. Treated concrete with calcium benzoate on different covering after 27 days
a) AP, b) GBP, c) THIN and d) THICK

Figure 4. Treated concrete with zinc salicylate on different covering after 27 days
a) AP, b) GBP, c) THIN and d) THICK
Conclusions
This study has concluded that at optimum humidity (90%) temperature (30°C) in indoors promoted *A. niger* growth easily. The results obtained in this study provide valuable knowledge on the abilities of antifungals to remediate *A. niger* were inoculated on the concrete substrate. The concrete substrate was covering with THIN and treated by PS antifungal was found more efficiency compares to other antifungals. Therefore, further work is needed to compare the total *A. niger* growth before and after application variables of antifungals in buildings for the real application to determine the accurate effectiveness of PS.

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