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In Vitro Inhibitory Assay of an Isolated Indoor Airborne Fungus from an Institutional Building of Computer Education by Using Potassium Sorbate

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Abstract. Currently, one of the main aspects of health and safety concern to facility owners and supervisors is indoor air quality. Meanwhile, pollution by airborne fungi in these facilities are acquiring more and more consideration due to its possible harmful side effects such as threats to occupiers' health and damage to building parts and furniture. One of the recommendations to solve these indoor fungi pollution is bioactive compound which can act as a biocide. However, assessment of this compound in the real environment is often time-consuming and impractical. In this study, a bioactive compound, potassium sorbate which is commonly applied in food manufacturing was assessed for its efficiency as a biocide to restrict the growth of an isolated airborne fungus using an *in vitro* inhibition assay. The fungus was isolated from a new building of tertiary education of computer studies. It was grown on both biocide-incorporated MEA and untreated MEA. The diameter of the fungal colonies was noted time to time. The diameter of the colony of the treated fungus was downsized by 41.25% averagely in comparison with the untreated fungus. It was shown that potassium sorbate can restrict the growth rate of the isolated airborne fungus.

Introduction

The quality of the air in a facility often affects the state of wellness of the people in that enclosed facility [1]. It is termed as indoor air quality (IAQ). IAQ of a facility is known to be important in determining occupants' satisfaction, comfort, and productivity. This is because indoor setting is closest to human beings in terms of day-to-day communication and activities as a large portion of human's daily life is spent here. Nevertheless, there are a lot of places on our Mother Earth, such as our dwellings, schools, colleges and dorms are seriously polluted by airborne fungi. As heterotrophic organisms, fungi easily inhabit on and within organic building materials that can be digested to become their energy sources, such as wallpapers and paint coatings [2]. Fungal pollution in a building has been linked to a lot of health problems such as headache, breathing problems, fungal infection, skin irritation and many others [3]. Indoor occupants' healthiness can also be affected by indoor fungal growth due to the ability of fungi to release poisonous mycotoxins, allergens or spores. Mold spores travel in the indoor air and alight on surfaces of water-damaged or wet building materials to establish new colonies for reproduction. The microscopic size of these released spores allows them to infiltrate deep into the human's breathing system and may reproduce there to cause a variety of illnesses eventually [2]. Youngsters, senior citizens and immuno-compromised people are at bigger dangers [4].

High temperature and high relative humidity are among the indoor environmental factors that contribute to the higher occurrence of indoor fungi [5]. Excess dampness is always the reason for

indoor mold or fungi proliferation. The moist air can be condensed by the high dampness and warm climate on cool surfaces of interior building parts. As a result, this phenomenon gives rise to the indoor mold problems. Proliferation of fungi occurs easily in a building over an extensive temperature range as long as there are adequate humidity and nutrients [5]. Temperature between 15°C and 30°C is the comfortable zone for most of the fungi [5]. On the other hand, the rest of the fungi favour temperatures lower than or higher than this range [5]. For example, the temperature range between 35°C and 50°C is suitable for propagation of thermophiles [5]. This phenomenon has created an inevitable problem of indoor fungal pollution in nations such as Malaysia, Singapore, Indonesia or other nations in ASEAN (Association of Southeast Asian Nations) which possess hot and moist weather all the time. This was proven in our earlier investigation where the proliferation of fungi was detected in the air of a chamber with high relative humidity and a temperature of about 25°C at a higher institutional building of computer education following treatments like washing and exchanging of the mold-overwhelmed ceiling panels [6]. This gives us an insight that ordinary treatments cannot provide a durable solution to the air pollution by fungi in buildings.

Indoor mold contamination in a higher institutional building of computer education is dangerous. It is proposed that the utilising of shared computers in an institutional setting create huge opportunity for the spread of dangerous fungal pathogens [7]. This is because of the using and touching of computers and its accessories which are not regularly disinfected by countless students day-to-day [7]. The situation may become worsen and uncontrollable if the killer pathogens present on these devices and cause an outbreak of deadly infectious disease. Besides, the comfortability, emotion and feeling of the professors, lecturers, tutors, instructors and learners might be influenced by the poor IAQ of the institutional buildings [8]. Indirectly, the educational process will also be affected [8]. Due to perennial hot and damp climate, higher institutional buildings in nations such as countries in ASEAN require an environmental friendly and enduring solution to this kind air pollution in institutional buildings.

Potassium sorbate is a type of salt of sorbic acid. A variety of manufacturing industry has incorporated sorbates to preserve their products such as in food and beverages, cosmetics, medicinal, and tobacco products since the 1940s [9]. Recently, this bioactive compound had been revealed to successfully restrict the proliferation of two species of fungus obtained from an interior wall surface [2]. It is recommended that this bioactive compound can be an environmentally friendly biocide and long-lasting remediation to solve indoor air pollution by fungi. However, to authors' knowledge, potassium sorbate's ability to remediate the air pollution by fungi has not been assessed yet. This might be due to it is time-consuming and impractical to evaluate the effectiveness of this biocide in real environment. Therefore, the purpose of this study is to assess the effectiveness of potassium sorbate to treat an indoor airborne fungus isolated from a higher institutional building of computer education using an *in vitro* inhibitory assay.

Materials and Methods

Isolation of Indoor Airborne Fungus. The samplings of the indoor air was carried out with a single-stage air sampler (SKC, USA) in order to isolate an airborne fungus. The indoor air samples were collected from a fungi-affected site of a higher institutional building of computer education in a Johor-based university in Malaysia.

Suspension of Fungal Spores Preparation. A physiological mixture consisting of Polysorbate 20 and sodium chloride was prepared accordingly to Bellotti *et al*. [2]. The spores of fungus were obtained from the actively growing isolated airborne fungus grown in Malt Extract Agar (MEA) and inoculated into the physiological mixture to become the suspension of fungal spores. A hemocytometer (Hirschmann EM Techcolor, Germany) and technique of serial dilution were used to tune the suspension's concentration to between 0.3×10^6 spores/ml and 0.5×10^6 spores/ml [2].

***In Vitro* Inhibitory Assay.** Three tests (Test A, Test B and Test C) were executed to evaluate the efficiency of potassium sorbate concurrently on the same day. A single drop of about 10^2 spores of the prepared suspension of fungal spores were deposited at the middle of the petri dish of each test. The MEA incorporated with potassium sorbate 0.03% w/v was then poured into these petri dishes. The procedure was repeated with two MEA controls that were free of biocide. All the five samples including controls were cultured in a 37°C incubator and the diameter of the fungal colonies produced were logged daily over a period of ten days as described by Bellotti *et al.* with minor modification on the duration of incubation [2].

Results and Discussion

According to *Bellotti et al.* [2], efficiency of potassium sorbate was evaluated by an *in vitro* inhibitory assay of fungal proliferation as the proliferation of a fungus species can be measured by recording diameters of the fungal colonies formed on culture media against duration of incubation (Figure 1).

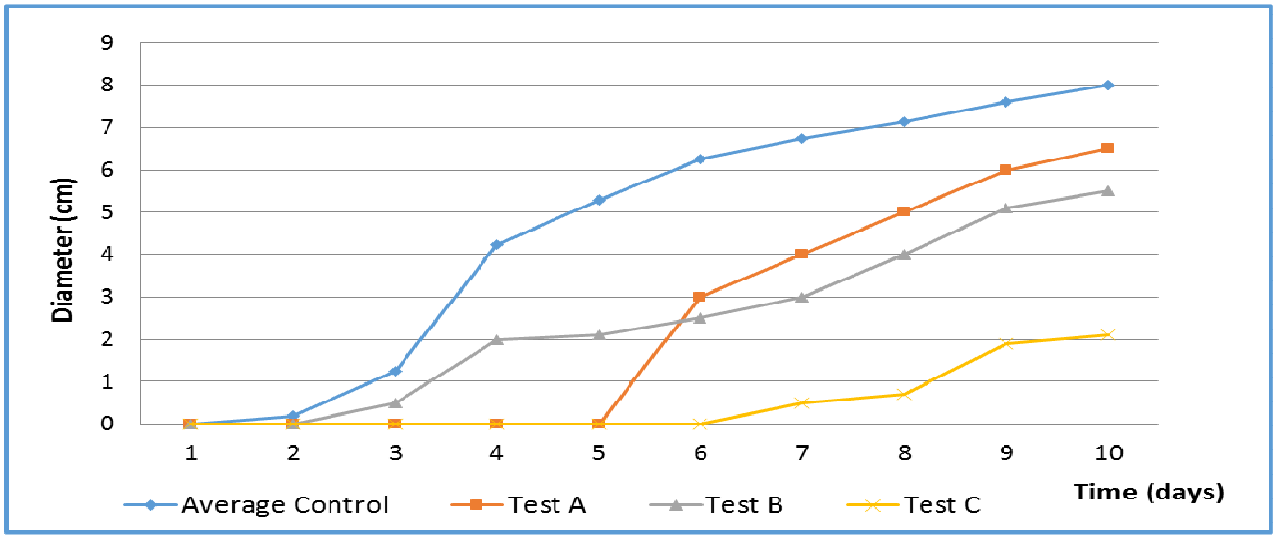


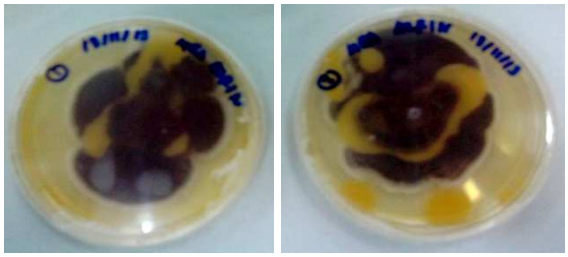
Figure 1: Overall measurement of indoor fungal colony growth diameter during biocide treatment

Potassium sorbate was revealed to be a potent biocide which exhibited high antifungal activity towards the isolated indoor airborne fungus as the colony diameter of the biocide-treated fungus was effectively controlled and reduced. The reduction is 41.25% on average in contrast to the control samples. There was no growth of the isolated fungus found on the first five days of assay in the biocide-incorporated culture media of Test A and that of Test C and slow growth of the fungus was found after that. This shows that the biocide remained highly effective at the early stage of the treatment and its effect was slowly diminishing with time. The results are considered consistent with a 42-week *in situ* study conducted in Brazil where the fungal colonization on the biocide-treated painted wall surface remained undetected during the initial phase of research till the 31st week, and thereafter, it rose to high levels till the end of the research [10]. Results of this study were possibly due to the slightly acidic nature of potassium sorbate that inhibited the growth of the microbes by penetrating the cytoplasmic membrane of the microbes when dissolved in water [11]. Photographs taken with the biocide-treated and control fungus at the 7th day of incubation are shown in Figure 2.

The fungus growth in Test C was apparently slower than that in Test A and Test B. The growth of fungus in Test C was found only after about a week of incubation. Moreover, the growth after that was found to be extremely slow compared to that of Test A and Test B. Therefore, it is assumed that the inhibitory activity of potassium sorbate can last for a longer time in this test. This

is possibly owing to the well mixing of biocide and media agar in this test (test C) compared to the other two tests.

This study shows that the biocide remains effective against the isolated fungus from the indoor air for at least ten days or longer. Ten days is a good duration for this assay because the colony of the control fungus reached the maximum size of the petri dishes. Further incubation will not show accurate results as the control fungus cannot grow bigger anymore. However, the fungus samples treated by potassium sorbate continued growing slowly by the end of the course of this assay. This is probably due to the biocide in the growing media had depleted.



(a) Control 1 (b) Control 2

(c) Test A (d) Test B (e) Test C

Figure 2: Untreated fungus (a)–(b) and biocide-treated fungus (c)-(e) after 7 days at 37˚C incubation.

Conclusion and Recommendation

It is proven that potassium sorbate gives a good indication to suppress the growth of the isolated indoor airborne fungus. The *in vitro* inhibitory assay demonstrated in this paper fits to be a low cost and convenience test for preliminary assessment of the effectiveness of a biocide. It is recommended that alternative methods of higher technology such as polymerase chain reaction (PCR) to be sought to test for the durability of this biocide.

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