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The biocidal effect of potassium sorbate for indoor airborne fungi remediation

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ABSTRACT

Indoor air contamination by fungi is common nowadays. It creates health problems, especially in people with asthma. Approximately 80% of these patients are allergic to fungus. In this study, a bioactive compound, potassium sorbate which is actively used in the food industry for antifungal purposes was evaluated for its ability to treat indoor airborne fungal contamination in two higher educational buildings at a university in Johor, Malaysia. Indoor air samplings of fungi were carried out at three different sites in each building using malt extract agar (MEA), which were incorporated with the mentioned biocide as culture media. It was shown under indoor and outdoor conditions that fungi were able to colonize untreated MEA. The effectiveness of the biocide to prevent the growth of fungi on treated MEA was compared with that of untreated MEA. It was clearly shown that the biocide can effectively prevent the airborne fungal growth at all six sites as the number of colony forming units was drastically reduced by more than 88% averagely on the treated culture media as compared with that of the untreated media.

Keywords: Indoor air quality; Occupational safety and health; Mold; Biocides; Airborne fungal pollution

1. Introduction

Nowadays, various pollutants from a number of sources have made environment sustainability a serious public health issue [1]. Air is essential for the survival of all forms of life on earth, along with water [2]. Every individual concerns about the air quality around them, especially indoor air quality (IAQ) since most of the people spend more time in an indoor environment, where air pollution can be far more serious than outdoors [3]. Good IAQ within an enclosed building or environment can maintain the well-being and healthiness of its occupants [4] and thus, the quality of performance in an organization. Nevertheless, IAQ is often jeopardized by indoor fungal or mold contaminations, especially in countries with a

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permanent humid climate, such as South-East Asia countries. Fungi are heterotrophic organisms, which commonly colonize and metabolize organic building materials such as wall coatings [5]. They can degrade surrounding building materials, weakening the structures, and become significant pathogens of humans. The indoor fungal pollution has been associated with a variety of diseases including asthma, headache, allergy and irritant effects, respiratory problems, mycoses, and several other non-specific health problems [6]. Besides, air-transmitting diseases caused by airborne fungi such as invasive aspergillosis, acute allergic alveolitis, and histoplasmosis are life-threatening to immunocompromised indoor occupants. Under most circumstances, these individuals are not aware of this inheritance trait. From an educational standpoint, a fungal contamination in the indoor environment of a university's educational building may hamper the process of teaching and learning by destroying the IAQ and the healthiness of lecturers and students [7].

Approximately, 10% of people worldwide and 5% of the population in industrial countries have fungal allergies [8,9]. Some indoor fungi are able to produce mycotoxins and have been associated with sick building syndrome [6]. These mycotoxins had been confirmed to associate with carcinogenic, immunesuppressive, and other unhealthy properties through animal studies [10]. All indoor growing fungi are potentially harmful to human beings because inhalation of fungal spores is implicated as a contributing factor for organic dust toxic syndrome and non-infectious fungal indoor environmental syndrome [11,12]. These floating tiny spores were released by fungi to the air and eventually land on other locations to reproduce. When they settle on moist surfaces, the spores can form new mold colonies. If they are inhaled into our respiration system, they will be lysed and the human body thereby exposed to the primary and secondary metabolites [13]. Inhalation exposure has been suggested to cause acute kidney failure, damage of the upper respiratory tract, and central nervous system damage [14].

Indoor fungal contamination is not a rare incidence in warm and humid countries like Malaysia and other South-East Asian countries due to the ability of fungi to grow over a broad temperature range with sufficient nutrients and dampness [15]. A recent study found an apparent growth of airborne fungi in a damp room (relative humidity of 87%) of a higher educational building of computer studies, which had been remediated conventionally using detergent cleaning and changing of the affected ceiling boards [16]. This study suggested that the conventional methods of remediation couldn't provide long-lasting effect to circumvent the indoor airborne fungal contamination. Besides, fungal particles can be aerosolized and spread during the remediation [17]. Frequent changing of building materials, such as ceiling boards, due to mold contamination is neither practical nor cost-effective. Therefore, green solutions are needed to reduce the viability of the airborne fungus, and thus, existence of secondary metabolites of fungi in indoor setting in order to secure the quality of teaching and learning among educators and students in a university building.

Bioactive compounds have long been used as an antimicrobial agent, especially in food and pharmaceutical industry. These compounds are naturally produced substances, which have a biological activity if it has a direct effect on a living organism and normally occur in small quantities in plant and food products. These compounds are gaining attention recently due to their potential applications which are beneficial to human health such as reduction of the incidence of some diseases, antioxidant, antimutagenic, antiallergenic, anti-inflammatory, and antimicrobial effects [18–20]. The usage of these natural compounds in the realm of the indoor environment and indoor air quality has not been given the full address yet.

Recently, potassium sorbate, a bioactive compound from the food industry, had been proven to be able to inhibit the growth of two fungus species (*Chaetomium globosum* and *Alternaria alternate*), isolated from an indoor waterborne coating [5]. It is suggested to be effective against airborne fungi too. Thus, the goal of this study is to evaluate the ability of potassium sorbate as a biocide, to render the viability, and thus slow down the growth of indoor airborne fungi in two higher educational buildings of a university in Johor, a southern state of Peninsular Malaysia using a direct biocidal efficacy testing method through air sampling and incubation.

2. Material and methods

2.1. Selection of testing sites

The air samplings of indoor airborne fungi were carried out at two new commissioning higher educational buildings of a university in Johor, Malaysia. Both of these buildings had been identified to be infected by microbial growth from previous study [16]. Three sites from each building were determined after walk-through inspection. They were each to represent microbial-contaminated sites, relatively mildly-contaminated sites and their respective outdoor environments of the buildings. The locations for outdoor air samplings were as close as possible to the outdoor air intake for the primary air handling system for the buildings [21].

2.2. Direct testing of the biocidal activity

The antifungal activity of the potassium sorbate was evaluated by a direct testing method, which includes air samplings of total airborne fungi, incubation, and counting of colonies formed. These procedures include using of biocide-treated and untreated culture media, and takes into account that the concentration of the viable fungi can be represented by colony-forming unit (CFU) analysis as the calculation shown in Eq. (1) below:

$$\mathrm{CFU/m^3} = [\text{Number of colonies} \times 1,000] / [\text{Sampling time (min)} \times \text{Flow rate (L/min)}] \quad (1)$$

where CFU is the colony-forming unit, m^3 is the volume of the air intake during each air sampling, min is the sampling time measured in minutes, and L/min is the volume of nominal air flow (Liters) per minute.

A BioStage single-stage viable cascade impactor (SKC, USA) was attached to a SKC QuickTake 30 Sample Pump (SKC, USA). According to the requirement of National Institute of Occupational Safety and Health (NIOSH) of Malaysia stated in method NIOSH Manual Analytical Standard Method (NMAM 0800), this equipment was operated at a flow rate of 28.3 L/min for air sampling of airborne fungi onto MEA plates incorporated with 0.03% (w/v) potassium sorbate as biocide over 5 min period for each sample. The impactor was located at a height of 1.0 meter above the floor at the center of each testing site. The air sampling using biocide-treated MEA was conducted in triplicate at each site for each building. The same procedure was carried out with untreated MEA as controls. All air samplings with biocide-treated MEA and untreated MEA of a particular site were executed on the same day during university's office hours, which are from 8 am to 5 p.m. in the presence of indoor occupants including lecturers, students, researchers, and staffs. The air samplings at different sites were carried out on different weeks. The samples were analyzed for total viable airborne fungi count by incubating them at 37˚C for 5 d and counting of the colony formed was done every day until the fifth day of incubation.

3. Results and discussion

The higher educational buildings involved in this study were buildings of computer studies and of engineering studies. The mean concentrations of total airborne fungi at two higher educational buildings were reduced by 88.2% averagely on MEA treated with potassium sorbate if compared with the untreated MEA (Fig. 1). Potassium sorbate was shown to exhibit highest biocidal efficacy at outdoors of two buildings by reducing 91.2% of the viability of total airborne fungi. However, the lowest mean concentrations of the viable airborne fungi after treatment were found at the mildly contaminated sites of two buildings, which are 21.2 CFU/m^3. The mildly contaminated sites had the lowest total airborne fungi, 176.7 CFU/m^3 on untreated media compared to the other sites. At contaminated sites, the viability of the total airborne fungi was reduced by 85.5% from 657.3 CFU/m^3 on untreated MEA to 95.4 CFU/m^3 on biocide-treated MEA.

These results have shown that potassium sorbate are effective against total airborne fungi at higher educational buildings in this southern Peninsular Malaysia's university. The high efficiency shown at outdoors and mildly contaminated sites of the buildings indicated that the biocide is suitable to be applied when the buildings are yet to be polluted by these microbes. These findings are consistent with previous findings and suggestions [22]. It can be used simultaneously with conventional remediation, such as detergent cleaning, to prevent the recontamination by indoor fungi. It is also suggested that the biocide can be applied at the entrances, windows, or the outdoor primary air handling systems, so that the outdoor air can be treated prior entering the buildings.

Although the allowable limit of the indoor airborne fungi concentrations set in Industry Code of Practice on Indoor Air Quality (ICOP-IAQ, 2010) by the Department of Occupational Safety and Health Malaysia is 1,000 CFU/m^3, the average readings of approaching 700 CFU/m^3 found in the contaminated sites of the two higher educational buildings were considered high enough to be risky to human health. This is due to unknown species of fungi present in the airborne fungi reservoir. Some of the species can produce a diversity of toxin or might be carcinogenic if inhaling them [23]. The mean concentrations of the after treatment, viable indoor airborne fungi at three testing sites of the two buildings were successfully reduced to below 100 CFU/m^3. Thus, it is suggested that this biocide is effective to maintain the indoor airborne fungi at an acceptable healthy level in indoor environment.

The growth curve of the viable total airborne fungi with and without biocide treatment clearly shows that the growth cycle of the fungi was successfully disturbed by the introduction of potassium sorbate in the growth medium (Fig. 2). The untreated, total indoor

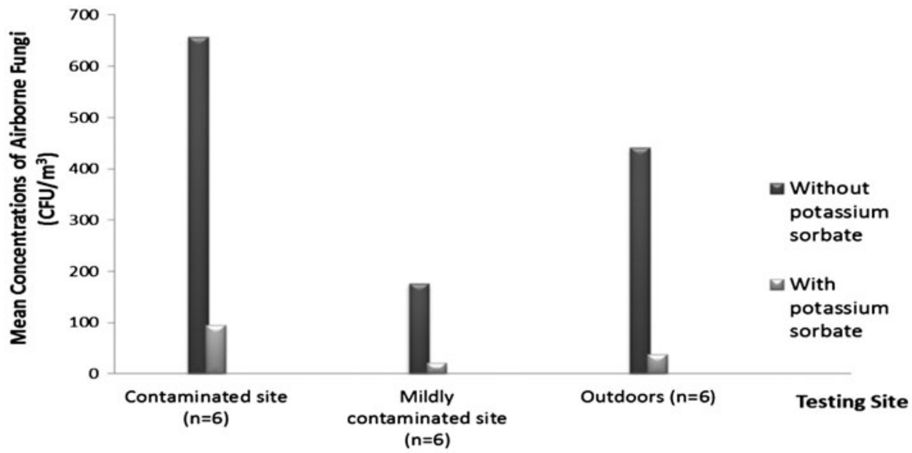

Fig. 1. Comparison of viability of total airborne fungi on biocide-treated and untreated MEA at both buildings.

fungi show a normal microbial growth curve with exponential growth in the first three days of incubation. While, the growth curve of the indoor airborne fungi on the biocide-treated MEA did not show log phase and stayed below 10 colonies during the incubation period. Therefore, it is suggested that the potassium sorbate is able to interrupt the exponential growth phase of total indoor fungi and thus, prevent their proliferation in indoor environments. This might be due to the ability of the potassium sorbate to render the fungi incapable of metabolizing and thereby proliferating [24]. Potassium sorbate can inhibit the growth, and metabolism of fungi is largely due to its ability to inhibit enzyme and cell transport processes of fungi [25].

Both buildings have been used for educational and research purposes. They were operated five days a week. The building of computer studies contained mainly the equipment of information and communication technology. The facilities in the building of engineering studies include all types of chemical used for teaching and laboratory purposes, expensive laboratory analytical instruments, and teaching and learning equipment. Anderson & Palombo [26] suggested that the possibilities of spreading of contaminating microbes through using of computers in these types of educational buildings is high as the computers were not disinfected despite high usage by numerous users every day. Airborne fungal contamination in these buildings does not only affect users' health and

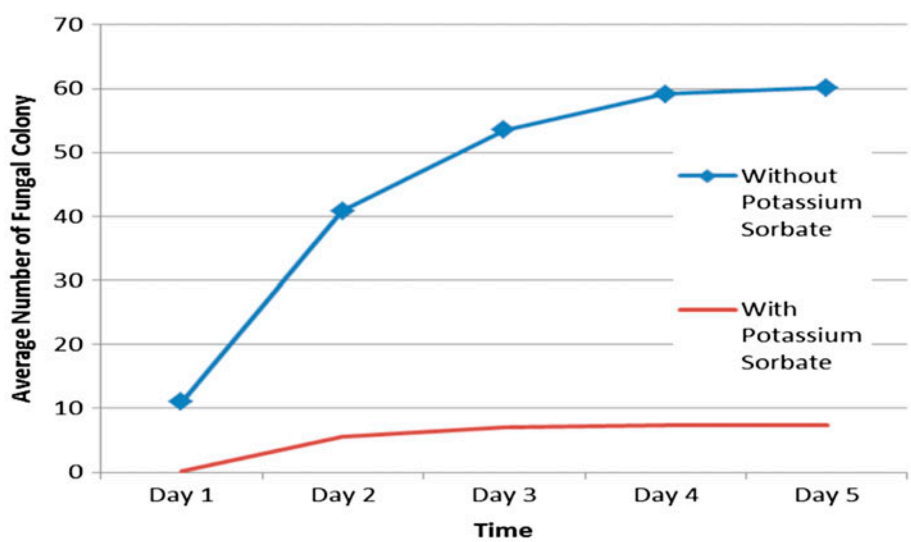

Fig. 2. Total fungal growth with and without biocide treatment at the two higher educational buildings.

learning processes, but also incur an unnecessary maintenance fee when the mold problem occurs in building materials such as ceiling boards and wall paints. The maintenance will be an obstruction to ongoing research progress in these facilities as the analytical instruments are highly sensitive. Therefore, this study provides a green alternative for indoor airborne fungi treatment. To our knowledge, including our previous reports [22,27], these are among the first few descriptions of the use of potassium sorbate to treat indoor airborne fungal contamination in higher educational buildings at a university.

4. Conclusion

The bioaerosal sampling is an assessment of possible proliferation and dissemination of fungi from building reservoirs. The results from a direct evaluation test like this represent the effectiveness of the biocide, potassium sorbate against the general microenvironment of airborne fungi from the testing site and building. Thus, we can conclude that potassium sorbate is an effective biocide to reduce the growth of total airborne fungi in higher educational buildings of universities in the southern region of Peninsular Malaysia. Through this reduction, the IAQ of the buildings of universities can be maintained in a healthy and comfortable level for all indoor occupants.

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