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# Survivability of Isolated Indoor Fungi after Treatment with Antifungal Agents

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**Abstract:** Microbial growth in indoor environments can creates health problems, especially to people who have asthmatic issue. Three antifungal agents were tested against isolated indoor fungal available in campus's building. The fungal that has been collected was *Aspergillus niger* and the selected antifungal agents used are calcium benzoate, potassium sorbate and zinc salicylate. The fungal has been isolated on the MEA-agar and treated by the antifungal agents. All samples are stored inside the incubator with temperature 25ºC and relative humidity of 90%. The diameter, ASTM percentage growth, ASTM scales rating and percentage of reduction of indoor fungal has been measured. This study proved that the most efficient antifungal agent is zinc salicylate. It scores the least diameter growth (4.4 cm), lowest ASTM percentage growth (45%), low ASTM scale rating (3), and highest percentage reduction (44.26%) compared to others antifungal agents. It can be concluded that zinc salicylate is the best antifungal agent between calcium benzoate and potassium sorbate that prohibit the growth of indoor fungi. Nevertheless, the concentrations of the antifungal agent use recommended to be optimized for a long lasting prevention of indoor fungal.

**Keywords:** ASTM growth scale, antifungal, IAQ, indoor fungal.

## 1. Introduction

Molds usually grow on the expired bread or on the decaying fruits. It can cause biodegradation of natural materials, which can spoil the natural materials. Mold actually a fungus that grows in the form of multicellular filaments called hyphae [1]. Mold also can probably cause asthma in people who having low immune in respiratory system. The existence of molds inside the building may harm the occupants. People may breathe in mold particles if mold is disturbed or damaged. Tiny spores that release into the air will get inside our lungs as we breathe.

Molds inside the building can be control and reduce by applying the antifungal agent on the affected surface. This chemical substance can reduce the harm of molds by controlling the growth. Bio-active compounds have been used as an antimicrobial agent in food and pharmaceutical industry. The usage of antifungal agents to treat the growth of indoor fungi has been long studied. There is a lot of bio-compound that has been tried and test to inhibit the growth of the indoor fungal. Some of the bio-compound tested can harm human health and some of them are not. That is why the research are still continue to search for the capable, long lasting and human friendly bio-compound to control the growth of indoor fungi [2]. For this research, three bio compounds used that is calcium benzoate, potassium sorbate, and zinc salicylate. These bio compounds usually found in food industries and are not harming human health [3].

Calcium benzoate is usually used in a food as a preservative. Calcium benzoate is competent against molds, yeasts, and also certain types of bacteria. It will present as a white crystalline powder in its raw form. Calcium benzoate, $Ca(C_7H_5O_2)_2$ created when benzoic acid, $C_6H_5COOH$ reacts with calcium hydroxide, $Ca(OH)_2$. The efficiency of benzoic acid depends on the pH of the materials. Potassium sorbate comes in a variety of sources, and it is mainly comes in preservative in preprocessed foods. This is because potassium sorbate can helps to prevent mold, fungi, and yeast growth that can spoils the substrates. Because of it is can be easily produced and not so expensive, this chemical is the ideal choice for many industrial applications. Potassium sorbate is formed when potassium salt bonds with sorbic acid, and creating a fatty acid salt that is polysaturated. Meanwhile, zinc salicylate can be seen as a white crystalline odorless powder. This chemical is soluble in water and also in alcohol. The main raw material of zinc salicylate is salicylic acid. Salicylic acid is prepared by the reaction of phenol. This type of acid is a monohydroxybenzoic acid, which is a type of phenolic acid and a beta hydroxyl acid.

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The aim of this research is to determine the growth of fungal after incorporate with antifungal agents. Three antifungal agents were tested against isolated indoor fungal available in faculty's building located in Universiti Tun Hussein Onn Malaysia (UTHM). The fungal that has been collected was *Aspergillus niger* and the selected antifungal agents are calcium benzoate, potassium sorbate and zinc salicylate. All the antifungal agents are usually found to be used as an additive and preservative for food. The fungal has been isolated on the MEA-agar and treated by the antifungal agents. All samples are stored inside the incubator with temperature 25ºC and relative humidity of 90%. The diameter, ASTM percentage growth, ASTM scales rating and percentage of reduction of indoor fungal has been measured.

## 2. Material and methods

### 2.1 Preparation of agar

Malt extract agar (MEA) is favored for the isolation, detection, and enumeration of fungi, specifically for yeasts and molds. This media are able to recover fungi from a variety of indoor and outdoor sources. MEA would have good sporulation, semi-restricted colonies, suppression of aerial mycelium as well as the suppression of sulcation/umbonation. As for this research, antifungal agents need to put together with the mixture of MEA agar. There are three antifungal agents that is going to be mix together with the MEA mixture; calcium benzoate, zinc salicylate, and potassium sorbate [4]. Each mixture of MEA must mix together with 0.03% (w/v) of each antifungal agent. One liter of distilled water needs 68g of MEA. After putting the MEA and the antifungal agents inside the beaker containing 1 L distilled water, they need to be heat up inside the microwave oven for mixing the two medium. After that, the beaker needs to be put inside the autoclave machine for about 1 hour. Autoclave is a pressure chamber that being used to sterilize equipment and supplies by putting them to high pressure saturated steam at 121°C. After an hour, after the MEA beaker temperature has cool down, the MEA agar is ready to be pouring into the Petri dish

### 2.2 Preparation of samples

After getting the right concentration of spores and the MEA agar are ready, the samples are ready to be prepared. The concentration of $10^6$ spores of *A. niger* were inoculated in a single point located at the center of the MEA agar in the Petri dish [5]. This procedure applied to all MEA agars that consist of calcium benzoate, zinc salicylate, potassium sorbate and also control MEA. Each control and antifungal agents samples have 3 replicates. Average measurement was calculated when analyzing the data. In this case, n=3. All of the samples will be incubated at 25°C [6].

### 2.3 Diameter growth of indoor fungal

The diameter growth of each samples are recorded periodically. The diameters are taken by using normal ruler and measured in centimetres. The diameter growth of fungal is taken once in an interval of two days. After the percentage of the growth of fungal obtained measurement ASTM D5590-00 rating scale are used to determine the scale for the fungal growth.

**Table 1: ASTM rating scale (ASTM, 2001)**

| Observed Growth on Specimens | Rating |
|---|---|
| None | 0 |
| Traces of growth (< 10%) | 1 |
| Light growth (10 – 30%) | 2 |
| Moderate growth (30 – 60%) | 3 |
| Heavy growth (60% to complete coverage) | 4 |

## 3. Results and discussions

The rapid growth of fungal on Day 2 to Day 4 in every samples indicated that the fungal are inside the extending phase, which the hyphae is very efficient in extracting the nutrient from the agar. The Figure 1 shows the diameter growth of indoor fungal that treated by antifungal agents. At the stationery phases, the nutrients are redistributed within the mycelium from storage in the spores. These phases were observed on Day 6 to Day 14 as the growth keep increasing. The next phases hyphae are probably empty, and hyphae wall degenerating [6]. All control and antifungal agents sample having the same type of phases. The difference in diameter size is because of the effectiveness of the antifungal agents to control the growth of the fungal. Clearly in this study, zinc salicylate is the effective antifungal agent that can control the fungal growth.

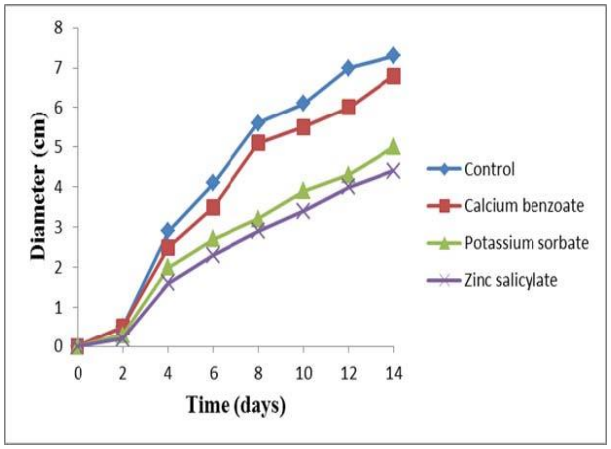


Figure 1 Diameter growth of indoor fungal treated by antifungal agents

Growth of indoor fungal is mainly influenced by the temperature of indoor environment. According to Ibrahim [7], the maximum growth of fungal attained at temperature 25ºC to 30ºC. The Figure 2 shows the ASTM (%) of indoor fungal growth treated by antifungal agents. As for this study, all the samples are incubated in 25ºC temperature. Besides that, relative humidity also plays a part in indoor fungal growth. High relative humidity (90% to 100% RH) will encourage

indoor fungal to growth [4]. All the samples are stored in 90% RH to encourage fungal growth. Zinc salicylate managed to control the ASTM growth percentage of the *A. niger* as it scored the lowest percentage compared to other antifungal agents. There is no argument about calcium benzoate has the bigger percentage because of difference in temperature and relative humidity because all the samples are stored in the same temperature and relative humidity.

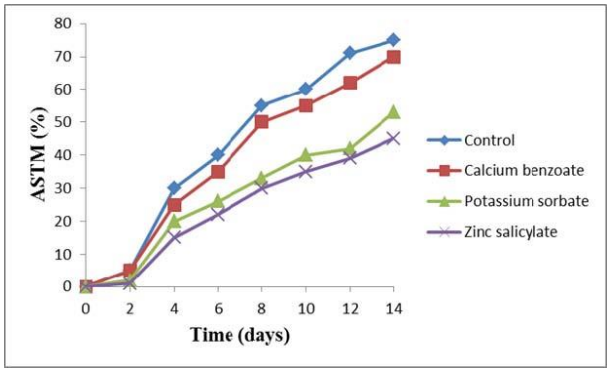


Figure 2 ASTM (%) of indoor fungal growth treated by antifungal agents

The maximum rating scale, that is scale 4, shows by the control and calcium benzoate samples at Day 10 to Day 14. Meanwhile, samples for potassium sorbate and zinc salicylate maintained at scale 3 from Day 8 to Day 14. The ASTM rating scale has the relation with ASTM percentage. The Figure 3 shows the ASTM rating scale of indoor fungal growth treated by antifungal agents. As the ASTM growth percentage increased, ASTM rating scale is also increased. Concentration of spores and antifungal agents probably are the factor in the increasing of rating scale [8-12]. According to Bellotti [5], the concentration of spores has to be in range between 0.3 – 0.5 x $10^6$ spores/mL. For this research, 1.5 x $10^6$ spores/mL have been used. Probably that is why the rating scale for control sample is increase suddenly on Day 4. As for zinc salicylate and potassium sorbate, they manage to control the rating because of their effectiveness in inhibiting the growth of agar that has the same concentration.

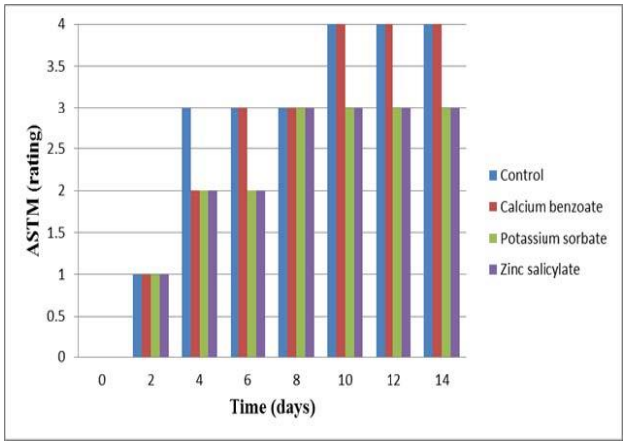


Figure 3 ASTM rating of indoor fungal growth treated by antifungal agents

Figure 4: Control sample at Day 14

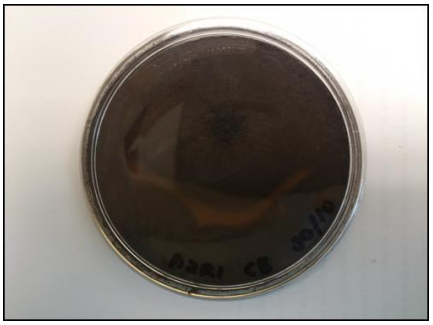

Figure 5: Sample of calcium benzoate at Day 14

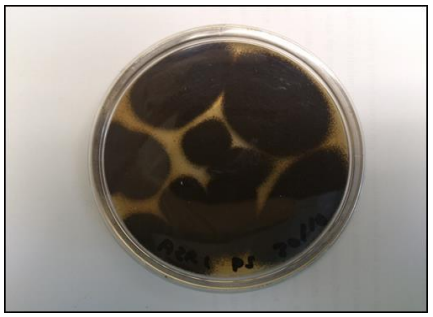

Figure 6: Sample of potassium sorbate at Day 14.

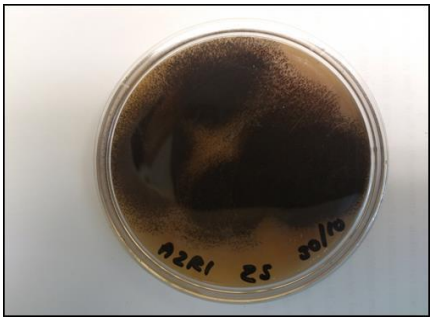

Figure 7: Sample of zinc salicylate at Day 14.

**4. Conclusion**

Indoor Air Quality (IAQ) has been known as one of the most critical environmental risk to public health. Microbial growth in indoor environments can creates health problems, especially to people who have asthmatic issue. The most efficient

antifungal agent is zinc salicylate. It scores the least diameter growth (4.4 cm), lowest ASTM percentage growth (45%), low ASTM scale rating (3), and highest percentage reduction (44.26%) on the last day of data collected compared to others antifungal agents. However, the growth of indoor fungal can only be controlled for a certain period of time. It can be concluded that zinc salicylate is the best antifungal agent between calcium benzoate and potassium sorbate that prohibit the growth of indoor fungi. Nevertheless, the concentrations of the antifungal agent are recommended to be optimized for a long lasting prevention of indoor fungal.

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