The Inhibitory Effect of Coating in Controlling Fungal Growth in Packaging

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Abstract. Packaging material needs to be packaged properly in order to protect it from contamination of other substances that can affect product quality. Moisture level of some products is high due to its moisture content and may result in the growth of Aspergillus niger fungus on the packaging. The use of thiabendazol in coating has been known as one of the methods to inhibit fungal growth on packaging. The purpose of this study is to determine the effect of coating on inhibition of fungal growth. Fungal resistance test was conducted by using Elmes method, disk diffusion and water absorption of packaging substrate (Cobb method). Carton as packaging material was coated using thiabendazol coating with various thicknesses (1μ, 2μ, 3μ, 4μ, 5μ and 6μ). Uncoated carton as control negative and nistatin as control positive. The optimal thickness of coating was 5μ with inhibition zone of 21 mm. As thickness of coating increased, then the larger zone of inhibition produced. These results indicate that the resistance of the antifungal coating was getting stronger. Coatings contain thiabenzol can inhibit growth of fungal on the packaging.

Key Words: Coating, fungi, packaging

1. Introduction

Packaging is a container or the outer part that wraps a product to protect it from weather, collisions, shocks and contamination from other substances, and also to increase the value and function of a product. Good quality of product packaging is influenced by the type and material of packaging, thereby it is gaining substantial attention during the selection of suitable packaging. Some products contain special ingredients that are very susceptible to certain conditions. Therefore, after printing, packaging is processed through the finishing stages, such as lamination, coating, sealing etc. One of main purpose of packaging is preservation and protection of all types of materials and their raw materials, mainly from oxidative, microbial and fungal spoilage and also the enhancement of shelf-life characteristics [1]

Packaging material needs to be packaged properly in order to protect it from contamination of other substances that can affect product quality. Moisture level of some products is high due to its moisture content. For example, bar soaps have high levels of moisture, containing about 15% water by weight in a bar soap. However, conventional paperboard packaging may cause number of potential problems associated with high moisture bar soaps. These problems include fungal growth on the paperboard packaging material especially Aspergillus niger and Penicillium chrysogenum. These problems
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Several damage, such as different colored stains, discoloration of pigments or inks and changes in the chemical and physical properties of paper, may occur caused by fungal growth on paper. [2] Carton packaging is very susceptible to microbial, fungal contamination and damage. Conventional microbicidal and fungicidal pose a serious health risk, even when microbes are no longer viable. The selection of antifungal agent will depend upon its properties such as no toxicity toward human, no adverse effects on carton, low cost, and stable chemical properties.

Several treatments such as gamma irradiation, low oxygen environments, UV radiation, temperature treatment can been used as an effective methods for preventing fungal growth. [3] The physical methods do not have long term action. Chemicals, as antifungal agents, have excellent antifungal activities, such as phenol derivatives [4], titanium dioxide [5], silver and zinc oxide composite [6,7], azole [8]. The Polybornoeactylate (PBA) coating also exhibits significant inhibition of spores germination.[9] Therefore in order to keep the paper spotless, the amount thereof is sufficiently 10 percent by weight of the coating concentration or a 19-µm infiltration of PBA.

The use of thiabendazole in coating has been known as one of the methods to inhibit fungal growth on packaging. [10]. Coating is the process of applying very thin surface to improve the property of the packaging. But simple coating does not inhibit growth of fungal on packaging and, therefore, it is necessary to look for alternatives. Adding said coating with antifungal agents (thiabendazole). Thiabendazole (2-(thiazol-4-yl) benzimidazole) is widely used as post-harvest fungicide on fruits before packing and transportation. [11] Azole compounds are used as antimonycotics for humans and as fungicides. [12] The aim of this research to determine the effect of coating using thiabendazole on inhibition of fungal growth.

2. Method
Materials that were used were carton 310 gsm, thiabendazole Distillates (petroleum) and hydrotreated heavy, coating, Aspergillus niger, PDA.

Instruments that were used were glass apparatus, petri dish, oven, Cobb tester, RI tester.

The stages of the research are as follows:

1. Antifungal coatings on paper. Antifungal coatings were printed onto carton using RI Tester. Coating with six different thickness (1µ, 2µ, 3µ, 4µ, 5µ and 6µ) were carried out to determine the optimal antifungal resistance.

2. Water absorption test using Cobb method to determine the amount of water absorbed by carton. The water absorption test used in this study was based on the standard SNI 0499:2008, requiring 60 seconds of testing time.

3. Grow Aspergillus niger fungus on new media.

4. Antifungal activity test was carried out according to Elmes Method and disk diffusion method. Aspergillus niger were used in this test. Aspergillus niger were added into antifungal coating on the surface of petri dish. Petri dish was incubated for 48 hours at 28º. After the incubation, zone of inhibition formed around packaging were observed. The inhibition zone appeared on packaging indicated the effectiveness of antifungal coating. A larger zone of inhibition means that antifungal coating is more potent in inhibiting the growth of fungal on packaging.

3. Discussion
Growth of fungal on carton surface was observed qualitatively and quantitatively. Disk diffusion was used for quantitative data analysis with measuring the diameters of the inhibition zones on the surface of petri dish. Elmes method was used for qualitative analysis by observing growth of fungal on carton surface in petri dish. At day 1, there was no growth of fungal seen on sample. At day 2, growth of fungal in the uncoated carton sample were visible (Figure 1). In the observation at day 3, growth of fungal was becoming increasingly visible (as picture shown below). Visual observation using Elmes method can be seen in Table 1.
Table 1. Visual observation growth of fungal.

| No | Treatment | Growth of fungal |
|----|-----------|-----------------|
|    |           | Day 1 | Day 2 | Day 3 | Day 4 |
| 1  | Non coating | -     | +++e  | +++   | ++++ |
| 2  | 1 µ        | -     | +++d  | +++   | ++++ |
| 3  | 2 µ        | -     | +++c  | ++    | ++++ |
| 4  | 3 µ        | -     | ++    | ++    | +++  |
| 5  | 4 µ        | -     | ++    | ++    | +++  |
| 6  | 5 µ        | -     | ++    | ++    | +++  |
| 7  | 6 µ        | -     | ++    | ++    | +++  |

* a (−) no inhibition
  b (+) mild inhibition
  c (+++) moderate inhibition
  d (+++ ) strong inhibition
  e (+++++) highly strong inhibition

On day 1 of observations, none of incubated petri dish showed growth of fungal or zone of inhibition. On day 2 of observations, growth of fungal was significantly visible. On day 3, the surface area was mostly white while *Aspergillus niger* were generally characterized by black spore pigment. Whereas in coated carton using thiabendazole, zone of inhibition was detected in petri dish but growth of fungal was not significantly visible on day 2 of observations. On day 3, the surface area was mostly white, while *Aspergillus niger* were generally characterized by black spore pigment, which showed decrease in growth of *Aspergillus Niger* spores with increasing coating thickness. On the carton coated with thiabendazole, the quantity of *Aspergillus niger* sporangia was significant reduced.

Quantitative test was used to measure the diameters of zone of inhibition and observe the growth of sample on incubated petri dish. Antifungal activity was considered as no inhibition with ≤ 5 mm zone of inhibition, moderately active (mild inhibition) with 6-10 mm zone of inhibition, highly active (strong inhibition) with 11-20 mm zone of inhibition, very highly active (very strong) with ≥20 mm zone of inhibition. [13,14]
As shown in Table 2, the zone of inhibition of uncoated carton was 6.8mm, surrounded by black spores of *Aspergillus Niger*. The inhibition zone diameter were calculated to be 12.1 mm, 14.5 mm, 16.8 mm, 19.1 mm and 21.7 mm at coating thickness $1 \mu$, $2 \mu$, $3 \mu$, $4 \mu$, and $6 \mu$ respectively. The calculated inhibitions were categorized as strong inhibition. Test result were obtained that carton without coating antifungal had no inhibiton. Whereas coated carton with thiabendazole has strong inhibition and very strong inhibition *Aspergillus niger*. Based on data above, antifungal showed very strong inhibitory effect at 5-6micron thickness as compared to nistatin as positive control positif (>20 mm). This results showed that the antifungal coating used has strong properties to inhibit growth of fungal because it contains Thiaben dalazol as antifungal chemical, as shown in figure 4. Using thiabendazole as antifungal shown that it inhibits the ergosterol biosynthetic pathway, which leads to affects change of sterol composition in the plasma membrane of fungi [15]

| No | Treatment       | Diameter (mm) |   |   |
|----|----------------|---------------|---|---|
|    |                | Day 1 | Day 2 | Day 3 |
| 1  | Control positive | 0    | 20.79 | 25.3 |
| 2  | Non coating     | 0    | 7.7   | 6.8  |
| 3  | 1              | 0    | 15.2  | 12.1 |
| 4  | 2              | 0    | 19.6  | 14.5 |
| 5  | 3              | 0    | 21.4  | 16.8 |
| 6  | 4              | 0    | 24.5  | 19.1 |
| 7  | 5              | 0    | 25.3  | 21.0 |
| 8  | 6              | 0    | 28.2  | 21.7 |

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**Figure 4.** Zone of inhibition on day 1.

**Figure 5.** Zone of inhibition on day 3

**Figure 6.** Zone of inhibition on day 4

Water absorption test results indicated capacity of water absorbed on carton and showed that the moisture level on carton, as packaging material, was influenced by the moisture content. Table 3 shows water absorption test results on uncoated and coated carton.
Table 3. Water absorption value.

| No | Treatment | Water absorption (g/m²) |
|----|-----------|------------------------|
| 1  | Non coating | 32.7                  |
| 2  | 1 µ        | 18.9                  |
| 3  | 2 µ        | 9.2                   |
| 4  | 3 µ        | 8.1                   |
| 5  | 4 µ        | 6.8                   |
| 6  | 5 µ        | 6.2                   |
| 7  | 6 µ        | 5.4                   |

The average of water absorption of uncoated carton was 32.7. While after coating with antifungal, the water absorption value was found to be decreased. The water absorption value were 18.9, 9.2, 8.1, and 5.4 at coating thickness 1µ, 2µ, 3µ, and 6µ respectively. These results showed that coating can remarkably decrease water absorption and inhibit the growth of fungal by reducing water content. Water absorption value decreased with increased in coating thickness. This caused the moisture content to decrease and it helped minimize growth of fungi. Fungi grow in environments rich in moisture and release spores as part of their reproductive process. [16] As thickness of coating increased, then the larger zone of inhibition produced and showed strong antifungal activity.

4. Conclusion
The optimal thickness of coating was 5µ with inhibition zone of 2.10 cm–21 mm. The coating that contains thiabendazol has properties to inhibit growth of fungal. As thickness of coating increased, then the larger zone of inhibition produced and showed strong antifungal activity. Water absorption decreased with increased in coating thickness. This caused the moisture content to decrease and it helped minimize growth of fungal.

5. References
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