



## THE EFFECT OF BILIMBI JUICE LEVELS AND SOAKING TIME ON THE GROWTH OF *Aspergillus flavus*, L. IN CORN KERNELS

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### ABSTRACT

*Aspergillus flavus* is one of the dominant fungi found in grains, especially corn. This fungus produces aflatoxin compounds that are hepatocarcinogenic. The study intends to analyze the effect of concentration and soaking time of corn kernels in bilimbi (*Averrhoa bilimbi*) juice on the growth of *Aspergillus flavus*. The experimental research, corn kernels soaked in star fruit juice concentrations of 5, 10, and 15% v/v, for 10, 20, and 30 minutes. Corn kernels are taken and planted in AFPA media. Microscopic observations every hour to observe the growth of *Aspergillus flavus* from planting to growth. Data were analyzed using two-way ANOVA, followed by LSD test with 95% CI. Research shows there is a decrease in pH caused by increased levels of star fruit juice. Concentrations of bilimbi fruit and soaking time can inhibit the growth of *Aspergillus flavus* in corn kernels. The optimal inhibitory potential was found at 15% v/v with a soaking time of 30 minutes.

**Keywords:** concentration, soaking, bilimbi, *Aspergillus*

### INTRODUCTION

*Zea mays* come from two languages, *Zea* (Greek) is a generic name for cereal and grains; some scientists believe that *Zea* stands for "sustaining life". *Mays* (Taino) means "the giver of life." maize "corn" is the connotation of the Spanish language "maiz" is one of the best ways to describe plants. Other synonyms are found *zea*, silk maize, [1]. Corn or maize (*Zea mays L.*) is ranked 3rd for the most widely used cereal in the world, after wheat and rice [2].

The world production of maize was 967 million metric tons (MMT), the United States produces 40 % of them, is known as the mother grain of Americans, and it is the driver

of the U.S. economy, followed by China and Brazil [3]. The various types of products, such as cornmeal, grits, starch, flour, tortillas, snacks, and breakfast cereals, maize flour, are used to make chapatis or flatbread [4]. The maize kernel contains carbohydrates 66.2%, protein 11.1%, fat 3.6%, minerals 1.5% and fibres 2.7%, there are also  $\beta$ -carotene, biotin, choline, pantothenic acid, folic acid, pyridoxine, thiamine, riboflavin, niacin, vitamin E, small amounts of vitamin C, N- (co-coumarin) tryptamine and N-ferulyltryptamine [5].

The maize is an essential source of various major phytochemicals, such as carotenoids, phenolic compounds, and

phytosterols [6,7,8]. Phytochemicals are one of the bioactive chemical compounds that are naturally present in plants to provide human health benefits and have the benefit of reducing the risk of major chronic diseases [9]. In traditional medicine, corn is used to remedy dysentery, diarrhea, prostatitis, lithiasis, angina, hypertension, urinary tract disorders, and tumors. This plant is used massively for hypoglycemic [10,11], anti-inflammatory [12], antioxidant [13,14] and diuretic properties [15].

*A. flavus* is known as a type of fungus that functions as the main producer of aflatoxin, which can cause contamination of grains, especially corn [16]. Aflatoxicosis is a disorder caused by aflatoxin, this is due to inhaling or consuming foods containing high amounts of aflatoxin. Aflatoxicosis, which can be harmful to humans who experience immune disorders [17]. Post-harvest Aflatoxin seed contamination is a major problem due to errors in improper storage procedures, insect activity and excessive moisture [18]. As a number of events in Kenya the occurrence of postharvest contamination that caused outbreaks of aflatoxicosis [19] and animal feed [20].

While aflatoxicosis (toxic effects on cells) is a growing cancer caused by long-term exposure to aflatoxin B1. Aflatoxin B1 is a powerful hepatocarcinogen and can induce tumors especially in the kidneys but also the lungs, liver and large intestine in humans and animals. Hepatocellular carcinoma is a type of primary liver cancer associated with the consumption of aflatoxin B1 which occurs in Africa and Asian countries [21]. this problem arises from contaminated maize killing

hundreds of people in Kenya This is also one of the big problems in developing countries, especially Asia and Africa [22, 23]

Several substances can inhibit or affect the production of aflatoxin including benzoic acid, sorbic acid, ferulic acid, propionic acid, oleuropein, vanillin, cinnamon [24], and other plants, but most as inhibitors of toxin production through inhibition of fungal growth. Pakki has studied clove leaf extract to control the growth of *Aspergillus flavus*. [25]. Various efforts to make corn seeds more durable for storage and to avoid contamination of *Aspergillus flavus*, among them using chemical antimicrobials, such as nitrate, nitrite, sodium benzoate, propionic acid, citric acid, platinum, sodium metabisulfite, formalin, etc., heating or cooling and fermentation techniques, enzymes (e.g., bromelain, papain) [26], radiation [27]. Long-term use of chemical preservatives can cause various diseases such as cancer and kidney disorders [28].

Some natural antimicrobials are useful as food preservatives, such as wood smoke, essential oils [29], *Allium tuberosum*, *Cinnamomum cassia* [30], *Curcuma zanthorrhiza* [31] which are effective for various bacteria. At the same time, their activity as mushroom extracts of shallots and red chillies inhibits the *Candida crucei*, and *Candida utilis* [32], leaves of *Salvia pratensis* can inhibit *Saccharomyces cerevisiae* [33].

Plants synthesize phenol compounds through secondary metabolism as a defense mechanism against microbial attacks, insecticides, and herbivores. Compounds that have antimicrobial activity are simple phenols, phenolic acids, quinones, xanthenes, flavonoids, tannins, and coumarins. The

antimicrobial properties of flavonoids are due to their ability to form complexes with bacterial cell walls, as well as extracellular proteins [34].

*Averrhoa bilimbi* is often used by the community as a traditional medicine to treat malaria, sore throat, diarrhea, ulcers, and asthma, inhibiting the growth of *Candida albicans* mushrooms, due to its flavonoid content [35], another ingredient, saponin, can lyse fungal cell membranes, so that inhibits growth, and has been used as a natural preservative in fresh tilapia [36].

Therefore, it is necessary to look for natural preservatives that can inhibit the growth of *Aspergillus flavus*. One alternative natural ingredient that can be used as a preservative that has antifungal activity is the *Averrhoa bilimbi*. Based on the description, it was observed the squeezed of *Averrhoa bilimbi* as a natural preservative of *Aspergillus flavus* on corn kernels.

## METHODS

### 1. Tools and materials

AFPA Media, Chloramphenicol, corn kernels, bilimbi fruit, stopwatch, magnifying glass, microscope, glassware, juicer.

### 2. Research Design

**Table 1.** Reserach Design

Groups	Treatment
Media control	Media
Growth control	Media + corn
Positive control	Media+corn+benzoic ac.
Negative control	Media + corn + aq.bides
Conc. 5 % v/v	Media + 5 % blimbi
Conc. 10 % v/v	Media + 10 % blimbi
Conc. 15 % v/v	Media + 15 % blimbi

Experimental research by giving treatment to each group, fungal growth was

observed microscopically every hour. Growth retardation is calculated after the completion of starfruit juice until signs of fungal growth are observed (Table 1).

## 3. Procedure

### a. Juice preparation

Weigh many bilimbi fruits and aquadest places into the juicer. Collect the juice and add aquadest quantum states to make juice concentration 5, 10, and 15% v/v.

### b. Mushroom growth test

Prepare *Aspergillus flavus* Parasitikus Agar (AFPA) media, add an antibiotic. Soak the Corn kernels in 5, 10, and 15% v/v juice of bilimbi fruit for 10, 20, and 30 minutes, respectively. Grab the corn kernels and place them in the AFPA media of positive control, negative control, media control, and treatment groups. Allow at room temperature and observer every hour until the fungus grows and repeated five times

### c. Identification of Flavonoid.

One ml of juice add methanol and heat, filter. Filtrate add H<sub>2</sub>SO<sub>4</sub>. Red color indicates flavonoids [37].

## 4. Data Analysis.

Data were analyzed by Shapiro-Wilk, homogeneity test, two-way ANOVA, and Post Hoc test using SPSS 15.0 for Windows with 95% of Confident Interval.

## RESULTS AND DISCUSSION

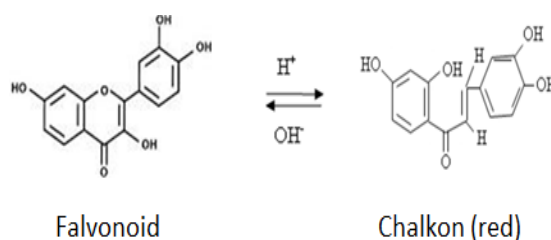
### 1. Plant Determination

Determination has been done in UPT Materia Medika Batu Malang, is :1b–2b–3b–4b–6b–7b–9b–10b–11b–12b–13b–14a–

15b–197b–208b–219b–220b–224b–225b–227b–229b– 230b–234b–235b–236b–237b–238b–1b–1a. *Averrhoa bilimbi* (Bilimbi fruit).

## 2. Identification of Flavonoid and Saponin.

The presence of flavonoid compounds in *A. bilimbi* fruit content is indicated by the formation of red color by reaction with sulfuric acid to form chalcone [38], flavonoids undergo decomposition by bases into molecules such as yellow acetophenone (Figure 1) [37]. The presence of saponins is indicated by foam formation if the sample is shaken out with HCl.



**Figure 1.** Identification of Flavonoid in *A. Bilimbi* Fruit

## 3. The pH of media.

Table 2 shows no effect of soaking time with medium pH, but increased concentration can reduce the pH of media.

**Table 2.** The average of pH based on immersion time

Groups	pH average (mean ± S.D.)		
	Immersion time (minute)		
	10	20	30
<b>Possitive</b>	4.22 ± 0.045	4.16 ± 0.055	4.30 ± 0.071
<b>Negative</b>	7.00 ± 0.045	7.10 ± 0.141	7.00 ± 0.000
<b>Conc. 5% v/v</b>	3.04 ± 0.134	3.02 ± 0.045	3.08 ± 0.045
<b>Conc. 10% v/v</b>	2.77 ± 0.134	2.88 ± 0.070	4.22 ± 0.000
<b>Conc. 15% v/v</b>	2.68 ± 0.045	2.54 ± 0.089	2.70 ± 0.000

As mentioned above, star fruit contains pantothenic acid and folic acid, so it has acidic ( $H^+$ ) properties. The data above, following Sorensen's theory, pH is a negative logarithmic function of the concentration of  $H^+$  ions in a solution and is formulated as follows.  $pH = -\log [H^+]$ ; therefore, the greater the concentration value of a solution, the pH will be smaller, and the degree of acidity stronger. But if the concentration gets smaller than the degree of acidity will approach neutral and can even be alkaline because the acidity is more than seven.

## 4. Observation of Growth Inhibition

Table 3. shows that the duration of immersion has an effect on the growth inhibition,

and the increase in concentration can increase the growth inhibition. This means that the immersion time and concentration of squeezed affect the inhibition of fungal growth. While the pH is relatively less influenced by the soaking time, otherwise the higher squeezed concentration causes a decrease in pH.

The factors that influence microbial growth consists of intrinsic factors (pH,  $A_w$ , potentiation-reduction, nutrient content, antimicrobial compound content, and biological structure), and extrinsic factors. Include temperature, relative humidity, and arrangement of gases in the environment.

Mushroom able to grow on a laboratory scale at pH range is quite wide, which is

between 4.5-8.0 with an optimum pH between 5.5-7.5 or depending on the type of fungus. According to [39], media pH is influential in the growth and production of enzymes. On mushrooms generally grow and produce various kinds of enzymes in the acidic pH range, the high N content can

increase the pH, and this will affect the ligninolytic ability [40]. Temperature also has an important role in the production of the enzyme lignin peroxidase. Each fungus has a different optimal, minimum, and maximum temperature for the growth of the enzyme lignin peroxidase [41].

**Table 3.** Coss table pH, soaking and inhibition time (hours)

Groups	Soaking time (minute)					
	10		20		30	
	pH	Inhibition	pH	Inhibition	pH	Inhibition
<b>Positive</b>	4.22 ± 0.045	20.8 ± 1.50	4.16 ± 0.055	21.0 ± 1.60	4.30 ± 0.071	21.4 ± 1.10
<b>Negative</b>	7.00 ± 0.045	28.2 ± 1.90	7.10 ± 0.141	29.0 ± 1.90	7.00 ± 0.000	29.4 ± 2.10
<b>Conc. 5 % v/v</b>	3.04 ± 0.134	21.8 ± 1.60	3.02 ± 0.045	23.4 ± 2.20	3.08 ± 0.045	24.2 ± 1.30
<b>Conc.10 % v/v</b>	2.77 ± 0.134	24.4 ± 1.10	2.88 ± 0.070	26.8 ± 1.10	4.22 ± 0.000	28.0 ± 1.20
<b>Conc. 15 % v/v</b>	2.68 ± 0.045	25.2 ± 1.30	2.54 ± 0.089	28.6 ± 1.80	2.70 ± 0.000	31.8 ± 1.80

**5. Statistical Analysis**

ANOVA two-way test shows there are significant differences between treatment groups,  $F_{\text{value.}}: 75.795$ ,  $p_{\text{value.}} : 0.000$ ; between immersion  $F_{\text{value.}}: 10.625$ ,  $p_{\text{value.}} : 0.000$  and treatment with immersion  $F_{\text{value.}}: 1.393$ ,  $p_{\text{value.}}: 0.128$ , CI: 95%, then tested post hoc, are shown in Table 4.

LSD test results showed a negative control with a concentration of 5%, 10%, and 15% showed a significant value of 0.001, 0.000 and 0.000 (p-value <0.005), which means that it has a significant difference.

**Table 4.** Post Hoc Test of concentration

Pairs	p-value	Difference
Negative vs. Positive	0.000	Sig.
Negative vs. Conc. 5%	0.001	Sig.
Negative vs. Conc. 10%	0.000	Sig.
Negative vs. Conc. 15%	0.000	Sig.
Positive vs. Conc. 5%	0.000	Sig.
Positive vs. Conc.10%	0.000	Sig.
Positive vs. Conc.15%	0.121	No Sig.
Conc. 5% vs Conc.10%	0.000	Sig.
Conc. 5% vs Conc.15%	0.000	Sig.
Conc. 10% vs Conc. 15%	0.000	Sig.

Note : Conc : concentration

The positive control is compared to a concentration of 15% has a significance value of 0.121 (p-value> 0.05), which means that it is not significantly different. This concentration has a comparable effect on the growth of the *Aspergillus flavus* on corn kernels. The negative control paired with concentrations of 5%, 10%, and 15% showed significant values (p-value <0.005), which means that they have a significant difference with negative (star fruit juice), so it is said to affect *Aspergillus flavus* growth in corn kernels. Positive control with a concentration of 15% (p-value> 0.05)that it is not significantly different or has an equivalent effect as an inhibitor of the growth of the fungus *Aspergillus flavus* on corn kernels.

**Table 5** Post Hoc Test of soaking time

Pairs	p-value	Difference
10 min. v 20 min	0.034	Sig.
10 min.v 30 min.	0.000	Sig.
20 min v 30 min	0.018	Sig.

Note : min.: minute; Sig: significant

The average inhibition of mushroom growth in a time of 30, 20, and 10 minutes was 26.9, 25.8, and 24.8 hours, respectively. It shows the more extended immersion using star fruit squeezed the longer the inhibitory growth of *Aspergillus flavus* (Table 5).

Antifungal effectiveness occurs because of the active compounds contained in star fruit, namely flavonoids and saponins. This compound can lyse fungal cell membrane so that it disrupts the growth of fungi or the fungus cannot grow, and in this case has a fungistatic effect. This research shows satisfactory results as a substitute or alternative to existing preservatives, especially when processing preserved agricultural grain products, especially preventing the growth of the *Aspergillus flavus* fungus on corn kernels, which is very economically detrimental and the impact of health problems caused by aflatoxin produced by *Aspergillus flavus*.

## CONCLUSION

Concentrations and soaking time of corn kernels in star fruit juice can inhibit the growth of *Aspergillus flavus*. The optimal inhibition potential was found at 15% v/v with a soaking time of 30 minutes.

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