

Durian Seed Flour (*Durio zibethinus* Murr) as an Alternative Medium for Fungal Growth

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Abstract

This study aimed to evaluate the potential of durian seed flour (*Durio zibethinus* Murr) as an alternative medium for the growth of *Candida albicans* and *Aspergillus niger*. The bleaching process utilized sodium metabisulfite at varying concentrations (600 ppm, 800 ppm, 1000 ppm) and immersion durations (15, 20, 25 minutes). The bleached samples were dried at 70°C for 3 hours. Durian seed flour media were formulated into three variants and compared with Potato Dextrose Agar (PDA) as the control medium. The activity test for *Candida albicans* used the pour plate method with incubation for 48 hours at 37°C, while *Aspergillus niger* was tested using the agar block method and incubated for 72 hours under the same conditions. Immersion duration significantly affected the flour's color, with longer durations producing whiter flour. Higher drying temperatures enhanced water evaporation efficiency, resulting in an average moisture content of 10.56%, compliant with national standards. The best medium formulation was F1 (10 g durian seed flour, 2 g sugar, 1.5 g agar, 0.25 g micronutrients, 100 mL distilled water), yielding 3.6×10^7 CFU/mL of *Candida albicans* colonies and an *Aspergillus niger* growth diameter of 28.5 mm after 72 hours of incubation. However, sporulation of *Aspergillus niger* was better on PDA media. Durian seed flour shows potential as an alternative fungal growth medium.

Keywords: *Aspergillus niger*; *Candida albicans*; Durian seed flour; Fungal growth medium.

INTRODUCTION

Durian season means one thing: durian seeds (*Durio zibethinus* Murr) are often unused and wasted. Consumers generally eat only the meat or salad part of the fruit, which makes up about 20–35% of the whole fruit. Meanwhile, the skin (60–75%) and seeds (5–15%) are not being maximally utilized (Sistanto et al., 2017). Durian seeds contain 51.5% water, 46.2% carbohydrates, 2.5% protein, and 0.2% fat (Damayanti et al., 2020).

The complete nutritional content of durian seeds can be utilized as a medium for mushroom growth. Fungi require nutrients such as carbon, nitrogen, and non-metallic elements such as sulfur and phosphorus, as well as metal elements such as Ca, Zn, Na, K, Cu, Mn, Mg, Fe, vitamins, water, and energy. Carbohydrates and their derivatives are the main substrates for carbon metabolism in fungi, with carbon being the most important element because it makes up 50% of the weight of microorganisms. Durian seeds, with their high carbohydrate content, can be a sufficient source of energy for fungal growth (Sundari et al., 2021).

A 100-gram serving of durian seeds contains 67 grams of water, 28.3 grams of carbohydrates, 2.5 grams of fat, 2.5 grams of protein, 1.4 grams of vitamin C, and

19.7 milligrams of potassium. Durian seeds have a fairly high starch content, so they have the potential to be used as flour (Lopulalan, 2016).

Fungi are cosmopolitan organisms that can grow in environments close to humans, such as air, soil, water, clothing, and even inside the human body (Hasanah, 2017). *Candida albicans* is a pathogenic microorganism that can cause disease when present in excessive amounts in the body (Ornay et al., 2017). *Aspergillus niger*, another fungal species, grows as a saprophyte on decaying vegetation and is also found in soil, dust, and food. Both fungi require sufficient nutrients to grow (Hasanah, 2017).

Media is a mixture of nutrients required for the growth of microorganisms and is used for isolation, inoculation, physiological and biochemical tests of microorganisms. One of the media for fungal growth is Potato Dextrose Agar (PDA). PDA is made from natural (potato) and synthetic materials (dextrose and agar). Potatoes are a source of carbon (carbohydrates), vitamins, and energy; dextrose is a source of sugar and energy; and agar components act as a solidifying agent (Qurrohman, 2021).

Previous research by Afriani et al. (2022) showed that the durian seed flour produced had a brownish-white

color. Treatment with the bleaching agent sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$) is required to produce whiter flour. $\text{Na}_2\text{S}_2\text{O}_5$, already widely used in the food industry, effectively inhibits the browning reaction and prevents the formation of melanoidin pigments (Akolo & Azis, 2018; Haryani et al., 2016).

In the study of Simanjuntak (2014), the complete randomized design (CRD) method was used with two (2) factors: drying temperature (50°C , 60°C , 70°C) and sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$) concentration (200 ppm, 400 ppm, 600 ppm). The results showed that treatment with 600 ppm $\text{Na}_2\text{S}_2\text{O}_5$ at 60°C produced durian seed flour with the best properties (Simanjuntak et al., 2014). Drying temperature affects the quality of flour, with low temperature reducing the rate of water evaporation and high temperature increasing the drying efficiency, reducing the moisture content below 10% to prevent growth.

MATERIALS AND METHODS

Chemical material

The materials used in this research were durian seeds (*Durio zibethinus* Murr), sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$), agar (Satellite), cane sugar (Gulaku), PDA instant media (Pronadisa), micronutrients, Mc Farland standard suspension, NaCl 0, 9%, 70% ethanol, distilled water, 3% HCl (hydrochloric acid), NaOH (sodium hydroxide), 3% CH_3COOH , Luff solution, 20% KI solution (potassium iodide), H_2SO_4 (sulfuric acid), cotton swabs, pH indicator strips, aluminum foil, plastic wrap (bagus), parchment paper, gauze.

Equipment

The instrumentations used in this research were Laminar Air Flow (Astec HLF 1200L), Incubator (Fisher Scientific), Oven (Mettler), Refrigerator (Toshiba), Refrigerator (Toshiba), Colony Counter, Blender (Philips), Digital Scale (Kern), Hot Plate (Stirrer), Sprayer, Basin, Tray, Flour Sieve, Mortar and Pestle, Erlenmeyer (Pyrex), Beaker (Pyrex), Measuring cup (Iwaki Pyrex), test tube, petri dish, porcelain cup, vernier, desiccator, distillation flask, Bunsen lamp, micropipette (Eppendorf), cork punch, tweezers, dropper, spatula, stirring rod, ose needle, knife.

Fungal Culture

The fungal cultures used in this research were *Candida albicans* and *Aspergillus niger* obtained from the Microbiology Laboratory of the Faculty of Medicine, Padang, West Sumatra.

Sterilization of tools and materials

All tools used were thoroughly washed with distilled water. Test tubes, beakers and conical flasks were covered with cotton. Petri dishes were wrapped in paper and sterilized by autoclaving at 121°C for 15 minutes.

The ose needle and forceps were sterilized with a Bunsen lamp. The medium was sterilized by autoclaving at 121°C for 15 minutes (Magfirah Abdullah et al., 2020).

Sampling

The samples used in this study were durian seeds (*Durio zibethinus* Murr) as much as 2 kg obtained from durian fruit plantations in Jorong Balai Sabuah, Balai Sabuah district, Batipuah Ateh, West Sumatra.

Plant identification

The identification of durian plants was carried out at its herbarium, Faculty of Biology, Andalas University, Padang, West Sumatra.

Sample preparation

Durian seeds that are still fresh and not rotten are selected, then washed with clean water and drained. Durian seeds are peeled off the outer skin, rewashed, drained, and then cut into pieces about 0.4 cm thick, then washed with running water until the sap is gone, and drained. The durian seeds are washed again with running water until the sap is gone and drained. Then the durian seeds are weighed according to the weight used for flour production (Simanjuntak et al., 2014).

Durian seed bleaching process

Sodium metabisulfite with 600 ppm, 800 ppm, and 1000 ppm were dissolved in 100 mL of distilled water, respectively. The bleaching solution was mixed with 100 grams of durian seeds in a glass beaker so that the entire surface of the durian seeds was immersed. The maceration process lasted for 15 minutes, 20 minutes, and 25 minutes, respectively. Afterward, the solution was separated from the durian seeds and dried using an oven at 70°C for 3 hours (Lastari et al., 2016).

Durian seed flour preparation

Durian seeds weighed according to weight are dried in an oven at 70°C for 3 hours. The dried durian seeds are pounded and crushed with a mortar and pestle until smooth, then sieved with a 100 mesh sieve (Simanjuntak et al., 2014).

Organoleptic test

An organoleptic test evaluates the color, shape, and smell of durian seed flour produced using the human senses (sensory) (Hevira et al., 2021).

Determination of moisture content

Moisture content is determined by the direct heating method or gravimetric method. The porcelain cup is dried in the oven at 100°C for 30 minutes, then cooled and weighed empty. Weigh 5 grams of durian seed flour in a cup, then dry it in an oven at 105°C for 3 hours. Cool the beaker and reweigh it. Record the weight of the sample before and after drying (Kiptiah et al., 2019).

Determination of carbohydrate content

Weigh about 3 g of durian seed flour into a 500 ml Erlenmeyer flask. Add 200 ml of 3% HCl solution. Simmer for 3 hours with the condenser upright, then cool and neutralize with saturated NaOH solution. Add a little 3% CH₃COOH to make the solution slightly acidic, then transfer the contents to a 500 ml volumetric flask up to the mark, then filter. Pipette 10 ml of filtered water into a 500 ml Erlenmeyer flask, add 25 ml of Luff's solution (with a pipette), a few boiling stones and 15 ml of distilled water. Bring the mixture to the boil for 3 minutes, then continue boiling for exactly 10 minutes (count from the start of boiling and use a stopwatch), then rapidly cool in an ice bath. After cooling, slowly add 15 ml of 20% KI solution and 25 ml of 25% H₂SO₄, immediately titrate with 0.1 N thio solution (use 0.5% starch solution indicator). Perform a blank determination (BSN, 2011).

Preparation of PDA media

Weigh 3.9 grams of Potato Dextrose Agar (PDA) and dissolve in 100 mL of distilled water using an Erlenmeyer flask. Place the media in a test tube lined with cotton and aluminum foil. Then, sterilized using an autoclave for 15 minutes at 121°C and left until the media is cool enough, aseptically poured into a petri dish as much as 20 mL (Mujipradhana et al., 2018).

Preparation of durian seed flour media

Durian seed flour-based media were prepared in 3 formulations with different durian seed flour compositions. The following is a table of each of durian seed flour-based fungal growth media formulation. Durian seed flour is weighed according to the formulation to be prepared, 1.5 grams of neutral agar and 2 grams of sugar are added and dissolved with 100 mL of distilled water in an Erlenmeyer flask. For the F1 formulation, 0.25 grams of micronutrients were added to the media solution. The media solution was heated to 90°C with homogeneous stirring until boiling. In addition, the pH was measured using pH indicator paper. The durian seed flour media solution was covered with cotton and aluminum foil, and then sterilized using an autoclave for 15 minutes at 121°C. Durian seed flour media was poured into Petri dishes, each concentration was made as much as three durian seed flour media and more for media stock.

Fungal inoculation on media

Candida albicans

Candida albicans culture stock was harvested using a sterile ose needle and then suspended in 10 mL 0.9% NaCl. The suspension was vortexed until the turbidity of the suspension was equal to the turbidity of the Mc Farland standard solution (10-8 CFU/mL). 1 mL of suspension was added to 9 mL of 0.9% NaCl solution and diluted up to 6-fold (10-6). Furthermore, the suspension preparation and *Candida albicans* culture

were inoculated on the surface of PDA sloped media and then incubated at 37°C for 48 hours (Septiani et al., 2017).

Aspergillus niger

Aspergillus niger culture was collected using a 5 mm diameter cork borer, then transferred to instant PDA and durian seed flour media and incubated at 37°C for 72 hours. The diameter of the fungal growth zone was measured every 24 hours using a caliper. The sporulation formed was visually observed. The test was performed with 3 replicates (Kwoseh et al., 2012).

Statistical data analysis

All data were statistically analyzed using one-way ANOVA followed by Tukey's HSD test. Tukey's HSD test was used to test differences between sample means for significance. A significance level of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The samples used in this study were durian seeds collected from Jorong Balai Sabuah, Balai Sabuah Batipuah Ateh District, West Sumatra. The samples were identified at the Herbarium of Andalas University (ANDA) to ensure the plant species used is (*Durio zibethinus* Murr).

Previous research by Afriani et al. (2022) showed that the durian seed flour produced was still brownish. This study used sodium metabisulfite (Na₂S₂O₅) as a bleaching agent to prevent browning reactions in durian seed flour. Sodium metabisulfite was chosen because it produces more whiteness than other bleaching agents such as ascorbic acid (vitamin C) and hydrogen peroxide (Haryani, 2016).

The bleaching process was performed by soaking durian seeds in Na₂S₂O₅ solution at concentrations of 600 ppm, 800 ppm, and 1000 ppm with soaking durations of 15, 20, and 25 min. The results showed that increasing the concentration and soaking duration produced durian seed flour with a whiter color (Purwanto et al., 2013). The drying temperature used was 70°C for 3 hours, as this temperature effectively reduced the moisture content without damaging the flour properties (Simanjuntak et al., 2014). After drying, the durian seeds were crushed until smooth and sieved using a 100 mesh sieve to produce flour with a very fine texture (Lopulalan, 2016).

Durian seed meal has a pH of 5, similar to the Potato Dextrose Agar (PDA) control medium, which also has a pH of 5. Environmental factors such as pH greatly affect mold growth, with low acidity (pH 4.5-5.6) being ideal for inhibiting the growth of bacteria that generally live at neutral pH (Etiek, 2020). Physically, durian seed flour is white, as opposed to PDA media, which is clear yellow. The consistency of these two media is both solid because both contain 1.5% agar, which allows the media to be

liquid at high temperatures and solid when cold. To further characterize the physical and chemical properties of the durian seed flour produced, an organoleptic evaluation and a comparative analysis with standard PDA media were conducted. The result of the organoleptic assessment is presented in Table 1, while the physical and chemical characteristic of both the durian seed flour media and PDA media are summarized in Table 2.

Table 1. Organoleptic of durian seed flour.

Sample	Immersion time	Color	Shape	Odor
Without bleaching	-	Brown	Powder	Typical
600 ppm	15	Brown	Powder	Typical
	20	Brown	Powder	Typical
	25	Brown	Powder	Typical
800 ppm	15	Brown	Powder	Typical
	20	Brown	Powder	Typical
	25	White	Powder	Typical
1000 ppm	15	Brown	Powder	Typical
	20	Brown	Powder	Typical
	25	White	Powder	Typical

Table 2. Characteristics of durian seed flour media and PDA (*Potato Dextrose Agar*).

Characteristic	Durian seed flour media	PDA (<i>Potato Dextrose Agar</i>)
Color	White	Yellow clear
Odor	Typical	Typical
Consistency	Solid	Solid
pH	5	5

The average moisture content of the durian seed flour produced was 10.56%, which meets the national standard requiring a moisture content of less than 14% (Kiptiah et al., 2019). This low moisture content is expected to improve the storability of the product, as low water content reduces the risk of microbial growth.

The carbohydrate content in durian seed flour without bleaching is 14.66%, the highest carbohydrate content is at a concentration of 1000 ppm in minute 25, which is 14.28%, while the lowest carbohydrate content is at 600 ppm. It can be concluded that the higher the concentration and soaking time of sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$), the higher the carbohydrate content in durian seed flour (*Durio zibethinus* Murr). Carbohydrates in flour generally show some changes with pre-treatment in the form of soaking time (Lastari et al., 2016).

Durian seed flour is used as an alternative medium for the growth of *Candida albicans* and *Aspergillus niger* fungi, which are isolates from Andalas University Laboratory, Padang, West Sumatra. These fungi were grown on durian seed flour media formulated in three concentration variations, namely formulation 1 (F1) 10

grams of durian seed flour with the addition of micronutrient (zinc), formulation 2 (F2) 8 grams of durian seed flour, and formulation 3 (F3) 10 grams of durian seed flour without the addition of sugar, each dissolved in 100 mL of distilled water, then heated and stirred until homogeneous and boiling. After that, it was sterilized using an autoclave at 121°C for 15 minutes.

The results showed that *Candida albicans* and *Aspergillus niger* were able to grow on durian seed flour media. *Candida albicans* formed white to yellowish-white colonies and oval-round shape after 48 hours of incubation. The highest number of colonies was found in F1 formulation (3.6×10^7 CFU/mL), while the lowest was in F2 (2.4×10^7 CFU/mL), while in PDA comparison media, the number of colonies was 46.7×10^7 CFU/mL. This shows that the concentration of durian seed flour affects the number of colonies that grow. The number of *Candida albicans* grown on various formulations of durian seed flour media compared to PDA is detailed in Table 3, Figure 1.

Table 3. Number of *Candida albicans* colonies on durian seed flour media and PDA media.

Media	Number of fungal colonies (CFU/mL)
PDA	46.7×10^7
F1	3.6×10^7
F2	2.4×10^7
F3	3×10^7

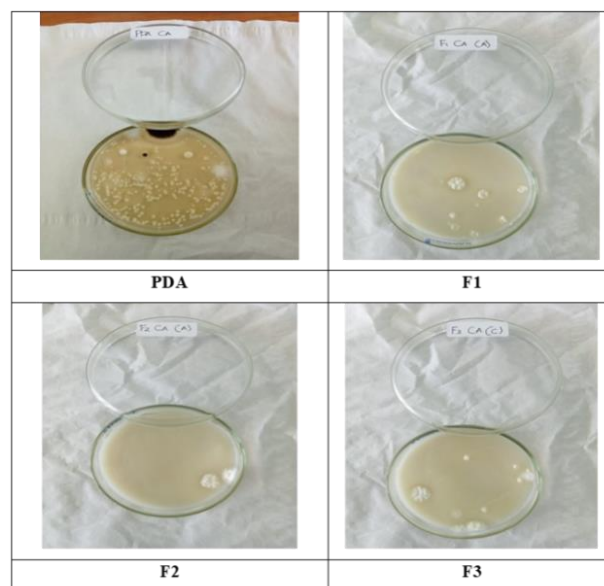


Figure 1. Colony count of *Candida albicans* on PDA media and durian seed flour media (PDA: Potato dextrose agar, F: Formula. PDA = 46.7×10^7 , F1 = 3.6×10^7 CFU / mL, F2 = 2.4×10^7 CFU / mL, and F3 = 3×10^7 CFU/mL)

Candida albicans fungal growth requires a low acid pH of 4.5-5.6. *Candida albicans* can grow well at 30-37°C. At the optimal temperature, chemical and enzymatic reactions in the cell occur faster, so the growth

rate increases faster. In durian seed flour media and PDA media, *Candida albicans* fungi have the same characteristics, the only difference is the amount of colony growth. During the incubation period of 72 hours, the *Candida albicans* fungi growing on Durian Seed Flour Media and PDA Media were yellowish white and even thin brown. This is due to the long incubation period and during this stage, the fungal colonies experience death (Basarang & Rianto, 2018).

In the research conducted by Afriani et al. (2022) on "Utilization of Durian Seed Flour (*Durio zibethinus* Murr) as a Mushroom Growth Media", the results obtained for the growth of the number of *Candida albicans* colonies were best in formulation 5 (F5), which was 4.5×10^7 CFU/mL.

Based on the comparison of several researchers, it can be concluded that durian seed flour (*Durio zibethinus* Murr) can be used as a substitute for PDA (Potato Dextrose Agar) because it is able to support the growth

of fungi that are unicellular and represented by *Candida albicans*.

Meanwhile, *Aspergillus niger* forms colonies with characteristic branched filaments, the initial color is white, which turns brownish black after the formation of conidiospores. The largest colony diameter was found on PDA media, where the sporulation grew thick and was black, while the sporulation on durian seed flour media was relatively thinner and blackish white, especially in F3, which did not contain sugar. The growth diameter of *Aspergillus niger* colonies on PDA media over different incubation periods is shown in Figure 2. Sugar is an important energy source for fungal growth (Sundari et al., 2021). Figure 4 presents the growth diameter of *Aspergillus niger* colonies on durian seed flour media formulation F2 and F3. Table 4 illustrates the colony diameters of *Aspergillus niger* grown on different durian seed flour media formulations and PDA, highlighting variations in growth.



Figure 2. Growth diameter of *Aspergillus niger* on PDA media A, B, C in storage time of 24 h, 48 h (17 mm), and 72 h (20.2 mm), respectively.

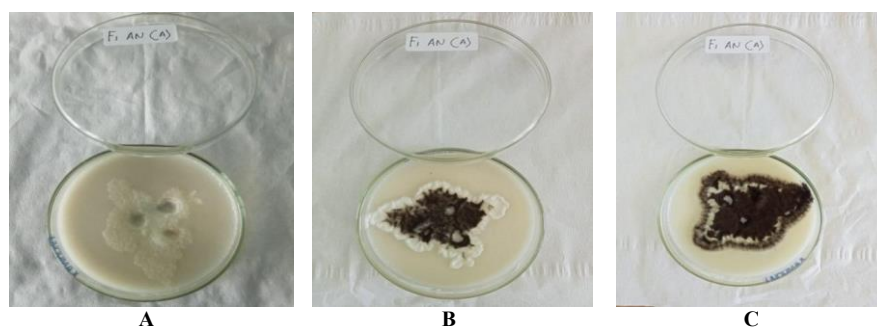


Figure 3. Growth diameter of *Aspergillus niger* on durian seed flour media F1 A, B, C in storage time of 24 h, 48 h (25.5 mm), and 72 h (28.5 mm), respectively

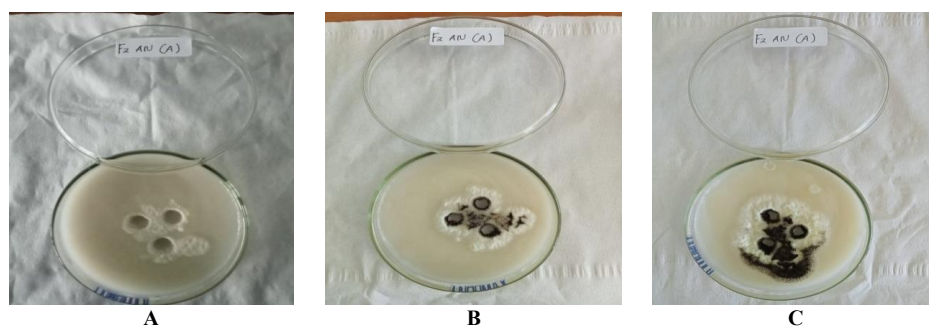


Figure 4. Growth diameter of *Aspergillus niger* on durian seed flour media F2 A, B, C in storage time of 24 h, 48 h (15 mm), and 72 h (20.5 mm), respectively.

Table 4. Diameter of *Aspergillus niger* colonies on durian seed flour media and PDA media.

No.	Media	Time	Average (mm)
1	PDA	48	17
		72	20.2
2	F1	48	25.5
		72	28.5
3	F2	48	15
		72	20.5
4	F3	48	20.2
		72	20.8

According to research conducted by Faradiana (2016), on "Utilization of Different Carbohydrate Sources (Suweg tubers and Kimpul tubers) as a Substitute for PDA (Potato Dextrose Agar) Media for Mushroom Growth" the results obtained are the growth of *Aspergillus niger* fungus has a different diameter. During the incubation period of 72 hours, the diameter of *Aspergillus niger* colonies on PDA media was 48.3 mm,

on suweg tuber media, the diameter of *Aspergillus niger* colonies was 31 mm, and on kimpul tuber media, the colony diameter was 30.6 mm.

Based on research by Indah et al. (2018) on "Taro flour as an alternative medium for the growth of *Candida albicans* and *Aspergillus sp.*" Shows the results of growth on the diameter of *Aspergillus niger* in taro flour media at a concentration of 2% by 20 mm, a concentration of 4% by 24.25 mm, a concentration of 6% by 26.50 mm, a concentration of 8% by 28.50 mm, and on PDA media was 27.75 mm. The results of research that has been done before, showing the diameter of *Aspergillus niger* colonies in durian seed flour media is less good than carbohydrate sources from tuber media and taro flour. Sporulation obtained on durian seed flour media is thinner than other media. This is due to the different nutrient content in each sample. The diameter of *Aspergillus niger* colonies on durian seed flour media formulation F3 is illustrated in Figure 5.

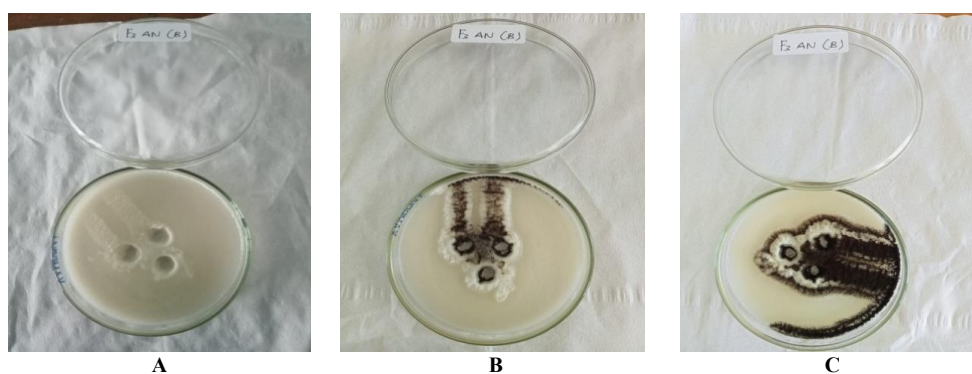


Figure 5. Growth diameter of *Aspergillus niger* on durian seed flour media F3 A, B, C in storage time of 24 h, 48 h (20.2 mm), and 72 h (20.8 mm), respectively.

Aspergillus niger requires nutrients for its growth, including carbon, nitrogen, non-metallic elements such as sulfur and phosphorus, and metallic elements such as calcium (Ca), zinc (Zn), sodium (Na), potassium (K), manganese (Mn), magnesium (Mg), iron (Fe), vitamins, and energy. Mushrooms grow optimally when nutrients support each other with appropriate growth environmental factors (Basarang & Rianto, 2018).

Fungi require organic compounds to obtain nutrients because they cannot produce food. *Aspergillus niger* fungus can grow during an incubation period of three or four days with an optimum temperature of 35-37°C (Fadhilah FR et al., 2020). According to Sundari et al. (2021), the *Aspergillus niger* fungus can grow well if there is nutrient content and qualifies as a growth medium derived from carbohydrates. Carbohydrates and their derivatives are the main substrates for carbon metabolism in fungi, as 50% of the weight of microorganisms is carbon. In addition to its ability to grow rapidly, *Aspergillus niger* is also a fungus that can produce cellulose enzymes, which are useful for

hydrolyzing cellulose into glucose for its metabolic process. During the inoculation process, *Aspergillus niger* requires enzyme-producing nutrients to optimize the enzyme production process. The production of enzymes must optimally take place (Abdillah et al., 2015).

Statistical analysis showed that the data were normally distributed based on the Shapiro-Wilk normality test with a p-value ≥ 0.05 . ANOVA test showed no significant difference between treatments, indicating that durian seed meal media performs close to that of PDA media as a control.

CONCLUSIONS

Based on the results of the research, durian seed flour (*Durio zibethinus* Murr) can be used as an alternative medium for fungal growth, both *Candida albicans* and *Aspergillus niger*. This medium has the potential to replace PDA as a fungal growth medium, with growth

characteristics supported by the content of carbohydrates and other nutrients contained in durian seed flour.

Competing Interests: The authors declare that there are no competing interests.

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