The use of coconut water (cocos nucifera l.) as alternative media to substitute Sabouraud Dextrose Agar (SDA) for the growth of aspergillus flavus

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Abstract. Media is a material composed by mixed nutrition to grow microorganisms, common media to grow fungi is Sabouraud Dextrose Agar (SDA). It contains 10 g of epton, 40 g of dextrose and 15 g of agar. This study used various concentration of coconut water (20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%). The aim of this study is to determine the concentration of coconut water that can be used to replace Sabouraud Dextrose Agar (SDA) for the growth of Aspergillus flavus and to determine the difference of colony diameter of Aspergillus flavus. Macroscopic observation of colony diameter (mm) has been done in 1 x 24 hours for 10 days using calipers with Lactophenol Cotton Blue (SDA) dyes for affirmation test. Data was analyzed statistically using One Way Anova continued by Post hoc test. It showed that the higher the concentration of coconut water, the bigger the diameter colony (sig. 0,00 < 0,05). Post hoc test showed that coconut water concentration of 20%, 30%, 40%, 50%, and 60% gave lower significant growth of the fungus than Sabouraud Dextrose Agar (SDA) media, while the concentration of 70%, 80%, and 90% gave non – significant value or similar towards control media Sabouraud Dextrose Agar (SDA). The concentration it can be concluded that coconut water can be used as an alternative media to substitute SDA (Aspergillus flavus) for the growth of Aspergillus flavus.

1. Introduction
Fungi is one of microorganisms that play some important roles in environment. They can cause diseases and contamination in many surfaces and in the other hand they are useful for human and the environment as well (Hasanah, 2017). One of them is Aspergillus flavus, a filamentous multicellular fungus. It produces aflatoxin (Amaike, 2010) that can cause cancer and liver destruction in human (Nuryati, 2017; Agnis, 2015). Aspergillus flavus is a mycotoxigenic fungus that possesses the ability to produce B aflatoxins. It can be readily distinguished morphologically by the production of a bright yellow-green conidial color, when cultured on malt extract agar or Czapek yeast extract agar (Jackson, 2016). To understand characteristics of fungi a study can be done by growing them in a growth media.
SDA (Sabouraud Dextrose Agar) is one of the media that is commonly used to grow fungi. Sabouraud Dextrose Agar or SDA was formulaed by Raymond Sabouraud in 1892. Sabouraud Dextrose Agar is used for the cultivation of fungi (yeasts, moulds), particularly useful for the fungi associated with skin infections (Aryal, 2020). It consists of peptone, dextrose and agar as an additional component (Isroi, 2009). Those media components are available also naturally. Coconut water is a natural product which is considered also as a waste product that contains energy, water, protein, fat, carbohydrate, cholesterol, vitamins, calcium and other minerals.

SDA is one kind of instant media for growing fungi. Its availability is rare in some areas and costly as well. Somehow it takes time to get the media needed for laboratory work. Using coconut water is studied to substitute the SDA media for the growth of Aspergillus flavus and to determine the optimum concentration of the coconut water in growing the fungus.

2. Methodology
This is an experimental study using coconut water in various concentrations ranging from 2% to 100% and SDA media as an experiment control. This study used post-only control group design. Every sample of experiment media uses agar and the pH is measured (between 5-6). Aryal, (2020) stated that this medium is also employed to determine microbial contamination in food, cosmetics, and clinical specimens. The pH is adjusted to approximately 5.6 in order to enhance the growth of fungi. A water-bath is used to homogenize the media and autoclave sterilization is used at 121°C for 15 minutes. 250 mg chloramphenicol antibiotic is added. The media is poured into petri-dishes and leave it to solid and cool. Aspergillus flavus is taken from a pure culture and inoculated in the middle of the media using a sterile needle. The inoculations are incubated at room temperature. The growth which is indicated by the addition of colony diameter is observed every 24 hours for ten days and measured using vernier caliper. Determination test is done by using Lactophenol Catton Blue (LPCB) staining. Data is analyzed by One Way ANOVA method.

3. Result and Discussion

|            | N  | Mean   | Std. Deviation | Minimum | Maximum |
|------------|----|--------|----------------|---------|---------|
| AK 20%     | 5  | 33.8800| 0.54955        | 33.10   | 34.60   |
| AK 30%     | 5  | 35.3200| 0.64962        | 34.60   | 36.10   |
| AK 40%     | 5  | 35.7000| 1.34350        | 34.10   | 37.80   |
| AK 50%     | 5  | 36.2800| 1.09636        | 34.90   | 37.90   |
| AK 60%     | 5  | 40.1600| 0.97877        | 39.10   | 41.30   |
| AK 70%     | 5  | 44.8200| 1.04259        | 43.60   | 46.30   |
| AK 80%     | 5  | 45.4600| 1.16103        | 43.70   | 46.50   |
| AK 90%     | 5  | 45.7800| 1.13885        | 44.10   | 46.90   |
| AK 100%    | 5  | 46.5400| 1.11937        | 45.40   | 47.90   |
| SDA        | 5  | 44.8800| 0.96021        | 43.60   | 46.10   |
| Total      | 50 | 40.8820| 5.13127        | 33.10   | 47.90   |

Note: AK = Coconut Water

Table 1 shows that there was various diameter measured at the tenth day for every treatment. The biggest diameter growth of Aspergillus flavus (46.5 mm) is found in the media with the use of 100% coconut water.
Figure 1. Growth curve of Aspergillus flavus colony diameter in various coconut water concentrations from day 1 to day 10.

Note: Hari Ke  : Day of
AK  : Coconut Water
Diameter colony : Colony Diameter

Both Table 1 and Figure 1 show that the growth of the fungi indicated by its colony diameter tends to increase as increasing time of incubation. Agustina (2016) said that coconut water contains complete nutrient composition such as carbohydrate and protein needed for the growth of fungi. This also proves that media play an important role in growing the fungi. One of nutrient needed is carbohydrate. Basalamah et al. (2018) mentioned that high carbohydrate content is needed in growing microorganisms, not only bacteria but also fungi. Optimum pH in the culture also supports the growth of the fungi as studied by Afifi et al. (2018). Growth of the fungus is largely unaffected by pH; it can grow over the entire pH range from 2.1 to 11.2, although growth rates are slower at pH<3.5, while it can grow at temperatures as low as 10–12°C and as high as 50–55°C, with optimal growth occurring at temperatures near 33°C (Jackson, 2016).

The result of the study shows that Aspergillus flavus needs 4 x 24 hours to through its lag phase growth as an adaptation stage. Aspergillus flavus needs time to breakdown complex nutrients to become simpler substances before entering its cell. At the end of measurement, colony diameter is measured 20.0 mm in control media. Coconut water concentration as alternative media that gives similar result with the control is the minimum concentration of 80% (21.04 mm). Cells in an appropriate media will form enzymes to break down the substrates at this stage (lag phase) (Gandjar, et al. 2006).

Table 2. Carbohydrate and protein content in coconut water

| No | Coconut water concentration | Carbohydrate (mL) | Protein (mL) |
|----|------------------------------|-------------------|--------------|
| 1  | 20%                          | 1.61              | 0.41         |
| 2  | 30%                          | 2.42              | 0.62         |
| 3  | 40%                          | 3.22              | 0.83         |
| 4  | 50%                          | 4.03              | 1.04         |
| 5  | 60%                          | 4.84              | 1.25         |
| 6  | 70%                          | 5.64              | 1.46         |
| 7  | 80%                          | 6.45              | 1.67         |
| 8  | 90%                          | 7.26              | 1.88         |
| 9  | 100%                         | 8.07              | 2.09         |

Table 2 shows the composition of carbohydrate and protein in each coconut water concentration. Coconut water also contains sucrose, dextrose, fructose and vitamin B complex. Protein in form of amino acids is needed for the growth of microbes, including fungi (Yulisma, 2018). There are some other substances in coconut water. Many natural fruit extracts contain phytohormone that can induce
the growth of microorganisms such as gibberellin. Coconut water also contain some phytohormone beneficial to increase the growth of microbes (Andriani, 2020).

After passing adaptation stage (lag phase), the fungi continue growing at acceleration (logarithmic) phase. The study shows that the cells start to multiply at the fifth to seventh day. Its metabolic activity increases until reaches its maximum at eighth and tenth day. At this point the colony diameter of the fungi in control reached 44.88 mm. The media of coconut water which produces similar growth with the control at this stage is the one with minimum concentration of 70%. At the exponential phase, the growth of fungi keeps increasing with green color observed. Jackson (2016) studied that it can be readily distinguished morphologically by the production of a bright yellow-green conidial color, when cultured on malt extract agar or Czapek yeast extract agar. Cell multiplication is enormous at exponential stage as a result of appropriate nutrient availability in the media and its environment including temperature and humidity. The growth of Aspergillus flavus growth is influenced by different factors (water activity, incubation temperature, protein and fat concentration, and pH) as studied by Kosegarten, et al. (2016).

As waste products, coconut water also contains some beneficial substances as that can be found in soybean waste used in making fermented soybean. Protein content in the waste products is good to be used in media to increase the growth of microbes (Anisa et al., 2018). The result of ANOVA One Way test showed that significance value of coconut water is 0.000 (p-value < 0.05). This means all alternative media concentrations have significantly affect the growth of Aspergillus flavus. Each treatment shows different significant value with the control showed by Post Hock Test. The test also shows that coconut water concentrations to grow the fungi at optimum growth are 70%, 80% and 90% while the maximum concentration is 100%.

4. Conclusion
Coconut water can be used as alternative media of SDA to grow Aspergillus flavus. The optimum concentration of coconut water for the growth of the fungi is 70%, while its maximum growth reached in the alternative media using 100% concentration of coconut water.

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