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PISANG NANGKA (*Musa paradisiaca*) AS AN ALTERNATIVE MEDIA FOR *Aspergillus niger* GROWTH

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ABSTRACT

Pisang Nangka (*Musa paradisiaca*) is one of the most popular bananas in Indonesia. Therefore, the production of waste will increase along with the use of bananas. *Pisang Nangka* peel and flesh can be used as an alternative media for mushroom growth because of its high carbohydrate content. The purpose of this study was to observe the growth of *Aspergillus niger* on alternative media made from *Pisang Nangka* with concentrations of 8%, 9%, 10%, 11% and 12% and to determine the optimum variation and concentration of alernative media. *Aspergillus niger* was grown on *Pisang Nangka* flesh media, *Pisang Nangka* peel media and mix of *Pisang Nangka* peel and flesh as well as PDA (Potato Dextrose Agar) media as a positive control using the single dot method. Colony diameter and pigmentation were observed for five days. The variation of *Pisang Nangka* mix media with a concentration of 12% is the optimum variation and concentration to grow *Aspergillus niger*.

Keywords: Alternative media, *Aspergillus niger*, *Pisang Nangka*

INTRODUCTION

Mycological study depends on the ability grow and maintain fungi in the suitable environmental condition. Media is an important component to cultivate fungi. Media must meet nutritional requirements to grow fungi. Potato Dextrose Agar is commercial media that commonly used in the lab. Unfortunately, this media is expensive and not easy to get in some area in Indonesia (Rohmi et al., 2019). Therefore, many researchs has been done to find an alternative media. These researchers mostly used tubers, bean and fruit since it had a lot of carbohydrates. Aini & Rahayu (2015) used canna tubers, yam and arrowroot as an alternative media for *Candida albicans* and *Aspergillus niger* growth. While Padhi (2022) used tomato as an alternative media for *Aspergillus niger* growth.

Indonesia is one of the largest banana producers in the world. In 2020, Indonesia produced 8.182.756 tons of banana (FAO, 2020). Nutritionally, banana is rich of carbohydrates, mineral and vitamin (Nuramalia et al., 2020). Therefore, banana is one of the potential fruit for alternative media due to its abundance and nutrition. Muthmainnah et al., (2019) has used ambon banana as media alternative for *Aspergillus niger* and Mulyawati et al., (2019) has used ambon, kepok, raja and candi banana as alternative media for *Aspergillus niger*. However, there is no studies using *Pisang Nangka* (*Musa pradisiaca*) as alternative media. Increasing production and consumption of *Pisang Nangka* in Indonesia has become potential candidate for *Pisang Nangka* as an alternative media (Nuramalia et al., 2020). Therefore, this study focused on *Pisang Nangka* as an alternative media.

Higher consumption of *Pisang Nangka* produce peel as a waste. Banana peel contains of carbohydrates that can be broken down by microorganisms (Saheed et

al., 2013). So that, peel and flesh of banana can be used as an alternative media. Nail et al., (2020) and Mulyawati et al., (2019) used banana peel broth for fungi growth. No studies used powder of peel and mix of peel and flesh powder as an alternative media. Therefore, this study used banana peel powder, banana flesh powder and mix of peel and flesh powder as an alternative media

Aspergillus niger is one of the important fungi for industry because its ability to ferment natural resources to produces citric acid (Dhunne & Apastambh, 2022). Many enzymes like amylase, protease, cellulose and lipase are produced by *Aspergillus niger* (Rasit et al., 2022). Beside its advantages, *Aspergillus niger* can be a pathogen for human. *Aspergillus niger* is widely distributed in soil and wide variety of organic matter. Inhalation of its spore can lead to sinusitis or pulmonary disease (Procop et al., 2017). Hence, this study aims to determine optimum alternative media for *Aspergillus niger* growth from peel, flesh and mix of *Pisang Nangka* peel and flesh.

METHODS

This study was an experimental study that conducted in Microbiology Laboratory, Faculty of Health Science and Technology, Jenderal Achmad Yani University on February – April 2022. *Pisang Nangka* (*Musa paradisiaca*) was obtained from Pasar Antri Cimahi. Old *Pisang Nangka* with black dot on the peel was chosen. Peel was peeled and the flesh was cut transversely. Peel and flesh were made into starch.

Flesh was steamed for 10-20 minutes and soaked in sodium metabisulfite ($Na_2S_2O_5$) for 5 minutes. Flesh was dried under sunlight for 36 hours and dried in oven for 24 hours. Dried flesh was chopped into powder with blender. Flesh powder was sieved with 100 mesh sieve and stored in container. Banana peel was washed and cut into 1x1 cm. Peel was dried under sunlight for 36 hours and dried in oven for 24 hours. Dried peel was chopped with blender and sieved with 100 mesh sieves. Peel powder was stored in closed container. Banana media was made into three categories i.e. flesh media, peel media and mix of peel and flesh media with concentration 8%, 9%, 10%, 11% and 12% (w/v). Ratio flesh and peel starch 1:1 (w/w) for mix media. Bacteriological agar was used as solidifier with concentration 1,5% (w/v). Potato Dextrose Agar (PDA) was made as control. Chloramphenicol 1% (v/v) was added to inhibit bacterial growth after sterilization on autoclave.

Aspergillus niger stock was grown in PDA. *Aspergillus niger* was transferred into banana media and PDA with single dot method. Media was incubated in room temperature for 5 days. Colony diameter and pigmentation was observed once a day. Microscopic characteristics was observed with microscope in the end of incubation. Data was analyzed using statistical method. Two-way anova was used to compare fungi growth from various concentration of banana media and control.

RESULT AND DISCUSSION

Macroscopic characteristics was observed by measuring colony diameter and pigmentation of *Aspergillus niger* in banana flesh media, banana peel media and mix media with concentration 8%, 9%, 10%, 11% and 12%. *Aspergillus niger* growth on banana flesh media, banana peel media, and mix media in various concentration was shown in table 1, table 2, and table 3 respectively. The result showed that colony diameter increased along with increasing of media concentration. All of banana media 12% showed the biggest colony diameter in the end of incubation day. The research of Mulyawati et al., (2019) showed that the biggest concentration of ambon, kepok, raja and candi banana media had the biggest growth of *Aspergillus niger*. Similarly with the research of Muthmainnah et al., (2019) *Aspergillus niger* grew optimally in the biggest concentration of ambon banana media. *Aspergillus niger* growth was very correlated with carbohydrates content on the media. *Aspergillus niger* used

carbohydrates as sole source of carbon and energy for cell growth and metabolism (Hamad et al., 2014).

Colony diameter of banana flesh media were bigger than colony diameter of banana peel media. Flesh of *Pisang Nangka* contained 84,06% carbohydrates, 3,3% protein, 0,6% fat, 2% ash and 2% fiber (Rosalina et al., 2018). While peel of *Pisang Nangka* contained 64,62% carbohydrates, 8,69% protein, 7,45% fat, and 12,5% ash (Bakar et al., 2018). Carbohydrates content of flesh was bigger than peel. Therefore, *Aspergillus niger* growth on flesh was faster than peel. *Aspergillus niger* growth in all banana media were insignificantly different (p>0,05) but the growth in mix media was faster than other media. This shown by colony diameter that bigger than other media. Although the amount of carbohydrates in the mixed media was not as much as in the fruit flesh media, other nutrients contained in the flesh would complement each other with the nutrients contained in the peel, so that mix media was the richest nutrients than other media. Therefore, *Aspergillus niger* growth is faster than growth in other media.

Colony diameter of PDA media was the smallest colony compared to all banana media colony in the end of the incubation day. *Aspergillus niger* growth in all banana media were insignificantly different with the growth in PDA (p>0,05). This indicated that all banana media could grow *Aspergillus niger* well.

Table 1. *Aspergillus niger* colony diameter of banana flesh media and PDA

Incubation time (day)	**Colony diameter (mm)**					
	8%	**9%**	**10%**	**11%**	**12%**	**PDA**
0	0	0	0	0	0	0
1	8,08	8,68	9,18	10,34	11,16	7,72
2	26,72	28,02	30,08	32,12	33,62	19,72
3	44,58	46,32	47,88	49,92	52,12	30,12
4	63,22	65,22	71,92	73,72	76,42	43,22
5	72,62	79,58	83,18	83,62	85,62	52,62

Table 2. *Aspergillus niger* colony diameter of banana peel media and PDA

Incubation time (day)	**Colony diameter (mm)**					
	8%	**9%**	**10%**	**11%**	**12%**	**PDA**
0	0	0	0	0	0	0
1	9,28	9,30	9,72	9,92	10,22	7,72
2	25,82	25,82	26,22	27,62	28,22	19,72
3	43,72	45,52	47,22	48,72	50,48	30,12
4	62,92	65,16	70,38	75,52	73,22	43,22
5	79,18	81,88	82,42	86,92	90	52,62

Table 3. *Aspergillus niger* colony diameter of mix media and PDA

Incubation time (day)	**Colony diameter (mm)**					
	8%	**9%**	**10%**	**11%**	**12%**	**PDA**
0	0	0	0	0	0	0
1	9,18	10,92	10,76	11,96	11,98	7,72
2	25,12	28,32	31,02	32,74	33,22	19,72
3	47,22	47,20	50,62	52,22	55,82	30,12
4	67,62	68,82	70,62	74,34	76,22	43,22
5	77,52	80,28	90	90	90	52,62

Aspergillus niger pigmentation in banana flesh media, banana peel media and mix media in various concentration was shown in table 4, table 5, and table 6 respectively. The result show that dark pigmentation increased along with increasing time. All concentration showed black pigmentation on banana flesh media and mix media colony in day 3 while colony on PDA still in white color. Pigmentation on peel media showed black in day 4 while colony on PDA still in brown color. Conidial pigment in *Aspergillus niger* is called aspergillin. Black pigment of *Aspergillus niger* results from the combination of brown and green pigments which absorbs light across the entire visible spectrum. This pigment is formed by hexadroxyl pentacyclic quinoid (HPQ) and melanin pigment (Jørgensen et al., 2011). Melanin pigment is synthesized via 1,8-dihydroxynaphtalene (1,8-DHN) pathway. This pathway used acetyl-CoA and malonyl-CoA to produced melanin (Chang et al., 2020). Acetyl-CoA and malonyl-CoA were produced from sugar metabolisms (Chen et al., 2018). Therefore, increasing sugar concentration in media would increase pigment production of *Aspergillus niger*.

Table 4. *Aspergillus niger* pigmentation on banana flesh media and PDA

Incubation time (day)	**Pigmentation**					
	8%	**9%**	**10%**	**11%**	**12%**	**PDA**
0	No growth	No growth	No growth	No growth	No growth	No growth
1	White	White	White	White	White	White
2	Brown	Brown	Brown	Brown	Brown	White
3	Black	Black	Black	Black	Black	White
4	Black	Black	Black	Black	Black	Brown
5	Black	Black	Black	Black	Black	Brown

Table 5. *Aspergillus niger* pigmentation on banana peel media and PDA

Incubation time (day)	**Pigmentation**					
	8%	**9%**	**10%**	**11%**	**12%**	**PDA**
0	No growth	No growth	No growth	No growth	No growth	No growth
1	White	White	White	White	White	White
2	White	White	White	White	White	White
3	Brown	Brown	Brown	Brown	Brown	White
4	Black	Black	Black	Black	Black	Brown
5	Black	Black	Black	Black	Black	Brown

Table 6. *Aspergillus niger* pigmentation on mix media and PDA

Incubation time (day)	**Pigmentation**					
	8%	**9%**	**10%**	**11%**	**12%**	**PDA**
0	No growth	No growth	No growth	No growth	No growth	No growth
1	White	White	White	White	White	White
2	Brown	Brown	Brown	Brown	Brown	White
3	Black	Black	Black	Black	Black	White
4	Black	Black	Black	Black	Black	Brown
5	Black	Black	Black	Black	Black	Brown

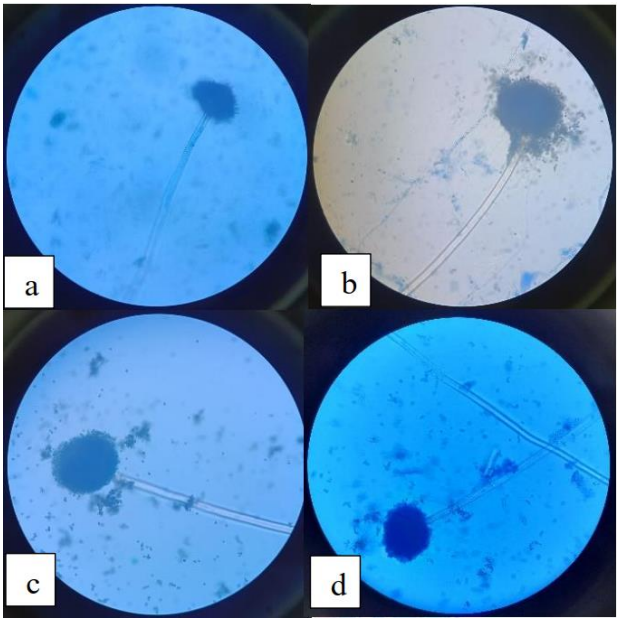

Figure 1. Microscopic examination of *Aspergillus niger* in a) banana flesh media, b) banana peel media, c) mix media, d) PDA

Microscopic characteristics of *Aspergillus niger* were very distinguishable from other type of Aspergillus especially on conidia type. Microscopic examination was found long conidiophore with dense and arose jet-black conidia around entire globose vesicle. These characteristics belonged to *Aspergillus niger* (Procop et al., 2017). These characteristics were found in all banana media and PDA (Fig. 1).

CONCLUSION

Based on the results, peel and flesh of *Pisang Nangka* could be an alternative media for *Aspergillus niger* growth. Optimum variation for *Aspergillus niger* growth was mix of *Pisang Nangka* peel and flesh with concentration 12%. Further research could analyse nutrient composition in *Pisang Nangka* media variation and this media could be applied to another fungi.

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