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Utilization of Rice Bran (*Oryza sativa L.*) Situ Bagendit Variety as an Alternative Media for Fungal Growth *Trichophyton mentagrophytes*

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ABSTRACT

Identification of fungi requires culture or propagation through a growth medium. Media commonly used is Potato Dextrose Agar (PDA) including instant media made by factories or companies in ready-to-use form, is expensive and can only be found in certain places so that an alternative medium that is easier to make and easy to obtain is rice bran media (*Oryza sativa L.*) Situ Bagendit variety. The utilization of rice bran as a growth medium for microorganisms is based on the nutritional components needed by the microorganisms. Rice bran (*Oryza sativa L.*) Situ Bagendit variety can be used as an alternative medium for the growth of *Trichophyton mentagrophytes*, the average diameter of growth of fungal colonies on rice bran media and PDA media, the effectiveness of fungal colony growth on rice bran media compared to PDA. Pre-experimental research with Static Group Comparison research design. Research subjects *Trichophyton mentagrophytes* with the research object of rice bran (*Oryza sativa L.*) Situ Bagendit variety. The results of measuring the diameter of the *Trichophyton mentagrophytes* colony on rice bran media mean 75.77 mm, the average colony diameter on PDA media is 75.52 mm. The difference in the mean colony diameter in rice bran media compared to PDA media was 0.25 mm or 0.33%. The effectiveness of growth is very effective. Rice bran (*Oryza sativa L.*) Situ Bagendit variety can be used as an alternative medium for the growth of *Trichophyton mentagrophytes* with 10% concentration.
Keywords: Effectiveness; Rice Bran; Alternative Media; Growth of *Trichophyton mentagrophytes*

1. INTRODUCTION

Indonesia has a tropical climate so it is very possible for the development of infectious diseases caused by fungi[1]. Fungi can cause infection in humans. One of the fungal infections with the highest incidence is dermatophytosis[2].

Dermatophytosis is a fungal disease of tissues that contain horn or keratin substances, such as nails, hair and the stratum corneum of the epidermis caused by dermatophyte fungi. The most important of these are the dermatophytes, a group of about 40 related fungi belonging to three genera namely Microsporum, Trichophyton and Epidermophyton[4]. One of the most infecting dermatophyte species is *Trichophyton mentagrophytes*[2]. This group of fungi can digest skin keratin because it has an attraction to keratin (keratinophilic) so that this fungal infection can attack the layers of the skin from the stratum corneum to the stratum basalis, hair, and nails.

Diagnosis of dermatophytosis is generally done clinically, can be confirmed by microscopic examination, culture, and examination with Wood's lamp in certain species. Identification of fungi required culture or propagation through growth media. The cultivation, growth and observation of fungi require different techniques from bacteria. Mushroom cultivation requires the use of selective media such as Sabouraud Agar (SA) or Potato Dextrose Agar[6].

Potato Dextrose Agar (PDA) is a common medium for fungal growth in the laboratory because it has a low pH (pH 4.5 to 5.6) which inhibits bacterial growth which

requires a neutral environment with a pH of 7.0 and an optimum temperature for growth between 25-30° C[6].

Mushrooms are often grown on Potato Dextrose Agar media which is a semi-synthetic medium. PDA media, including instant media made by factories or companies in ready-to-use form, are expensive and can only be found in certain places, which is often a problem for researchers. Instant media which is relatively expensive and the abundance of natural sources both containing carbohydrates, proteins, and fats encourage researchers to find alternative media from materials that are easily available, do not require expensive costs, and at the same time can reduce the overall costs incurred in research[7]. Natural resources are very abundant and have not been widely used, such as rice bran.

Rice bran is a fine waste obtained from the rice grain milling process8. Utilization of rice bran as a medium for the growth of microorganisms is based on the content of the nutritional components needed by microorganisms. Rice bran contains high carbohydrates, protein, fat, vitamins, and crude fiber. Rice bran (*Oryza sativa* L.) Situ Bagendit variety is rich in carbohydrates. The carbohydrate content of rice bran (*Oryza sativa* L.) Situ Bagendit variety is 58.69%. In addition to carbohydrates, rice bran (*Oryza sativa* L.) Situ Bagendit variety also contains other nutrients such as protein 10.39%, minerals 15.02%, fiber 27.55% and water 10.31 %[10].

Research conducted by Naim bran can be used as an alternative medium for the growth of Aspergillus sp with the results of Aspergillus sp growth being more fertile when compared to SDA media. In addition, Herawati et al. also succeeded in conducting research on alternative media for white rice bran (ricebran) as the growth of the fungus Candida albicans.

2. MATERIAL AND METHOD

This research is pre-experimental research with Static Group Comparison research design. The results of this observation were measurement of colony diameter, macroscopic and microscopic observations of *Trichophyton mentagrophytes* fungal colonies on rice bran (*Oryza sativa* L.) medium Situ Bagendit variety and then compared with the results of observations in the control group on PDA (Potato Dextrose Agar) media.

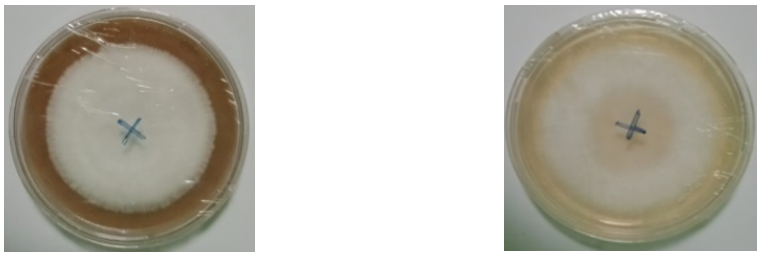
This research was conducted at the Parasitology Laboratory of the Health Analyst Department of the Health Polytechnic of the Ministry of Health, Yogyakarta. The time of the study began in February 2021-March 2021. The subject in this study was the fungus *Trichophyton mentagrophytes* ATCC 9533 purchased at the Yogyakarta Health and Calibration Laboratory and the object of this research was rice bran (*Oryza sativa* L.) Situ Bagendit variety obtained from farmers in Karangsewu Village, Galur, Kulon Progo. The quality of the bran is in accordance with predetermined criteria, namely smooth, not lumpy, cream, or brown in color and does not smell rancid. Rice bran is obtained from the milling process and sieved using a 100 mesh sieve repeatedly.

The production of rice bran (*Oryza sativa* L.) media for Situ Bagendit variety used a 10% concentration. Observation of the growth of the fungus *Trichophyton mentagrophytes* on rice bran (*Oryza sativa* L.) Situ Bagendit and PDA media was carried out on day 9, colony diameter was measured using a millimeter scale on each medium. Then it was painted with Lachtophenol Cotton Blue for microscopic observation.

Data processing was carried out using tables that were processed descriptively with pictures of microscopic observations and data on measuring the diameter of the fungus *Trichophyton mentagrophytes*. Analytical analysis was carried out by calculating the percentage and effectiveness of the growth of the *Trichophyton mentagrophytes* based on the mean colony diameter on rice bran (*Oryza sativa* L.) Situ Bagendit variety and PDA media.

3. RESULTS AND DISCUSSION

The results obtained 32 data on the growth of the fungus Trichophyton mentagrophytes, consisting of two groups, namely 16 replicates on rice bran (Oryza Sativa L.) medium Situ Bagendit variety and 16 replicates on Potato Dextrose Agar (PDA) media as a comparison. in Figure 1. Figure 1 shows that the morphological characteristics of Trichophyton mentagrophytes colonies on rice bran media (Oryza Sativa L.) Situ Bagendit variety, colonies are large, round, white, thick, the surface is like a pile of cotton, while on Potato Dextrose Agar (PDA) the colonies are large. , round, creamy white and thinner. The results of Lactophenol Cotton Blue (LPCB) staining microscopic observations of the fungus Trichophyton mentagrophytes are shown in Figure 2. Figure 2 shows that Trichophyton mentagrophytes fungal cells have septate hyphae, sometimes spiral hyphae, have microconidia that are round like grapes. Macroconidia are cylindrical, rare and few in number. On Potato Dextrose Agar (PDA) media, more microconidia were seen when compared to rice bran (Oryza Sativa L.) Situ Bagendit variety. The results of measurements and calculations of the mean colony diameter of Trichophyton mentagrophytes on rice bran (Oryza Sativa L.) medium Situ Bagendit variety and on Potato Dextrose Agar (PDA) media are shown in Table 1.



a B

Figure 1. The results of macroscopic observations of the growth of Trichophyton mentagrophytes fungal colonies. *a. Rice bran media (Oryza Sativa L.) Situ Bagendit variety b. Potato Dextrose Agar (PDA) Media*.

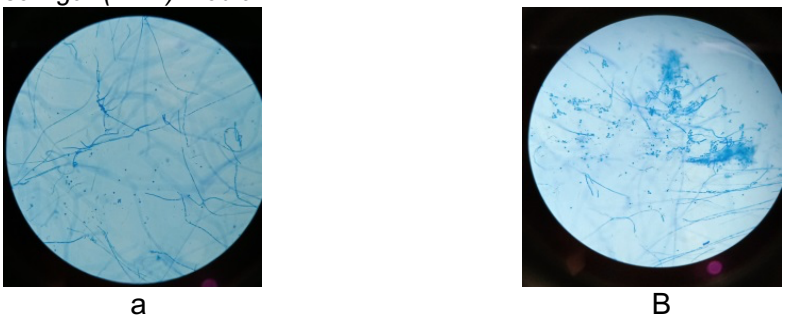


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Figure 2. The results of microscopic observations of the growth of Trichophyton mentagrophytes fungal colonies. *a. Rice bran media (Oryza Sativa L.) Situ Bagendit variety b. Potato Dextrose Agar (PDA) Media*

Table 1. Results of Measurement of Colony Diameter of *Trichophyton mentagrophytes* Fungus Colonies

Repetition	Colony diameter of Trichophyton mentagrophytes (mm)	
	Rice bran media (Oryza sativa L.) Situ Bagendit variety concentration 10%	Media *Potato Dextrose Agar*
16	75.77	75.52

Table 1 shows that the mean colony diameter of Trichophyton mentagrophytes on rice bran (Oryza Sativa L.) Situ Bagendit variety was 75.77 mm while the average diameter of Trichophyton mentagrophytes on Potato Dextrose Agar (PDA) media was 75.52 mm. The difference in the mean colony diameter on rice bran (Oryza Sativa L.) medium Situ Bagendit variety compared to Potato Dextrose Agar (PDA) media was (75.77 : 75.52) mm, 0.25 mm or 0.33%.

The average diameter of Trichophyton mentagrophytes colonies on rice bran (Oryza Sativa L.) medium Situ Bagendit variety was compared with Potato Dextrose Agar (PDA) media, the percentage of effectiveness (75.77 / 75.52) x 100% was 100.33% which is very effective.

Based on observations, it was found that the *Trichophyton mentagrophytes* fungal colony the longer the incubation time, the larger the diameter of the colony. This is in accordance with the statement that one of the growth parameters is the increase in cell volume. The increase in cell volume is irreversible, meaning that it cannot return to its original volume. Colonies are generally used as a criterion for growth, because the cell mass originates from one cell, in the form of fungal spores or conidia, into a mycelium or visible colony[11].

The macroscopic morphology of *Trichophyton mentagrophytes* colonies are white to cream in color with a surface like a pile of cotton[12]. Macroscopic observations of *Trichophyton mentagrophytes* showed differences in rice bran (*Oryza sativa* L.) Situ Bagendit variety, namely the white fungal colonies were thicker than the fungal colonies on Potato Dextrose Agar media with thinner colonies and cream pigment visible. According to the statement that colony diameter, characteristics (texture, surface and reverse coloration, zoning) and sporulation of the test fungus were strongly influenced by the type of growth medium used[13].

The nutritional content of rice bran (*Oryza sativa* L.) Situ Bagendit variety, namely carbohydrates 58.69%, protein 10.39%, minerals 15.02%, fiber 27.55% and water 10.31%[10]. The content of these nutrients can be utilized by the fungus *Trichophyton mentagrophytes* to grow and develop. This is evidenced by the presence of *Trichophyton mentagrophytes* fungal colonies growing on rice bran (*Oryza sativa* L.) Situ Bagendit variety media. Carbohydrates and their derivatives are the main substrates for carbon metabolism, fungi also have the ability to decompose proteins in their environment and use them as nitrogen and carbon sources[11].

In addition to adequate nutrients, fungal growth and development also requires appropriate environmental factors, such as the degree of environmental acidity (pH), temperature and humidity. In this study, the pH of the media used was 5.5 and incubated at room temperature ranging from 25-30oC with a humidity of 70-80%. This is in accordance with the statement that the fungal growth medium requires low acidity (pH 4.5-5.6). At the optimum temperature, chemical and enzymatic reactions in cells take place more quickly so that growth increases faster as well.

The growing colonies of *Trichophyton mentagrophytes* were observed microscopically under a microscope with a magnification of 400x. The microscopic picture of this fungus has the form of septate hyphae, sometimes spiral hyphae, has microconidia that are shaped like grapes, macroconidia are generally rare and few in number[12].

On rice bran media (*Oryza sativa* L.) Situ Bagendit variety showed fewer microconidia and no macroconidia were found when compared to Potato Dextrose Agar media so that it was not as optimal as on Potato Dextrose Agar media. However, the growth of hyphae on rice bran (*Oryza sativa* L.) media of Situ Bagendit variety was the same as that on Potato Dextrose Agar media, which had the form of septate hyphae, sometimes forming spiral hyphae. This can happen because the nutrients in rice bran (*Oryza sativa* L.) Situ Bagendit variety are more complex than Potato Dextrose Agar media. Rice bran has more complex carbon and nitrogen sources than other media. This is confirmed by the statement that the complex content in the media causes the enzymes released by the mycelium in the test mushrooms to take longer to decompose into simple components[11].

Fungi need a source of nutrients in the form of simple compounds to be easily absorbed by the mycelium for growth. Mycelium will secrete extracellular enzymes (carbohydrase and protease) into the substrate to degrade complex compounds into simple compounds[14].

Rice bran media (*Oryza sativa* L.) Situ Bagendit variety with a concentration of 10% had an average diameter of *Trichophyton mentagrophytes* fungus colonies that were larger than those on Potato Dextrose Agar media. The percentage of effectiveness obtained is 100.33% which is included in the very effective category. This research is in accordance with previous research conducted by Naim bran can be used as an alternative medium for the growth of Aspergillus sp. characterized by the formation of fungal colonies on the bran media.

Based on the results of macroscopic, microscopic observations and the calculation of the average diameter of the growing fungal colonies, it was proven that the rice bran (*Oryza sativa* L.) media of 10% concentration of Situ Bagendit variety could be used as alternative media for the growth of *Trichophyton mentagrophytes* fungi. However, in this study, the results of microscopic growth of *Trichophyton mentagrophytes* were not better than Potato Dextrose Agar media. This can happen because Potato Dextrose Agar media is one of the most commonly used culture media because it's simple formulation is the best medium because of the ability to support the growth of various fungi[15].

4. CONCLUSION

a. Rice bran media (Oryza sativa L.) Situ Bagendit variety can be used as an alternative medium for growth of the fungus Trichophyton mentagrophytes at a concentration of 10%
b. The average growth diameter of the fungus Trichophyton mentagrophytes on rice bran (Oryza sativa L.) medium Situ Bagendit variety was 75.77 mm
c. The average growth diameter of the fungus Trichophyton mentagrophytes on Potato Dextrose Agar (PDA) media is 75.52 mm
d. The effectiveness of Trichophyton mentagrophytes colony growth on rice bran (Oryza sativa L.) Situ Bagendit medium compared to Potato Dextrose Agar (PDA) media was very effective.

REFERENCE

1. Hayati I. Identifikasi Jamur *Malassezia furfur* Pada Nelayan Penderita Penyakit Kulit di RT 09 Kelurahan Malabro Kota Bengkulu. Jurnal Gradien. 2014;10(1).
2. Brutel GF, Morse JS. Mikrobiologi Kedokteran Edisi 23. Jakarta: EGC, 2008.
3. Harahap M. Ilmu Penyakit Kulit. Jakarta: Hipokrates, 2000.
4. Jawetz, Melnick, Adelberg. Mikrobiologi Kedokteran. Alih Bahasa : Brahm U Pendit. Jakarta: EGC, 2017.
5. Siregar. Penyakit Jamur Kulit. Jakarta: EGC, 2004.
6. Cappuccino JG, Sherman N. Manual Buku Laboratorium Mikrobiologi Edisi 8. Alih Bahasa : July M, Henrita V. Jakarta: EGC, 2014.
7. Aini N. Media Alternatif untuk Pertumbuhan Jamur Menggunakan Sumber Karbohidrat yang Berbeda. Surakarta: Universitas Muhammadiyah Surakarta. 2015.
8. Nursalim Y, Razali ZY. Bekatul Makanan yang Menyehatkan. Jakarta: Agro Media Pustaka, 2007.
9. Dewi C, Tjahjadi P, Artini P. Produksi Gula Reduksi oleh *Rhizopus oryzae* dari Substrat Bekatul. Jurnal Bioteknologi. 2005; 2(1).
10. Luthfianto D, Retno Dwi N, Indah K. Karakterisasi Kandungan Zat Gizi Bekatul pada Berbagai Varietas di Surakarta. The 6th University Research Colloquium. 2017.
11. Gandjar I. Mikologi Dasar dan Terapan. Jakarta: Yayasan Pustaka Obor Indonesia, 2006.
12. Setia ET. Pengaruh Penambahan Tetelan Daging Sapi dalam Media Sabouraud Dextrose Agar pada Pertumbuhan *Trichophyton Mentagrophytes*. 2015.
13. Sharma G, Pandey RR. Influence of Culture Media on Growth, Colony Character and Sporulation of Fungi Isolated From Decaying Vegetable Wastes. Jornal of Yeast and Fungal Research. 2010;1(8):157-164.
14. Chang ST, PG Miles. Mushroom: Cultivation, Nutritional Value, Medicinal Effectsand Environmental Impact 2nd Ed. CRC Press Boca Raton, 2004.
15. Saha A, Mandal P, Dasgupta S, Saha D. Influence of Culture Media and Enviromental Factors on Mycelia Growth and Sporulation of Lasiopdiploda theobromae (Pat.) Griffon and Maubl. Journal of Enviromental Biology. 2008;29(3):407-410.