Utilization of rice wastewater as an alternative medium for growth and quality of Shiitake mushrooms (*Lentinula edodes*)

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**Abstract.** Multiplication of Shiitake (*Lentinula edodes*) as a pure breeding commonly uses Potato Dextrose Agar (PDA) as the medium. Further, the cost of its basic material, which relatively expensive needs an alternative medium to produce pure brood stocks for Shiitake and to reduce the cost of producing pure brood stocks such as utilizing rice wastewater. Rice wastewater known to have composition as complete as PDA. The research aimed to find the utilization of rice wastewater as Shiitake mycelial growth medium and determine the best concentration of rice wastewater medium on the growth rate and quality of Shiitake mycelium. The research used an experimental with descriptive analysis method consisting of 11 treatments of rice wastewater concentration i.e. 50%, 55%, 60%, 65%, 70%, 80%, 85%, 90%, 95%, 100%, and PDA as the control, all treatments were replicated three times. The results showed that various concentrations of rice wastewater affected on the growth rate of Shiitake mycelium. The concentration of 100% rice wastewater produced the best growth and quality of mycelium compared to other treatments. Therefore, the rice wastewater can be used as an alternative medium to multiply pure broodstocks of Shiitake mushrooms.

1. **Introduction**

Shiitake known to be the popular edible and medicinal mushroom among other family of mushrooms. It has high protein content, antimicrobial and mineral composition, and high fibers content, and these chemical contents are available in both fresh consumption and dried processing [1–3]. Thus, human can get the benefits of its nutritional contents.

The most important step of mushroom seedling is producing pure culture. The mycelial growth needs glucose, sucrose, and dextrose as carbon sources, ammonium chloride and lignocellulosic substrate sources as the best condition medium to grow [4]. Mycelial growth using Potato Dextrose Agar (PDA) gives the optimum condition for mycelium to grow [5,6]. However, the cost of PDA basic material contents are economically high, so it needs an alternative medium to obtain pure culture of Shiitake mushrooms and reduce the cost of producing pure culture by utilizing rice wastewater.

The rice wastewater is potentially utilize as a medium of pure culture of mushrooms. Rice wastewater consist of carbohydrates, mineral, vitamin [7], which is suitable for Shiitake’s mycelial growth condition [8]. The 90% of rice wastewater is the best concentration to produce the highest growth of the white oyster mushroom [9]. This study used ten concentrations of wastewater and PDA as the control. The aim
of the research was to know the utilization of rice wastewater and the best concentration on mycelial growth of Shiitake mushroom.

2. Methods
The experiment was carried out at the Integrated Laboratory of Faculty of Science and Technology UIN Sunan Gunung Djati, Bandung on July 2017 – July 2018. The treatments arranged as concentration of rice wastewater i.e. 50%, 55%, 60%, 65%, 70%, 80%, 85%, 90%, 95%, 100% and Potato Dextrose Agar (PDA) as control. Each concentration and control replicated three times. The material used are: IR 64 variety as the rice wastewater, Granola variety as PDA medium, Alcohol 75%, aquades, agar powder, sugar, spawn of Shiitake. The PDA medium produced by boiling 200 g potatoes in 1000 ml water, and adding 20 g of glucose and 8 g of agar. The rice wastewater made by washing 1 kg of rice in 1000 ml of aquades, dividing the rice wastewater into the treatment concentrations (each concentration made into 100 ml solution), and adding 2 g of glucose and 0.8 g of agar. The isolates isolated from the brood stocks of Shiitake. The brood stock was cutted off vertically, then sliced approx. 1 cm in the middle part between the cap and the stalk/stipe of Shiitake fruiting body.

Colony diameters determined by the average of the colonies diameter (cm) on horizontal and vertical direction (using a slide caliper). The mycelial growth rate (cm/day) calculated by:

\[
 v = \frac{\text{diameters at last observation day} - \text{diameter at first observation day}}{\text{day range}}
\]  

[1]

The quality of mycelium determined by visually color change of mycelium. Analysis of all the data used descriptive analysis.

3. Results and discussion

3.1. Colony diameters
The results in table 1 showed the effect of rice wastewater concentrations to colonies diameter of Shiitake mushrooms. It showed that the highest growth rate after 4 to 20 days of incubation was 100% concentration of rice wastewater.

| Treatments | 4 DAI\(^a\) | 10 DAI | 16 DAI | 20 DAI |
|------------|-------------|--------|--------|--------|
| PDA        | 0.60        | 2.05   | 3.00   | 3.00   |
| 50%        | 0.30        | 1.83   | 2.86   | 3.00   |
| 55%        | 0.30        | 2.30   | 2.86   | 3.00   |
| 60%        | 0.93        | 4.20   | 5.60   | 6.00   |
| 65%        | 0.06        | 0.60   | 1.40   | 2.26   |
| 70%        | 0.50        | 2.56   | 3.00   | 3.00   |
| 75%        | 0.13        | 1.30   | 2.60   | 2.86   |
| 80%        | 0.00        | 0.00   | 0.00   | 0.00   |
| 85%        | 0.00        | 1.43   | 2.66   | 3.00   |
| 90%        | 0.60        | 3.93   | 4.50   | 4.83   |
| 95%        | 0.00        | 0.00   | 0.00   | 0.00   |
| 100%       | 0.96        | 3.93   | 5.66   | 6.00   |

\(^a\) DAI = Days After Inoculation
Each concentration of rice wastewater indicated the difference of concentration could affect the growth of mycelium as well as its nutritional content availability. Mycelial growth medium requires nutrition substance i.e. carbohydrates, mineral, and vitamins [10]. Hence, it could be assumed that medium containing dextrose or glucose supported mycelial growth [11]. Mycelial growth affected by carbon sources containing in glucose, molasses, and 1-5% sucrose [4]. Utilization of sorghum meal had mycelial growth rate 69.9 mm in six days after inoculation [11]. In addition, the rice wastewater added to the varies medium substrates give the optimum effect for mycelium growth [12]. Whereas, this research obtained maximum growth in twenty days after inoculation as much as 6 cm on 60% and 100% of rice wastewater medium (Table 1).

This result indicates that medium made from a substrate extraction is more effective for mycelial growth compare to rice wastewater. Rice wastewater contain only nutrition outside the rice substrate. When the rice washed physically, the carbohydrates, mineral, and vitamins remained inside the rice. The rice wastewater contains only a little amount of nutrition. Otherwise, the temperature (23°C) and 3.00 - 3.36 pH applied on the medium affects the mycelium growth of Shiitake [10].

3.2. Mycelium growth rate
According to equation, the mycelial growth rate calculated by its diameters in first day and last day then divided by the range of days [1]. The concentration of 100% rice wastewater showed the highest mycelial growth rate (Table 2). According to this result, the difference of rice wastewater concentrations might affect the colonies diameter and mycelial growth rate of Shiitake mushroom.

| Treatments | Colonies diameter (cm) | Mycelial Growth Rate (cm/day) |
|------------|------------------------|-----------------------------|
|            | 4 DAI | 20 DAI |                      |
| PDA        | 1.80  | 9.00   | 0.18                |
| 50%        | 0.90  | 9.00   | 0.20                |
| 55%        | 0.90  | 9.00   | 0.20                |
| 60%        | 2.80  | 18.00  | 0.39                |
| 65%        | 0.20  | 6.80   | 0.16                |
| 70%        | 1.60  | 9.00   | 0.18                |
| 75%        | 0.40  | 8.70   | 0.21                |
| 80%        | 0.00  | 0.00   | 0.00                |
| 85%        | 0.00  | 9.00   | 0.23                |
| 90%        | 2.00  | 14.50  | 0.32                |
| 95%        | 0.00  | 0.00   | 0.00                |
| 100%       | 2.90  | 18.00  | 0.39                |

Mycelium growth rate is depend on the type of substrate used as the medium. This research applied on in vitro method using 0.08% agar and rice wastewater medium in a petri dish. Despite the rice wastewater containing 80-90% carbohydrates, in a liquid medium the carbon absorption is slower than a solid medium [13]. In a solid medium, such as yam dextrose agar, sweet potato dextrose agar, and malt extract agar give the good mycelium growth of oyster mushroom, and at 1-3% sucrose concentration, mycelium colony diameter was achieved the maximum value [4]. Otherwise, each medium has different porosity, which affect the mycelium growth rate. The medium porosity is depend on medium characteristics, thus affects oxygen absorption by the mycelium [14].

3.3. Mycelial quality
Mycelium is the hyphae colonies that form a tissue. Mycelium is a part of mushroom body that serves to form new organ of mushroom. The quality of mycelium can be observed by its color: white color and uniform spread [15]. The result showed the maximum quality of mycelium obtained in twenty days after
inoculation. In addition, the 100% rice wastewater concentration showed the maximum quality within 16 days after inoculation. In the 16 days after inoculation, 100% concentration of rice wastewater has the white color of mycelium with uniform spread (Figure 1). Mycelium turns the color into brown, if the nutritional content in the medium decreased and low oxygen content. Mycelial growth of Shiitake requires sufficient oxygen supply [16]. The quality of mycelium obtained by purifying, as in vitro, into the new culture medium.

**Figure 1.** Mycelial quality of Shiitake in PDA and rice wastewater. a) PDA in 16 days after inoculation, b) PDA in 20 days after inoculation, c) 60% rice wastewater in 16 days after inoculation, d) 60% rice wastewater in 20 days after inoculation, e) 90% rice wastewater in 16 days after inoculation, f) 90% rice wastewater in 20 days after inoculation, g) 100% rice wastewater in 16 days after inoculation, h) 100% rice wastewater in 20 days after inoculation.

4. Conclusion
The concentration of 100% rice wastewater obtains the best growth and quality of mycelium compared to other treatments. Therefore, rice wastewater can be utilized as an alternative medium to produce pure broodstocks of Shiitake mushrooms.

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