

The Growth of Oyster Mushroom on Ramie Chip Waste-Derived Media was Accelerated by Rice-washed Water

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Abstract. Rice-washed water is wastewater resulted from the rice cleaning process that is rich in nutrients. The use of ramie chip waste can be considered a potential substrate due to its lignocellulosic nature, similar to other wastes studied. This study aimed to examine the effect of rice-washed water on the mycelium and pinhead growth of white oyster mushrooms grown in the ramie chip waste as the growing medium. The experiment was conducted for three months using a completely randomized design with four rice-washed water treatments and six replications. The rice-washed water concentration treatments were 0% (K0), 25% (K1), 50% (K2), and 100% (K3), which were added to the ramie chip waste as the growing medium for white oyster mushroom. The time of mycelium propagation velocity, the time the first pinhead appearance, the number of mushroom bodies, and the largest cap diameter were observed. The results showed that ramie chip waste-derived media was suitable to cultivate white oyster mushrooms. Adding 100% rice-washed water to the media significantly affected the speed of mycelium propagation (16.51 days) compared to control (30 days) and the appearance of the first pinhead (31.5 days) compared to control (38.5 days) as well as the number of fruiting bodies of mushroom (8.8) compared to control (6.2). However, the rice-washed water application did not significantly affect the diameter of the largest mushroom cap.

Keywords: oyster mushrooms, ramie chips, rice-washed water

Citation

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INTRODUCTION

Rice-washed water is wastewater resulting from the rice cleaning process that contains carbohydrates, protein, cellulose, hemicellulose, and vitamin B derived from pericarp and aleurone, which are eroded from the grain (Alfianti et al., 2021). Due to its content, Rice-washed water can be used as plant organic fertilizer (Akib et al., 2014; Nabayi, Sung, Zuan, & Paing, 2021). Although large amounts of rice-washed water are produced due to the high rice consumption in Indonesia (Mangku, 2017), many people are unaware of its potential use, so they dispose of it as wastewater (Nabayi, Sung, Zuan, Paing, et al., 2021).

Ramie (*Boehmeria nivea*) is a fiber-producing plant known as China grass (Liu et al., 2012). Ramie fiber is obtained through the decortication process that produces waste in the form of scraps from ramie trees that are not utilized for ramie fiber. Ramie decortication produces a considerable quantity of waste. One hectare of ramie fields can produce 27.6 tons of dry leaves and 15 tons of ramie wood chips yearly. Therefore, proper management is necessary to utilize environmentally friendly ramie waste (Shu et al., 2020). An alternative use of ramie waste is as a mushroom-growing medium because of its organic content, such as lignin, hemicellulose, and cellulose (Xie et al., 2017).

The oyster mushroom (*Pleurotus ostreatus*) is one of the most widely edible mushrooms cultivated in the world, especially in tropical and subtropical countries (Bulam et al., 2022; Deepalakshmi & Mirunalini, 2014; Ejigu et al., 2022; Sánchez, 2010; Xie et al., 2017). Oyster mushrooms contain 19-35% protein with nine types of amino acids (Egra et al., 2018). In Indonesia, people cultivate oyster mushrooms because of their efficacy, high economic value, and provide good income (Egra et al., 2019). The Central Bureau of Statistics reports that in 2017, the level of mushroom consumption in Indonesia reached 47,753 tons, while its production was only 37,020 tons. Every year, the need for oyster mushrooms

increases by 10% due to consumer demand.

Mushroom cultivation commonly uses agricultural organic waste as the growing media (Pérez-Chávez et al., 2019) such as sawdust, plant stems and twigs, straw, bagasse, coconut coir, dried banana leaves, rice-washed water, and various other organic materials (Kamthan & Tiwari, 2017). Oyster mushrooms require growing media that contains carbohydrates, protein, vitamins, carbon, cellulose nitrogen, hemicellulose, and lignin (Latif et al., 2023; Rambey et al., 2018). Ramie waste is rich in nutrients, such as protein, lignin, cellulose, and some essential elements (Suryanah et al., 2017; Susanti et al., 2015). Therefore, it can potentially be used as a novel growing medium for mushroom cultivation. Adding rice-washed water into the ramie chip waste-derived media is expected to increase the growth of white oyster mushrooms. Previous studies showed that adding rice-washed water into agarose, rice blog, and grass clippings growing media increased the mycelium length and the fruit body size of white oyster mushrooms. In this study, rice-washed water was added into the ramie chips waste derived as a major medium for growing the white oyster mushrooms as the novel waste material utilized for growing media of mushrooms.

MATERIALS AND METHODS.

Material and research design

The mycelium source of white oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm) was obtained from a mushroom cultivation center in Cianjur, West Java, Indonesia. Ramie chips were obtained from a ramie cultivation center in Wonosobo, East Java. The study used a completely randomized design consisting of four treatments of rice-washed water with six repetitions. The rice-washed water concentration (w/v) was based on the rice-to-water (R:W) ratio that was used in washing rice grain. They were 0% (K0) as control, 25% (K1), 50% (K2), and 100% (K3).

Baglog for growth media and sterilization

Baglog for mushroom growth media was made from a mixture of 80% ramie chips (w/w), 15% (w/w) rice bran, 3% (w/w) corn bran, 1.4% lime (w/w), and 0.6 gypsum % (w/w). The media was mixed until evenly distributed, and then 60% (v/w) water was added and remixed until the mixture was homogeneous. The media that had been mixed was compacted using a filler machine and put into 1 kg of polypropylene plastic each. After that, the media was sterilized by steaming in a container for 8-9 hours at a temperature of 900C to eliminate pathogenic microbes (Piryadi, 2013).

The application of rice-washed water

The rice-washed water was obtained from the first washing process of white rice. Rice washing used 1000 ml of water with pH 8 for 250, 500, and 1000 g of rice to produce the rice-washed water concentrations used in this study of 25%, 50%, and 100%. Rice was stirred 50 times until the water was thick white (homogeneous). The control (0%) was the water not used for rice washing. Rice-washed water application was carried out by injecting 100 ml of 0%, 25%, 50%, and 100% rice-washed water using a syringe into each baglog of growth media at ten different spots. Rice-washed water injection was conducted seven days after the mushroom mycelium began to grow during incubation.

Mushroom growth and parameter observation

The mycelium that thoroughly colonized the baglog was ready to initiate mushroom pin head growth. Stimulation was carried out by opening the cover of the baglog to allow the mushrooms to grow. The growth parameters measured in this study were the time

the mycelium expanded fully covered the baglog (the day after inoculating, DAI), the time of appearance of the first pinheads/pri-mordia (DAI), the number of mushroom fruit bodies, and the diameter of the mushroom cap.

Data analysis

The data of Oyster mushroom growth parameters were analyzed using one-way Anova test and was followed by Duncan Multiple Range Test (DMRT) using SPSS version 24.0. Prior to Anova test, the normality homogeneity tests were carried out.

RESULTS AND DISCUSSION.

Mycelium growth

Figure 1 shows the mycelium of an oyster white mushroom (*Pleurotus ostreatus*) that completely covered the baglog of growth media derived from ramie chip waste with application of different concentrations of rice-washed water. The application of rice-washed water accelerated the expansion of the mycelium throughout the growth medium surface. Table 1 shows the effect of Rice-washed water at various concentrations on the time required for the mycelium to expand and fully cover the growth media surface. The ramie chip waste-derived media can grow the mycelium of white oyster mushrooms, as shown in the K0 treatment. Adding rice-washed water into the ramie chip waste-derived growth media significantly accelerated the time required by mycelium to expand and fully cover the surface media compared to the control (K0). However, K1 and K2 had no significant difference with K0. It was assumed that the 25% and 50% rice-washed water concentrations provided inadequate nutrients to accelerate mycelium growth using ramie chip waste-derived media.

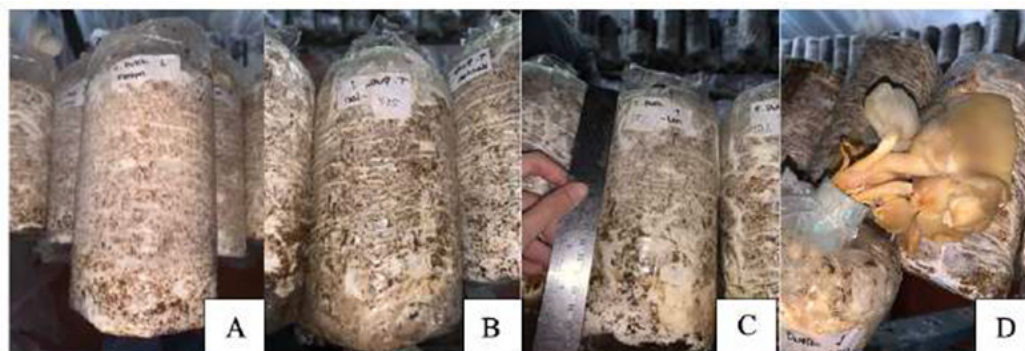


Figure 1. Mycelium of white oyster mushroom (*Pleurotus ostreatus*) expanded to fill the baglog of growth medium at 37th day after inoculation in control (A), 25% (B), 50% (C), and 100% (D) of rice-washed water

The ramie chip waste-derived growth media that was treated with 100% rice-washed water accelerated the time required for the mycelium to expand and fill the entire baglog surface compared to other treatments that contained fewer nutrients at other rice-washed water concentration treatment. Rice-washed water contains many vitamins, minerals, and elements such as N, P, K, and C (Dewi et al., 2022; Limbongan, 2023). The differential impact of 25% and 50% concentrations of rice-washed water, in comparison to the 100% concentration, on the growth of oyster *ostreatus* mycelium can be ascribed to the availability of nutrients and the physiological requisites of the mycelium. The 100% concentration of rice-washed water presents a more copious supply of essential nutrients

including nitrogen, phosphorus, potassium, and carbon, which are imperative for the rapid growth and metabolic activities of the mycelium. Diminished concentrations may fail to fulfill the optimal nutrient threshold necessary for accelerated growth, resulting in a deceleration of mycelial expansion. This physiological rationale is corroborated by the nutrient-dependent growth patterns documented in various studies pertaining to mushroom cultivation. The 100% rice-washed water concentration provides a superior nutrient density, which is critical for the metabolic processes and growth of the mycelium. Lesser concentrations may not furnish adequate nutrients to sustain optimal rates of growth (Shifriyah & Badami, 2012; Tamba et al., 2024).

Table 1. The time occupied by white oyster mushroom (*Pleurotus ostreatus*) expanded through out a baglog of growth media treated with various concentrations of rice-washed water. The different letters above bars indicate significantly different effects among treatments based on DMRT analysis, $\alpha = 0.05$.

Treatment	Rice-washed water treatment in growth media	Time of mycelium expanded fully covered the surface of growth media (day)
K0	0%	30.04 ^a
K1	25%	30.27 ^a
K2	50%	29.05 ^a
K3	100%	16.51 ^b

The ramie chip waste-derived growth media that was treated with 100% rice-washed water accelerated the time required for the mycelium to expand and fill the entire baglog surface compared to other treatments that contained fewer nutrients at other rice-washed water concentration treatment. Rice-washed water contains many vitamins, minerals, and elements such as N, P, K, and C (Dewi et al., 2022; Limbongan, 2023). The differential impact of 25% and 50% concentrations of rice-washed water, in comparison to the 100% concentration, on the growth of oyster *ostreatus* mycelium can be ascribed to the availability of nutrients and the physiological requisites of the mycelium. The 100% concentration of rice-washed water presents a more copious supply of essential nutrients including nitrogen, phosphorus, potassium, and carbon, which are imperative for the rapid growth and metabolic activities of the mycelium. Diminished concentrations may fail to fulfill the optimal nutrient threshold necessary for accelerated growth, resulting in a deceleration of mycelial expansion. This physiological rationale is corroborated by the nutrient-dependent growth patterns documented in various studies pertaining to mushroom cultivation. The 100% rice-washed water concentration provides a superior nutrient density, which is critical for the metabolic processes and growth of the mycelium. Lesser concentrations may not furnish adequate nutrients to sustain optimal rates of growth (Shifriyah & Badami, 2012; Tamba et al., 2024).

Rice-washed water encompasses essential elements such as nitrogen, phosphorus, and potassium, which are vital for cellular functions and energy production within the mycelium. The diminished availability of these elements at 25% and 50% concentrations could hinder growth (Desisa et al., 2024). The growth of mycelium is significantly contingent upon the availability of nutrients

that facilitate its metabolic activities. An elevated concentration of nutrients can adequately satisfy the heightened metabolic demands encountered during phases of rapid growth. Empirical studies have demonstrated that nutrient-enriched environments substantially augment the growth rate and yield of mushrooms, suggesting that nutrient concentration exerts a direct influence on physiological growth parameters (Sruthi et al., 2024). In this regard, the 100% concentration appears to reside within the advantageous range for the expansion of *Pleurotus ostreatus* mycelium. The treatment of 100% rice-washed water provided adequate nutrients that help the development of white oyster mushroom mycelium to expand the mycelium spread and cover the entire baglog. In contrast, the 25% and 50% concentrations treatments of rice-washed water have no effect compared to 100% concentration due to having lower content of nutrients such as NH_4^+ , NO_3^- , N, P, K, Mg and Cu dissolved. These nutrients are essential to support plant growth and development. The oyster mushroom mycelium needed nutrition, such as cellulose, hemicellulose, and protein, to support its growth and development (Ejigu et al., 2022). The mycelium of mushroom proliferation in baglog was influenced by internal factors such as genetics and external factors such as media composition and concentration (Elawati et al., 2022). Cellulose, hemicellulose, and lignin can accelerate the growth of mushroom mycelium (Rambey et al., 2020; Wu et al., 2023; Yang et al., 2013).

Pinhead/Primordia Appearance

Figure 2 shows that pinhead white oyster mushrooms (*Pleurotus ostreatus*) can grow on the media that utilize ramie chip waste with or without rice-washed water treatment as additional nutrients. Ramie chip waste was suitable as the basal growth media for white oyster mushrooms (K0). Moreover, adding

rice-washed water into the media affects the first pinhead's emergence time. The time of the first oyster mushroom primordia appearance was different between control and rice-washed water -treated media (Table 2). The application of rice-washed water into growth media that uses ramie chip waste affects the appearance

of white tyrant fungus primordia. The time required for the appearance of the first primordia of white oyster mushrooms in the control was 38.5 days, significantly different from the K3 and K4 treatments, which required a shorter time, 35.3 and 31.5 days, respectively.

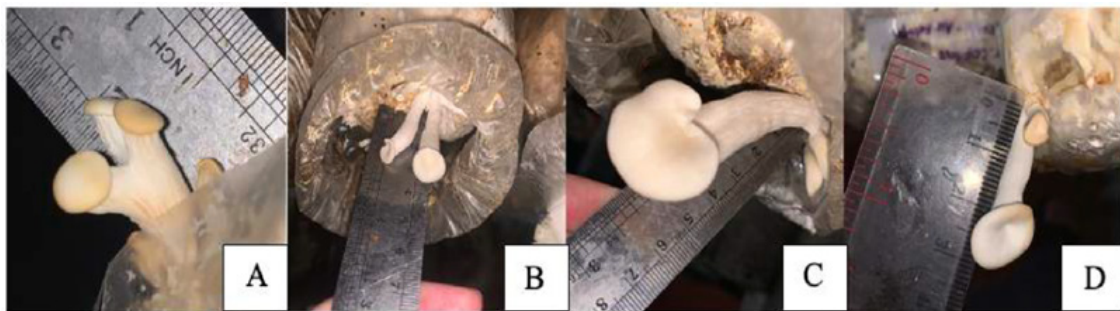


Figure 2. Pinhead white oyster mushroom (*Pleurotus ostreatus*) grown in hemp chip waste media treated with rice-washed water of 0% (A), 25% (B), (c) 50% (C), and 100% (D)

Table 2. The time appearance (days after inoculation) of the first pin head of white oyster mushroom grown in ramie chips with different rice-washed water concentrations. The different letters above bars indicate significantly different effects among treatments based on DMRT analysis, $\alpha = 0.05$.

Treatment	Rice-washed water treatment in growth media	Time of primordia appearance (days)
K0	0%	38.5 ^a
K1	25%	38.0 ^a
K2	50%	35.3 ^b
K3	100%	31.5 ^c

Different letters at the same row indicate significant difference at $P < 0.05$.

The treatments of 50% and 100% rice-washed water into the growth medium significantly accelerated the pinhead appearance compared to other treatments. The nutritional content of rice-washed water in both treatments was sufficient to stimulate the appearance of a mushroom pinhead. The formation of mushroom pinheads was correlated with the growth of mycelium covering the surface of the mushroom growth media in this study. The mushroom fruit body emerged more quickly if the maximum mycelium development was achieved throughout the growth media. The

sooner the pinhead appears, the sooner the fruit body can be obtained (Tasnin et al., 2015).

Additional nutrients are also needed to optimally meet the needs of mushroom growth, including protein, potassium, and nitrogen contained in the media (Muswati et al., 2021). Potassium content in the growth media influenced the formation of pinheads and the development of mushroom fruit bodies. Hence, the possibility of forming pinheads was better as the provision of rice-washed water increased the growth of oyster mushroom pinheads because rice-washed water was rich in nutrients,

such as nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, and vitamin B1 (Rambey et al., 2020). Lignocellulose contained in ramie chip waste was a source of nutrition for mushrooms because of its ability to degrade these compounds and make it a source of energy for mushroom growth (Kumla et al., 2020; Lestari & Priambodo, 2020).

Mushroom Fruiting Body

Figure 3 shows the fruiting bodies of white oyster mushrooms growing on ramie chip waste media with 0, 25, 50, and 100% rice-washed water treatment. Growth media derived from ramie chip waste was suitable for cultivating white oyster mushrooms. Adding rice-washed water to the growth medium did not significantly affect the total number of mushroom fruit bodies formed, except for the 100% rice-washed water concentration treatment (Table 3). In this study, the rice-washed water was submitted once at the beginning of the incubation step. No additional rice-washed water was injected into the growing media during incubation, causing the nutrients in the growing media provided by rice-washed water to be much decreased during the mycelium's growth phase. Applying rice-washed water with a concentration of less than 100% in this study did not provide sufficient additional nutrients into ramie chip media to initiate the higher number of fruit bodies formed. The treatment of 100% concentration of rice wash water offers an abundant supply of key nutrients essential for mycelial growth. Lower concentrations may not meet the necessary nutrient levels for optimal growth, hindering mycelial expansion. This is supported by research on nutrient-dependent growth in mushroom cultivation. The 100% rice-washed water concentration is crucial for mycelial metabolic processes and growth. Reduced concentrations may lack sufficient nutrients for optimal growth rates (Tamba et al., 2024).

Several essential plant nutrients have been reported contained in the rice-washed water, such as 35.4 -115 ppm of total N, 25.3 – 98.0 ppm of P, 112.9 – 153.1 ppm of K, 13.5 – 36.5 ppm of Mg, and 0.02 – 0.14 ppm of Zn. These elements easily leached out from 1.4 to 35.2% in rice washing processing (Dewi et al., 2022; Nabayi, Sung, Zuan, Paing, et al., 2021). The nutrients contained in rice-washed water were influenced by the rice-and-water ratio (R:W) and the intensity of washing (Nabayi, Sung, Zuan, & Paing, 2021). Therefore, the higher the ratio of R:W or the higher the concentration of rice-washed water, the higher the nutrient content dissolved. In this study, the 100% concentration of rice washing water (1000 g of rice-washed with 1000 ml of water) contained more nutrients than other treatments and was sufficient to support significantly more fruit bodies of oyster mushroom formed. After the incubation process, the mushroom-growing media lost nutrients, so it is necessary to add nutrients for the growth of the fruit bodies so that the nutrient content was maintained in the media (Bellettini et al., 2019; Girmay et al., 2016).

Nutritional requirements for mushroom growth include cellulose, hemicellulose, lignin, calcium, and nitrogen. The high C/N ratio allows nutrients to form larger fruit bodies. Insufficient N in the media caused the edible mushroom fruit bodies small (Cesur et al., 2022; Muswati et al., 2021; Sakamoto, 2018). According to Nabayi, Sung, Zuan, Paing, et al. (2021), the higher concentration of rice-washed water, higher content of N dissolved. This study suggests that higher concentrations of rice-washed water result in greater N content due to the easy dissolution of plant-available nitrogen compounds, leading to a lower C/N ratio at 100% concentration compared to lower concentrations. The carbon-to-nitrogen (C/N) ratio is a pivotal factor in mushroom cultivation, influencing the size and yield of

fruiting bodies. A balanced C/N ratio ensures optimal nutrient availability, promoting larger fruit bodies. Different mushroom species require specific C/N ratios for optimal growth, such as in *Lentinus* species, the mycelial growth was optimal at C/N ratios of 10:1 and 40:1 (Dulay et al., 2020), while, in *Pleurotus pulmonarius* showed the highest yield reached

at a C/N ratio of 120:1 (Osunde et al., 2019). A higher C/N ratio is beneficial for superior weight, stipe length, and pileus diameter compared. Therefore, the C/N ratio content for each treatment in this study is needed to further investigation to reveal the optimum ratio in the growth media for *Pleurotus ostreatus*.

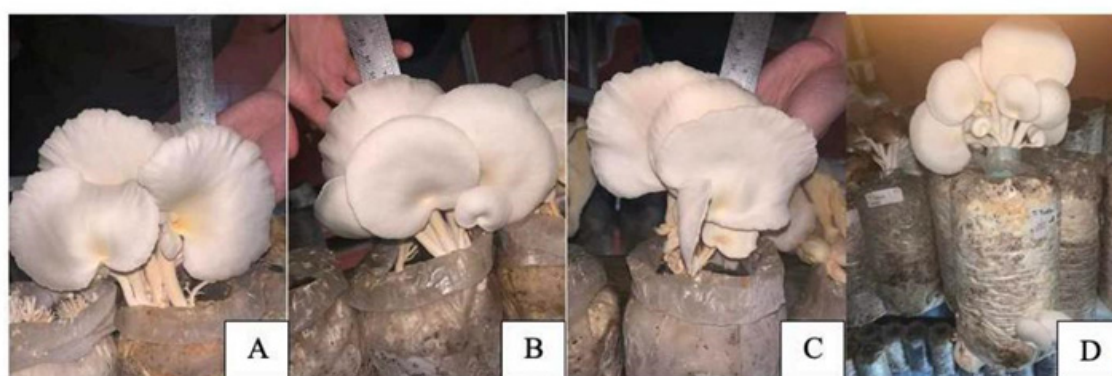


Figure 3. The fruit bodies of white oyster mushroom (*Pleurotus ostreatus*) were grown in the ramie chips waste with the addition of rice-washed water of 0% (A), 25% (B), 50% (C), and 100% (D).

The fruiting bodies of the white oyster mushrooms produced in this study had an excellent appearance. The high content of complex carbohydrates in the growth media as a carbon source influenced pinhead growth optimally, forming relatively more fruit bodies (Wahidah & Saputra, 2015). A large num-

ber of fruit bodies formed were caused by a large number of growing pinheads (Hendri, 2015) that correlated to the nutrients contained in the growth media distributed to each pinhead that formed the fruit body of a mature mushroom (Muswati et al., 2021).

Table 3. The average number of white oyster mushroom fruit bodies in the ramie chip waste-derived media with the addition of various rice-washed water concentrations

Treatment	Rice-washed water treatment in growth media	Total number of mushroom fruit bodies
K0	0%	6.17 ^a
K1	25%	7.50 ^a
K2	50%	6.75 ^a
K3	100%	8.83 ^b

Different letters at the same row indicate significant difference at $P < 0.05$.

The Mushroom Cap Size

Figure 4 shows white oyster mushrooms with caps grown on media derived from ramie chip waste with different concentrations of rice-washed water. Ramie chip waste was suitable as a primary medium for oyster mushroom cultivation, as shown by the growth of mushrooms, including cap width ranging from 8.18 to 10.62 cm. This result was comparable to the size of oyster mushroom cups using primary media derived from corncob and rice, empty palm fruit bunch waste (Apri-

yani et al., 2019; Elawati et al., 2022; Yang et al., 2013) and was more extensive than using sawdust, twigs (Herliyana, 2023). The ANOVA test showed that adding rice-washed water to the ramie chip waste-derived growth medium did not significantly affect the diameter of the mushroom cap (Table 3). The result indicated that the nutrients supplied by one application of rice-washed water could not significantly increase the diameter of the cap on growing media derived from raie chip waste.

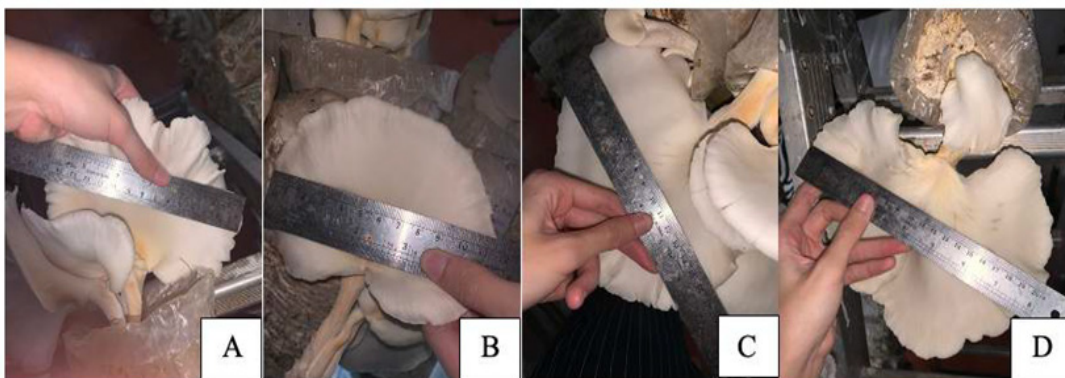


Figure 4. White oyster mushroom cap (*Pleurotus ostreatus*) grown on media derived from hemp chip waste with the addition of 0% (A), 25% (B), 50% (C), and 100% (D) rice-washed water

Ramie contains 16.35-29% of crude protein, 6.36-8.56% of crude oil, 13.61-29% crude fiber, and 4.00-8.50% lignin (Despal et al., 2011; Suryanah et al., 2017). The nutrients in the ramie chip waste-derived growth media were able to meet the needs of nutrients, especially nitrogen as a source of protein needed

to compose actively growing tissues so that it can support the maximum development of the mushroom fruit bodies and enlargement of the mushroom cap diameter (Laksono, 2019). Therefore, adding rice-washed water to the growth media did not significantly affect the size of the oyster mushroom cap.

Table 4. The largest diameter of the cap of white oyster mushroom grown in the ramie chip waste-derived media with the addition of various rice-washed water concentrations

Treatment	Rice-washed water treatment in growth media	Width of the mushroom cap (cm)
K0	0%	9.78 ^a
K1	25%	8.18 ^a
K2	50%	9.83 ^a
K3	100%	10.62 ^a

Different letters at the same row indicate significant difference at $P < 0.05$.

The diameter of the mushroom cap was affected by temperature, humidity, and nutrient content in the media. The optimum temperature for the growth of white oyster mushrooms is between 22 – 28 0C with a humidity of 80 – 90%. Inappropriate environmental factors, such as temperature and humidity, will inhibit the growth and formation of the mushroom cap optimally (Sakamoto, 2018). The growth room condition in this study possessed a temperature of 23.5 - 28.5 0C and a humidity of 75 - 90%. These conditions were suitable for mushroom development so that the cap could grow normally. In this study, mushroom caps with larger diameters were observed in the media that contained fewer mushroom bodies. This result was in accordance with (Muswati et al., 2021; Tasnin et al., 2015) that the larger the mushroom cap size, the fewer mushroom bodies are formed.

CONCLUSION

The use of rice-washed water in ramie chip waste media significantly influenced the growth parameters of white oyster mushrooms. Mycelium colonization and pinhead development times were notably reduced in rice-washed water-treated media. The number of pinheads increased significantly in the rice-washed water media. However, there was no substantial improvement in the diameter of the largest mushroom cap. A 100% concentration of rice-washed water produced optimal results for mycelium growth, primordia emergence timing, total mushroom yield, and the diameter of the largest cap.

AUTHOR CONTRIBUTION

R.B., R.H, and J.K. designed the research and supervised all processes, H.A.W. conducted the experiment and collected data, R.H. analyzed the data and wrote the manu-

script. M.N., T.S. and A.Z.M. helped analyze the data.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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