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OPTIMIZATION OF WHITE OYSTER MUSHROOM (*Pleurotus ostreatus*) GROWTH USING VARIOUS MEDIA ENRICHED WITH MILLET FLOUR

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ABSTRACT

This study aims to evaluate the best growing medium for the mycelium growth of F1 oyster mushroom (Pleurotus ostreatus) seeds using millet flour as a nutritional supplement. A Completely Randomized Design (CRD) was applied with two factors: the type of growing medium (corn, sawdust, rice husks) and the concentration of millet flour (0%, 25%, 50%, 75%). Observed variables included mycelium growth (cm), mycelium density, and daily growth on the 7th, 14th, and 21st days after inoculation. The results indicated that corn without millet flour (J1P0) produced the best mycelium growth, while adding 25% millet flour to corn (J1P1) and sawdust (J2P1) enhanced mycelium density. However, higher millet concentrations (50% and 75%) tended to reduce growth efficiency due to excessive moisture content. This study recommends using millet flour at low concentrations to improve the efficiency of sustainable F1 oyster mushroom seed production.

Kata kunci:
jagung, jamur tiram putih, media tanam, sekam padi, serbuk gergaji, serbuk jewawut.

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi media tanam terbaik bagi pertumbuhan miselium jamur tiram putih (*Pleurotus ostreatus*) bibit F1 dengan tambahan serbuk jewawut sebagai suplemen nutrisi. Rancangan Acak Lengkap (RAL) digunakan dengan dua faktor, yaitu jenis media tanam (jagung, serbuk gergaji, sekam padi) dan konsentrasi serbuk jewawut (0%, 25%, 50%, 75%). Variabel yang diamati meliputi pertumbuhan miselium (cm), kerapatan miselium, dan pertumbuhan harian pada hari ke-7, 14, dan 21 setelah inokulasi. Hasil analisis menunjukkan media jagung tanpa jewawut (J1P0) memberikan hasil pertumbuhan miselium terbaik, sedangkan penambahan jewawut 25% pada jagung (J1P1) dan serbuk gergaji (J2P1) meningkatkan kerapatan miselium. Namun, konsentrasi jewawut yang tinggi (50% dan 75%) cenderung menurunkan efisiensi pertumbuhan akibat kadar air berlebih. Penelitian ini merekomendasikan penggunaan jewawut pada konsentrasi rendah untuk meningkatkan efisiensi produksi bibit F1 jamur tiram secara berkelanjutan.

INTRODUCTION

The white oyster mushroom (*Pleurotus ostreatus*) is a high-value biological food source widely consumed due to its nutritional content. With a nutritional profile that includes 16 grams of protein, 0.9 grams of fat, 64.6 milligrams of carbohydrates, as well as essential vitamins and minerals, this mushroom has become a favorite among consumers (Kumar, 2020; Kaisun et al., 2022). The global demand for white oyster mushrooms continues to rise, making it the second most cultivated mushroom after the button mushroom (*Agaricus bisporus*). In Indonesia, mushroom production has increased significantly from 1,796 tons in 2014 to 3,442 tons in 2018, highlighting a substantial market opportunity (Rizki et al., 2022).

The increased production of this mushroom directly impacts the demand for high-quality F1 spawn, which is typically developed using grain-based media such as corn. This media is chosen due to its nutritional content, including carbohydrates, protein, and fiber, which support optimal mycelium growth (Sandeep et al., 2022; Istifanus et al., 2018). However, the limited availability of raw materials and the growing need for cost efficiency have driven the search for alternative media. Sawdust and rice husks, which are abundant organic waste materials in Indonesia (Aprianto et al., 2018), have become potential candidates to be combined with nutrient-rich materials such as millet flour (*Setaria italica*) (Nasution et al., 2022).

Millet, one of the cereal crops, contains up to 84.2% carbohydrates and 10.7% protein, making it an excellent nutritional source to support mycelium growth (Pasally et al., 2022). However, the utilization of millet in Indonesia remains limited, with most of it being used as bird feed (Kusumadewi & Ridwan, 2018). By utilizing millet as a nutritional supplement in cultivation media, mycelium growth is expected to accelerate, thereby supporting the efficient and high-quality production of F1 spawn.

The main challenge in F1 spawn production lies in the suboptimal formulation of cultivation media, which can slow down mycelium growth and reduce production stability (Sunandar et al., 2018). Therefore, it is essential to develop innovations such as the use of millet flour as an additive in corn-based media, sawdust, and rice husks. This study aims to evaluate the combination of millet flour with various types of media and its impact on the mycelium growth of white oyster mushrooms.

This study introduces a novel approach by exploring the potential of millet as a nutritional supplement in cultivation media. There is limited research on this topic, making this approach a potential innovation in developing cultivation media that not only supports optimal mycelium growth but also promotes sustainable organic waste management and strengthens sustainable agricultural systems.

RESEARCH METHODOLOGY

Time and Place of Research

The research was conducted from June to July 2024 at the Integrated Laboratory of the Faculty of Agriculture, Animal Husbandry, and Fisheries, Universitas Muhammadiyah Parepare. This location was chosen due to its comprehensive facilities, which support media sterilization and the observation of mycelium growth.

Research Tools and Materials

This study utilized primary tools such as a laminar airflow cabinet to maintain sterility during inoculation, an autoclave for media sterilization, and an analytical balance to ensure precise measurement of materials. Additional tools included a spatula, ruler, and sprayer. The materials used were F1 spawn of white oyster mushrooms (*Pleurotus ostreatus*), yellow corn kernels, sawdust, rice husks, millet flour, water, 70% alcohol, media bottles, aluminum foil, and food-grade plastic covers.

Research Design

The study used a Completely Randomized Design (CRD) with two factors: the composition of millet flour and the type of media. The media type factor (J) included J1 (corn), J2 (sawdust), and J3 (rice husks). The millet flour composition factor (P) consisted of P0 (0%), P1 (25%), P2 (50%), and P3 (75%). Each treatment combination had three replications, resulting in a total of 36 experimental units. The observed parameters included mycelium growth (cm), measured on the 7th, 14th, and 21st days after inoculation, and mycelium density based on visual assessment on the same days.

Research Procedure

Media Preparation

The media used in this study included corn, sawdust, and rice husks. These three types of media were soaked in water for 24 hours to remove impurities and ensure cleanliness before use. The corn media was boiled for 15–20 minutes until the outer layer softened, then drained and left to dry until it reached the optimal moisture content. Meanwhile, the millet flour was dried beforehand to ensure the appropriate moisture content before being mixed with the other media.

Media Sterilization

After mixing the media according to the predetermined treatments, the media were sterilized using an autoclave at a temperature of 121°C for 30 minutes. Additionally, all tools used for inoculation, such as spatulas and media bottles, were also sterilized to prevent contamination.

Inoculation Process

F1 spawn of white oyster mushrooms was inoculated into the media aseptically inside a laminar airflow cabinet to maintain cleanliness during the inoculation process. After inoculation, the media bottles were tightly sealed using aluminum foil and plastic wrap to preserve sterility.

Incubation Process

The inoculated media were stored in an incubator at a controlled temperature of 23–28°C. This temperature was maintained throughout the incubation period to ensure optimal conditions for mycelium growth.

Data Analysis and Interpretation

The research data included measurements of mycelium growth in length (cm) and visual assessments of mycelium density on the 7th, 14th, and 21st days after inoculation. The data were analyzed using Analysis of Variance (ANOVA) at a 5% confidence level. To determine significant differences between treatments, a post hoc Least Significant Difference (LSD) test was conducted. The results of this analysis were used to identify the most effective combination of media and millet flour concentration in supporting the growth of white oyster mushroom mycelium.

RESULTS AND DISCUSSION

Mycelium Growth Performance

Figure 2 shows the mycelium growth performance of white oyster mushrooms (*Pleurotus ostreatus*) on three types of media: corn, sawdust, and rice husks, with the addition of various concentrations of millet flour. Each medium was combined with different millet flour concentrations (0%, 25%, 50%, and 75%).

Corn + Millet Flour Sawdust + Millet Flour Rice Husk + Millet Flour

Figure 1. Mycelium growth of white oyster mushrooms on various media with the addition of millet flour.

Corn-based media without the addition of millet flour (J1P0) demonstrated the best mycelium growth, achieving 100% performance. This is attributed to the nutritional content of corn, particularly its carbohydrates and proteins, which optimally support mycelium growth. The addition of millet flour up to 25% (J1P1) still resulted in good mycelium growth, although it was not superior to the control (J1P0). A decline in performance was observed at higher millet flour concentrations (50% and 75%), likely due to changes in media structure that affected aeration and nutrient distribution.

Sawdust-based media without millet flour (J2P0) also achieved 100% performance. In the combination of sawdust with 25% millet flour (J2P1), mycelium growth remained favorable. However, increasing the millet flour concentration up to 75% (J2P3) resulted in slower mycelium growth. This may be due to excessive moisture content or uneven distribution of additional nutrients from the millet flour. Contamination also contributed to the decreased performance of media with higher millet flour concentrations.

Rice husk-based media without millet flour (J3P0) exhibited good mycelium growth performance with a value of 100%. However, the addition of millet flour at concentrations of 50% and 75% (J3P2 and J3P3) led to a decline in performance, with uneven mycelium growth observed. This decline was likely caused by excessive moisture content in the media, which hindered nutrient absorption and the metabolic activity of the mycelium.

Good mycelium growth in several treatments (J1P0, J2P0, J2P1, and J3P0) was associated with sterile media conditions and sufficient nutrient content. Conversely, poor growth at higher millet flour concentrations (50% and 75%) could be attributed to excessive moisture content, uneven nutrient distribution, and potential contamination. A study by Laila and Wardani (2020) supports these findings, stating that media contamination can slow mycelium growth. Additionally, optimal incubation temperatures (24–28°C) and humidity levels of approximately 60–70% were also crucial factors in supporting mycelium development (Wibowo & Damanhuri, 2019; Zhan et al., 2021).

Mycelium Density

Observations on the density of white oyster mushroom mycelium with the addition of millet flour as a nutritional supplement in the cultivation media were conducted on the 7th, 14th, and 21st days after inoculation. Based on the data in Table 1, the results show variations in mycelium density across different types of media and millet flour concentrations.

Table 1. Density of white oyster mushroom mycelium on various media and compositions at 7, 14, and 21 days after inoculation (DAI).

Treatment		Days After Inoculation (DAI)		
Media Type	Composition	7	14	21
J1	P0	+ + + + +	+ + + + +	+ + + + +
	P1	+ + + + +	+ + + + +	+ + + + +
	P2	+ + +	+ + + + +	+ + + + +
	P3	+ + + + +	+ + + + +	+ + + + +
J2	P0	+ +	+ + + +	+ +
	P1	+ + +	+ + + +	+ + + +
	P2	+ + +	+ + + +	+ + + + +
	P3	+ + +	+ + +	+ + + + +
J3	P0	+ +	+ +	+ +
	P1	+ + + + +	+ + + + +	+ + + + +
	P2	+ + +	+ + + +	+ + + + +
	P3	+ + +	+ + + +	+ + + + +

Corn-based media without millet flour (J1P0) and the combination of corn + 25% millet flour (J1P1) showed the best mycelium density results across all observation days (7 DAI, 14 DAI, and 21 DAI), with dense and thick mycelium growth (+++++). These media provided optimal results due to the high carbohydrate content in corn, which supplied sufficient nutrients for mycelium metabolic activity. Treatments with higher millet flour concentrations (50% and 75%) still exhibited good density but were not superior to J1P0 and J1P1.

Sawdust-based media without millet flour (J2P0) exhibited less uniform mycelium growth (++ on 7 DAI and 21 DAI) compared to the treatment of sawdust + 25% millet flour (J2P1). In the J2P1 combination, mycelium density improved, reaching a good level on 14 DAI and 21 DAI (++++). However, treatments with higher millet flour concentrations (50% and 75%) showed less uniform growth, particularly on 7 DAI (+++), before eventually achieving optimal density on 21 DAI (+++++).

Rice husk-based media without millet flour (J3P0) showed relatively low mycelium density across all observation days (++ on 7 DAI, 14 DAI, and 21 DAI). However, the combination of rice husks + 25% millet flour (J3P1) resulted in the best mycelium density across all observation periods (+++++). Adding millet flour at higher concentrations (50% and 75%) produced good density, but the growth was not superior to J3P1.

The combination of corn-based media + 25% millet flour (J1P1) showed the best mycelium density due to the carbohydrate and protein content in corn, which supports mycelium development. The addition of millet flour provided supplemental nutrients without significantly altering the physical characteristics of the media.

Sawdust has lower nutritional content compared to corn, making the addition of millet flour necessary to enhance the quality of the media. A study by Hamzah et al. (2022) indicated that corn-based media provided the best results for F1 mycelium growth. The addition of 25% millet flour (J2P1) improved mycelium density, reaching optimal conditions by 21 DAI. However, higher concentrations of millet flour (50% and 75%) resulted in uneven nutrient distribution and excessive moisture content, which hindered initial mycelium growth.

Rice husks, with their limited nutritional content, require the addition of millet flour to support mycelium growth. The results showed that the combination of rice husks + 25% millet flour (J3P1) produced the best density across all observation periods. Higher millet flour concentrations provided good results, but the physical properties of the media at higher concentrations could affect the balance of aeration and moisture content, resulting in less optimal growth compared to J3P1. Rice husks contain lignin and cellulose, which are beneficial for supporting mycelium growth (Hendri, 2018).

Mycelium Growth

The analysis of variance results showed that the type of media had a significant effect on the growth of white oyster mushroom mycelium in the third week, while the composition of millet flour and the interaction between media and composition had a highly significant effect. The mycelium growth results across different media and millet flour compositions are summarized in Table 2.

Table 2. Mycelium growth of white oyster mushrooms on various cultivation media and different compositions (cm) in the third week.

Media Type	Millet Flour Composition (%)				Mean
	P0 (0)	P1 (25)	P2 (50)	P3 (75)	
J1	8,67 (c)	4,50 (ab)	3,33 (a)	2,50 (a)	4,75 (A)
J2	6,17 (b)	2,83 (a)	3,50 (a)	4,00 (ab)	4,13 (A)
J3	12,00 (d)	4,17 (ab)	2,50 (a)	2,83 (a)	5,38 (A)
Mean	8,95 (B)	3,83 (A)	3,11 (A)	3,11 (A)	
LSD 0.01	2,3903				
LSD 0.05	1,7751				

The best medium was rice husks (J3), with the highest average mycelium growth of 5.38 cm, followed by corn (J1) with an average of 4.75 cm, and sawdust (J2) with 4.13 cm. Based on the LSD values, these three media fall into the same category (code A), indicating that although there are differences in the mean values, these media produced relatively comparable results.

The composition of millet flour had a significant effect on mycelium growth. The composition without millet flour (P0) showed the best results across all media, particularly on rice husk media (J3P0), with growth reaching 12.00 cm. Conversely, the lowest mycelium growth was observed in the 75% millet flour composition on corn media (J1P3) and the 50% millet flour composition on rice husk media (J3P2), both reaching only 2.50 cm.

The best interaction was found in the combination of rice husk media without millet flour (J3P0), with an average growth of 12.00 cm. In contrast, the lowest interaction occurred in the combinations of 50% millet flour on rice husk media (J3P2) and 75% millet flour on corn media (J1P3), each with a growth of 2.50 cm. The main factor contributing to the low mycelium growth at high millet flour concentrations was the moisture content in the media. According to the study by Suparti and Zubaidah (2018), the optimal moisture content for mycelium growth ranges between 70% and 75%. Excessive moisture content in the 50% and 75% millet flour compositions may hinder nutrient absorption by the mycelium, as supported by the findings of Arafat and Alamsyah (2018), which emphasized the importance of proper moisture levels.

CONCLUSION

Corn-based media without the addition of millet flour (J1P0) provided the best results for mycelium growth, followed by sawdust media with 25% millet flour (J2P1). Rice husk media (J3) without millet flour (P0) also demonstrated optimal results, with mycelium growth reaching 12.00 cm in the third week. The addition of millet flour at low concentrations (25%) significantly improved mycelium density in several media; however, higher concentrations (50% and 75%) tended to reduce growth due to excessive moisture content.

In general, the composition without millet flour (P0) showed the best growth results across all media, particularly in supporting optimal mycelium density and growth. Adjusting moisture content and ensuring proper nutrient distribution are critical factors in developing media with high millet flour concentrations to support optimal mycelium growth.

The use of media with the addition of millet flour should be limited to low concentrations (25%) to maintain a balance of moisture content and nutrients, with media formulations adjusted to prevent excessive moisture, especially at higher millet flour concentrations (50% and 75%). Additionally, regular monitoring of moisture content is necessary to ensure the media remains within the optimal range (70%–75%), as recommended for the growth of white oyster mushroom mycelium.

The sterilization procedures for media and tools must be carefully maintained to prevent contamination, especially in media with high nutrient content. Further research is needed to test variations in the addition of other organic materials or combinations of millet flour with other substances that can enhance nutrient availability without disrupting the physical structure of the media. Additionally, field-scale trials are necessary to evaluate the effectiveness of media formulations under real-world conditions.

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