Oil palm empty bunches as a growing media for white oyster mushroom (*Pleurotus ostreatus*)

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Abstract. The media used to grow oyster mushrooms is sawdust waste, such as sengon, gmelina, and candlenut. Therefore, several researchers conducted research to find alternative media to replace sawdust, such as mulberry pulp, sago pulp, coconut fibre, and bamboo pulp. This research utilizes waste of oil palm empty bunches as a medium used to grow oyster mushrooms. In addition, this study aims to determine the duration of immersion of oil palm empty bunches as a pre-treatment before being used as a growing medium for white oyster mushroom (*Pleurotus ostreatus*). Before being used as a medium, empty fruit bunches of oil palm are cut into small pieces and then made into powder. The stages in this research are mixing raw materials, composting, making baglog, sterilization, inoculation, incubation, maintenance, and harvesting. There were seven treatments applied in this study, namely immersion in cold water for five days (P1), for seven days (P2), and nine days (P3); immersion in 100 °C hot water for 1 hour (P4), for 2 hours (P5), and 3 hours (P6); without immersion or control (P7). The results obtained are empty oil palm fruit bunches with cold soaking for five days (P1) and hot soaking at 100 °C for two hours can be used as a medium for oyster mushroom cultivation.

1. Introduction

Oyster mushroom (*Pleurotus ostreatus*) is a type of wood fungus that grows on dead wood or plants. Generally, fungi that grow on woody stems are saprophytic. The community now widely cultivated Oyster mushrooms, either by using logs, rice husks, or sawdust. However, logs have begun to be abandoned because of the difficulty of obtaining raw materials and the relatively high price. Farmers are now starting to switch to using alternative raw materials by utilizing sawdust waste as a planting medium. Oyster mushrooms are relatively easy to cultivate as long as the growing medium has elements such as cellulose, glucose, and lignin that the fungus needs for its survival.

The community widely cultivates oyster mushroom cultivation, and it is necessary to think about other alternatives that can be used as growing media for oyster mushrooms. One of the ingredients used is oil palm empty bunches because it contains the elements needed to grow oyster mushrooms. Oil palm empty bunches are lignocellulosic materials that can replace sawdust as a growing medium for white oyster mushrooms. However, oil palm empty bunches have not been widely used and have become a solid waste for the palm oil industry. The increasing production of palm oil has increased the amount of waste produced. Therefore, it is necessary to find a good use for the waste generated. Crude palm oil production in Indonesia in 2020 is around 52 million tons [1]. Simultaneously, oil palm empty fruit bunches will be produced. This study aims to determine the best initial treatment in the
form of soaking oil palm empty bunches to be used as a medium for growing white oyster mushroom (*Pleurotus ostreatus*). 

2. Methods
The method used in this research is pre-treatment of oil palm empty bunches; mixing of raw materials; composting; making baglog; sterilization; inoculation; incubation; maintenance to harvest. First, oil palm empty bunches are chopped into small pieces. Next, this chop is dried by drying. The chopped oil palm empty bunches that have been dried are then ground using a hammer mill until they become powder. Furthermore, the oil palm empty bunches were sieved using 20 mesh and 40 mesh sieve machines to obtain a homogeneous powder size.

Oil palm empty bunches were then given different treatments before being mixed with other mediamaking materials. The treatments given were as follows: the powder was soaked in cold water for five days (P1); powder soaked in cold water for seven days (P2); powder soaked in cold water for nine days (P3); the powder is soaked in hot water at 100 °C for 1 hour (P4); the powder is soaked in hot water at 100 °C for 2 hours (P5); the powder is soaked in hot water at 100 °C for 3 hours (P6); and powder without immersion (P7). The powder that has been soaked is then drained.

The variables that will be observed in this study are:
1. Mycelium growth time on the growing medium after inoculation (days).
2. When the mushroom fruiting body grows, the baglog is filled with hyphae until the ovary has formed like a thumb (day).
3. Wet weight of mushrooms produced by each baglog, directly after harvesting (fresh), is calculated in grams.
4. The amount of mushroom production per baglog by recording the number of mushrooms that grow in one baglog during the first harvest.

Data were processed using statistical analysis of Completely Randomized Design with Duncan's further test.

3. Result and Discussion

3.1. Mycelium Growth Time of *Pleurotus ostreatus*
In this study, the initial immersion was carried out using hot and cold water. This immersion is intended to remove or dissolve the extractive substances in the raw materials to be used, in this case, the oil palm empty bunches. Compounds found in wood in small amounts are called extractives [2]. These substances can be separated from wood using water or organic solvents such as ether or alcohol without physically damaging the wood. Extractive substances soluble in hot water are fat, dyestuff, tannin, resin, and phlobatannin. The extractive substances that dissolve in cold water include glucose, fructose, carbohydrates, sugar, pectin, dyes, and certain acids.

The mycelium growth time of oyster mushrooms after being inoculated is presented in Figure 1. The results showed that in cold water immersion, the best growth was in baglog P2 and P3, where the mycelium growth was on days 22 and 23, respectively. However, on soaking in hot water, the treatment with the fastest mycelium growth was baglog P4 which was 30 days. Thus, the process of soaking the media using cold water affects the time of mycelium growth. In this study, the longer it was soaked in cold water, the faster the mycelium covered the baglog. Meanwhile, the longer the hot water immersion treatment was applied, the lower the mycelium's ability to grow on baglog. It is in line with research conducted by Ilyas et al. [3], that hot and cold water immersion had a significant effect on mycelium growth and cold water immersion had the fastest closing time than hot water. When viewed from all treatments, both with cold water immersion, hot water, and control, the treatment with the fastest mycelium closure was P2, which was soaked in cold water for 22 days. Typically, the mycelium covers the baglog about 40 days after inoculation—some media, even less than 40 days. The use of mulberry waste as a medium takes 36-49 days after inoculation [4]; sago
pulp as a medium takes 18-38 days [5], candlenut sawdust as a medium takes 29-40 days [6] and bamboo waste as a medium takes 39-40 days [7].

![Figure 1. Mycelium growth time of pleurotus ostreatus in immersion treatment.](image)

The ANOVA test shows an F-value (54.38) more significant than the F-critical value for the selected alpha level 5%. That means that immersion treatment in the media affects the mycelium growth time, so further tests need to be carried out. Duncan's Multiple Range Test ($\alpha = 0.05$) was obtained in baglog P1, significantly different from all treatments. On the other hand, the treatment of baglog P2 was not significantly different from that of P2 and P4. Meanwhile, the treatment of baglog P5 was not significantly different from that of baglog P7. The immersion treatment causes some water-soluble extractive substances to come out of the cell wall [8]. Immersion time affects the number of extractive substances that come out. The empty cavity previously filled with extractive substances made it easier for fungal hyphae to penetrate the cell wall and spread throughout the medium.

In addition to cellulose, hemicellulose, and lignin, some macro and microelements affect the growth of mycelium [9]. The elements are Potassium (K) and Nitrogen (N). Potassium plays a role in activating the enzymes needed to form starch and protein. Sodium functions to build mycelium, protein formation, and build enzymes stored in the body abundant in the cytoplasm. The resulting starch and protein will be degraded into simpler compounds which will then be used for mycelium growth and build enzymes stored in the body.

In this study, in some samples, some contaminants caused the mycelium to fail to develop. Initially, the mycelium of each baglog grew well, but then a pest attack appeared in the form of rats that boreholes in several baglogs. This rat pest is thought to carry the organism that causes the baglog to be contaminated. The contaminated baglog then changes colour from what should be white to green and black. Several organisms attack oyster mushroom plants [10]:

1. *Trichoderma* spp can inhibit the growth of oyster mushroom mycelium to thwart the growth of oyster mushroom fruiting bodies. The contamination characteristics caused by this fungus are the emergence of green spots or stains on the oyster mushroom growing media.
2. *Mucor* spp, mucor contamination is indicated by the appearance of black spots on the surface of the baglog media. This contamination causes competition with the growth of the oyster mushroom mycelium to inhibit or thwart the growth of the oyster mushroom fruiting body.
3.2. Growth Time of Pinhead White Oyster Mushroom.
Pinheads will grow after the mycelium spreads and covers the entire surface of the baglog. Pinheads grow from mycelium that overlaps each other and form small lumps or clumps like buttons. This pinhead will later develop into an adult mushroom from a closed hood and bloom in a semicircle like an oyster shell [11]. Figure 2 shows the growth of the fruiting bodies of each treatment calculated from the time of inoculation.

![Growth of pinhead (Days)](image)

P1: soaked in cold water for five days; P2: soaked in cold water for 7 days; P3: soaked in cold water for 9 days; P4: soaked in hot water at 100 °C for 1 hour; P5: soaked in hot water at 100 °C for 2 hours; P6: soaked in hot water at 100 °C for 3 hours; P7: powder without immersion.

Figure 2. Pinhead growing time of *pleurotus ostreatus* in immersion treatment.

Immersion treatment in cold water, baglog containing oil palm empty fruit bunches powder soaked in cold water for five days (P1) was the fastest-growing ovule (52 days). On the other hand, immersion treatment in hot water, the baglog with the fastest pinhead growth was baglog P4 (soaked in cold water for 1 hour) on the 56 days. Pinhead growth is affected by temperature. Meanwhile, the control has not shown any pinhead growth. The ANOVA test shows an F-value (108.15) more significant than the F-critical value for the selected alpha level 5%. That means that immersion treatment in the media affects the growth time of pinhead white oyster mushrooms, so further tests need to be carried out. Duncan's Multiple Range Test (α = 0.05) showed that the treatment in Baglog P5 was significantly different from all treatments.

Generally, pinheads will appear one or two weeks after opening the baglog [12]. In this study, pinheads grew between 8-35 days, but some baglogs had been covered by mycelium, but until the 99th day, no pinheads appeared. One of the factors that determine the growth of pinheads is the temperature and humidity of the maintenance room. The temperature required for pinheads to develop is 16 °C-22 °C with 80-90% humidity [13]. However, in this study, the room temperature ranged from 23 °C - 31 °C with a humidity of 65%-80%. Temperatures that are outside the pinhead growth temperature range cause the pinheads to take longer to grow.

3.3. Harvesting
Things that need to be considered in harvesting are harvesting done by pulling without leaving any part of the fungus, clean and not scattered. In addition, the harvested baglog are cleaned of the remains of fungi that are still attached to the baglog to ensure that the remaining mushrooms do not invite pests and diseases to come [14].
3.3.1. Harvest Time.
Harvesting is done after the growth of the fungus reaches its optimal level, which is large enough but not yet in full bloom—usually done five days after growing the mushroom candidate. The mushroom size is quite large, with an average diameter of 5-10 cm at that time. Therefore, harvesting should be done in the morning to maintain freshness. Harvesting is done by pulling out all the existing mushroom clumps that do not cut the base of the giant mushrooms because one mushroom clump has the same growth stadia. Therefore, if harvesting is only carried out on large mushrooms, small mushrooms will not grow much more significantly and may even die [15]. The harvest time of white oyster mushrooms in this study was calculated from the time of inoculation. Oyster mushroom harvest time in various treatments is presented in Figure 3.

P1: soaked in cold water for five days; P2: soaked in cold water for 7 days; P3: soaked in cold water for 9 days; P4: soaked in hot water at 100 °C for 1 hour; P5: soaked in hot water at 100 °C for 2 hours; P6: soaked in hot water at 100 °C for 3 hours; P7: powder without immersion.

Figure 3. Harvest time of pleurotus ostreatus in immersion treatment

The fruiting body growth time in this study was calculated from pinhead growth to harvest time; it was ranged from 2-3 days. This result follows Cahyana et al. [15] statement that could be harvest mushrooms after 2-3 days from the emergence of pinheads. Oyster mushroom harvest time ranged from 55-67 days after inoculation (Figure 3). Baglog P1 was the fastest at 55 days, while the slowest harvest was baglog P5 at 67 days. The ANOVA test shows an F-value (116.17) more significant than the F-critical value for the selected alpha level 5%. That means that immersion treatment in the media affects mushrooms' harvest time, so further tests need to be carried out. Duncan's Multiple Range Test ($\alpha = 0.05$) showed that the baglog P1, P2, P3, and P4 were not significantly different. Likewise, baglog P2 and P5 are also not significantly different. Meanwhile, baglog P5 was significantly different from baglog P1, P3, and P4.

3.3.2. Fruit Body Weight.
The weight of the fruiting body produced by each treatment was different. Fruit body weight was obtained by weighing fresh oyster mushrooms. The results of observations of fruit body weight are presented in Figure 4.
P1: soaked in cold water for five days; P2: soaked in cold water for 7 days; P3: soaked in cold water for 9 days; P4: soaked in hot water at 100 °C for 1 hour; P5: soaked in hot water at 100 °C for 2 hours; P6: soaked in hot water at 100 °C for 3 hours; P7: powder without immersion.

Figure 4. Fresh fruit bodyweight of *pleurotus ostreatus* in immersion treatment

Figure 4 shows the difference in fruit body weight from each treatment. The P1 treatment produced the highest fruiting bodyweight of 110 g, while the lowest was 65 g in the P5 treatment. The ANOVA test shows an F-value (13.89) more significant than the F-critical value for the selected alpha level 5%. That means that immersion treatment in the media affects fresh fruit body weight, so further tests need to be carried out. Duncan's Multiple Range Test (α = 0.05) showed that the baglog P2, P3, P4, and P5 were not significantly different. Likewise, baglog P1 and P2 are also not significantly different. Meanwhile, baglog P1 was significantly different from baglog P3, P4, and P5. The fresh weight of mushrooms is influenced by the diameter of the mushroom hood, the number of mushroom bodies, and the availability of nutrients in the media [16]. The results of the study are not always the more fruiting bodies, the heavier the results obtained. It can be seen in P2 treatment where the number of fruiting bodies is 16 but only weighs 87 g. It is thought to be due to insufficient nutrients for developing the oyster mushroom fruiting body, resulting in oyster mushrooms with smaller hoods.

3.3.3. The number of Fruiting Bodies.
The number of fruit bodies produced from each treatment was calculated after weighing the oyster mushrooms. Figure 5 shows the number of fruiting bodies produced from each treatment ranging from 6-16 fruiting bodies. The maximum number of fruiting bodies was 16 in treatment P2, while the least was six fruiting bodies in treatment P3.
P1: soaked in cold water for five days; P2: soaked in cold water for 7 days; P3: soaked in cold water for 9 days; P4: soaked in hot water at 100 °C for 1 hour; P5: soaked in hot water at 100 °C for 2 hours; P6: soaked in hot water at 100 °C for 3 hours; P7: powder without immersion.

**Figure 5.** The number of fruiting bodies of white oyster mushroom *pleurotus ostreatus* in immersion treatment

The ANOVA test shows an F-value (2.14) is smaller than the F-critical value for the selected alpha level 5%. That means that immersion treatment in the media does not affect the number of mushroom fruiting bodies. The difference in the number of fruiting bodies produced in each baglog can be caused by the ability of the mycelium to absorb nutrients so that it affects the pinheads produced. According to Nurjayadi [17], the optimal number of fruiting bodies reaches 8-10 fruit bodies. In this study, the number of fruiting bodies produced was 16 pieces.

4. Conclusion
Oil palm empty bunches have the potential to be used as growing media for oyster mushrooms. However, pre-treatment on oil palm empty bunches affects fungal growth. Therefore, oil palm empty bunches powder by cold-soaking for five days can be used as a medium for oyster mushroom cultivation.

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