Pine fruit as a media for planting white oyster mushroom 
(*Pleurotus ostreatus*)

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Abstract. Oyster mushroom is a wood fungus that can grow naturally on dead or weathered trees (saprophytes). The commonly used planting medium for oyster mushroom cultivation is sawn waste from various trees. However, considering the high rate of deforestation, ranging from illegal logging, forest burning, and conversion of forest land functions, it is necessary to make substitutions in terms of the leading media for the growth of oyster mushrooms. In this study, pine fruit is the primary medium for the growth of oyster mushrooms to add alternative materials for growing oyster mushrooms. This study aims to determine the potential of pine fruit as a growth medium for oyster mushroom (*Pleurotus ostreatus*) by the soaking method using two treatments, namely cold and hot water immersion. In cold water samples, the pine cone powder was soaked for five days, seven days, and nine days for hot water soaked for 1 hour, 2 hours, and 3 hours. The research procedure started preparing oyster mushroom growth media, inoculation, maintenance and growth, and harvesting. The results showed that oyster mushrooms could grow at media soaking in hot water for 2 hours (P2) and 3 hours (P3). Treatments P2 and P3 took 78 days and 105 days, respectively, to grow oyster mushrooms.

1. Introduction

Oyster mushroom is a wood fungus that can grow naturally on dead or decayed trees in tropical and subtropical areas. Oyster mushrooms have started to be favored by some people, apart from being relatively affordable, also because oyster mushrooms contain a lot of protein, vitamins, iron, and calcium. Oyster mushrooms have various benefits and potentials, so it is necessary to cultivate them, especially for the South Sulawesi area, which is still lacking. Currently, the commonly used planting media is sawn waste from various types of trees. However, seeing the high rate of deforestation, ranging from illegal logging, forest burning, and conversion of forest land functions, will impact the lack of production in the wood processing industry so that sawn waste begins to decrease. Therefore, it is necessary to make substitutions in terms of the primary growth medium for oyster mushrooms. Therefore, in this study, pine cones will be used as the primary medium for oyster mushroom growth to add alternative materials for oyster mushroom growing media.

Pine fruit is a substitute planting medium because pine fruit is very abundant in Indonesia. Moreover, it does not cost money to get it because there has been no effort to use it. Then in terms of content, pine fruit has cellulose and hemicellulose, which need to grow oyster mushrooms [1]. However, pinecones contain extractives and resins that can be a nutrient on the growth of oyster mushrooms, so that several treatments are needed to reduce them, one of which is soaking using hot water and soaking in cold water. Therefore, this study aims to determine the potential of pine fruit as a growth medium for white oyster
mushroom (Pleurotus ostreatus) by the soaking method using two treatments, namely hot and cold-water soaker.

2. Methods

The research procedure started from growth media preparation, inoculation, mushroom maintenance, and mushroom harvesting. The main stage of preparation for the growth of oyster mushrooms is the initial treatment for pine fruits, namely washing the pine fruit thoroughly and then arranging them, after being laid out, then dried in the sun to dry, and then ground using a hammer mill into powder. Next, soaking the pine fruit powder was carried out using hot water for 1 hour (P1), 2 hours (P2), and 3 hours (P3), and cold water for five days (P4), seven days (P5), and nine days (P6)—the control sample using pine fruit powder without soaking (P7) and sawdust of sengon (P8). The purpose of soaking pine fruit powder is to remove extractive substances. The soaked pine fruit powder is then dried in the sun to dry so that the powder can be used as a planting medium. To get an exact media composition, mixing pine fruit powder, bran, lime, and gypsum according to the dose. Then the growing medium was added with EM4 and water.

Furthermore, the baglog is sterilized in an autoclave sterilizer at a temperature of 120°C for three hours. The media has to sterilize then cool for 24 hours, and the inoculation process carries out. The inoculated media was stored in the incubation room until the mycelium filled the baglog; the baglog stood in a standing position, followed by observing the closure of the mycelium on the media. After the mycelium filled the baglog media, the baglog media was transferred to the kumbung and continued with observations of pinhead growth time, harvest time, wet weight of mushroom fruiting bodies, and the number of mushrooms harvested for each baglog.

3. Result and Discussion

3.1. Growth of Oyster Mushroom Mycelium.

Mycelium formation is an early phase in the development of oyster mushrooms. This mycelium will later form small nodules, which then develop into pinheads and eventually form the limbs and fruit bodies of the fungus [2]. Fungal mycelium branches and at the meeting points form a small nucleus called a sporangium which will grow into a bud or ovule of a mushroom fruiting body and eventually grow into a fungus. Mycelium is a collection of fungal hyphae that coalesce to form a network. Hyphae are loose or continuous cells to form insulated or non-insulated threads [3]. The mycelium growth process calculates starting from the baglog has been inoculated until the mycelium has filled the baglog. The use of sawdust as a medium takes approximately 40 days from inoculation until the mycelium covers the baglog; for example, using candlenut sawdust takes 29-40 days [4]. Some studies that did not use sawdust took 39-40 days for bamboo media [5], 18-38 days for sago pulp [6], and 36-49 days using mulberry waste [7]. In this study, the time required for the mycelium to cover the baglog was between 32-42 days (Figure 1.)

In Figure 1, it can see that the media of pine fruit powder soaked in hot water experienced the fastest mycelium closure process, namely baglog P2, i.e., 32 days. On the other hand, the medium of pine fruit powder soaked in cold water, the fastest to experience the overall mycelium closure process was baglog P6, i.e., 48 days. Meanwhile, baglog P4 did not experience the mycelium growth process at all. Thus, the results obtained in the treatment of soaking pine fruit powder using cold water are the same as the results using hot water, where the more extended the pine fruit powder is soaked, the faster the mycelium will cover the surface of the baglog.

However, several baglog were contaminated so that their mycelium growth became very slow, for example, in baglog P3. Contamination can be caused by non-aseptic conditions when isolating seedlings. Contamination can also cause by imperfect sterilization, namely the duration of sterilization and the inappropriate dosage of F0 media [8]. The causes of the failure of oyster mushroom cultivation include: the process of selecting seeds that are not good, making baglog that are not hygienic, the place used as a production house (kumbung) is not clean, the temperature and humidity of the kumbung are not suitable [9]. In this study, mushroom baglog overgrown by dark green mushrooms.
Figure 1. Mycelium growth of oyster mushroom (*Pleurotus ostreatus*) in various treatments. [P1: soaked for 1 hour in hot water, P2: soaked for 2 hours in hot water, P3: soaked for 3 hours in hot water, P4: soaked for five days in cold water, P5: soaked for seven days in cold water, P6: soaked nine days with cold water, P7: without soaking and P8: using sengon sawdust].

When compared between media soaked in hot and cold water, baglog P2 was the baglog that experienced the fastest mycelium closure (32 days). The media of pine fruit powder soaked in hot water had the fastest average mycelium closing time than the cold-water soaking treatment. Meanwhile, the mycelium closure process does not occur if the sample does not treat as in baglog 7. Ilyas et al. [10] said that the soaking of sawdust causes the cell wall to expand to facilitate fungal hyphae with the help of enzymes that break down cellulose, hemicellulose, and lignin to penetrate by perforating the cell wall. Enzymes digest wood compounds that are hollowed out and use them as a source of nutrients for the fungus.

Two baglog did not experience the mycelium growth process at all, namely baglog P4 and P7. The non-growth of mycelium in the two samples cause by extractive substances and resins that still contains in the pine cone powder. Cold soaking for five days is not enough to dissolve the extractives or resins possessed by pine cone powder. Meanwhile, it knows that the presence of extractives or resins in sawdust can inhibit the growth of fungal hyphae. Cahayana [11] state that one of the causes of the slow formation of mycelium is because the media used has sap, where this sap contains a lot of extractive substances. Extractive substances are non-structural components that will inhibit the growth of white oyster mushrooms. Extractive substances in wood are repellent to wood destroying microorganisms, one of which is a fungus.

The media that has been widely using for the growth of oyster mushrooms is sawdust of sengon. In this study, sengon media use as a control (P8). When compared between the use of sengon sawdust with pretreatment on pine cone powder (soaked in hot water for 2 hours), the mycelium closure on media soaked in hot water was faster (32 days) compared to the use of sengon powder (42 days).

The ANOVA test shows an F-value (166.5) 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 Distance Test ($\alpha = 0.05$) shows that baglog P2, significantly different from all treatments. On the other hand, the treatment of baglog P1 and P8 was not significantly different. Meanwhile, the treatment of baglog P3, P5, and P6 were not significantly different. It is because extractive substances that dissolve in water will come out of the cell wall during the immersion process. The amount of extractive substance that comes out is also influenced by the
immersion time [12]. The empty cavity in the pine cone, which was previously filled with extractive implications, is used by the fungus to be supplied by the hyphae so that the hyphae can easily penetrate the cell wall and spread throughout the media.

3.2. Growth of Oyster Mushroom Pinhead.

After the mycelium covers the baglog as a whole, the baglog cover opens to provide space to grow on the fruiting body (pinhead). The pinhead is a small clump consisting of a collection of mycelia that will develop into a fruiting body. Pinheads that develop gradually will become parts of the mushroom fruiting bodies, such as the hood and stalk not located in the middle of the hood [13]. Pinhead's growing time calculates from inoculation time until the pinhead appears. The pinhead growth time presents in Figure 2.

![Figure 2. Pinhead growth time](image.png)

In Figure 2, it saw that for the hot water soaking treatment, the growth of pinheads occurred in baglog P2 with a growing time of 42 days and baglog P3 for 53 days, while baglog P1 did not experience a pinhead growth process. For the cold-water treatment (P4, P5, and P6), none of them experienced the pinhead growth process. Meanwhile, for the control sample, the growth of pinheads only occurred in sample P8 (21 days), while P7 did not experience pinhead growth. The ANOVA test shows an F-value (1372.703) 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, so further tests need to be carried out. Duncan's Multiple Distance Test (α = 0.05) shows that baglog P2, P3, and P8 are significantly different from each other and significantly different with all treatments. According to Ilyas et al. [10], the time of growth of the mushroom fruiting body is directly proportional to the length of closure of the fungal mycelium. If mycelium growth is good, it will affect the speed of fruiting body formation (primordial). This statement is not in line with the results of this study. This study found the fastest mycelium closure in baglog P2 (Fig. 1), but the fastest pinhead growth was in baglog P8 (Fig. 2). It is possible that closure of the mycelium only occurs on the surface of the baglog, while in the baglog, there has not been a complete closure of the mycelium. It is thought to be one of the reasons why pinheads grow late on the surface of the baglog. Likewise, baglog that have not yet undergone the pinhead growth process need even longer time to grow pinheads. In addition, some baglog also experience dryness and compaction caused by the room temperature, often reaching more than 30°C and humidity below 40%. The growth factor of pinhead oyster mushrooms influences by temperature (22-28°C), humidity (60-70%), time, CO₂ content, and light. In addition, the life and development of fungi require food in the form of chemical elements such as nitrogen, phosphorus, sulfur, potassium, and carbon, which are
already available in wood tissue, although in small quantities [8]. Therefore, it is necessary to select the planting media and treatment of the appropriate growing media and the addition of nutrients from the outside, such as fertilizer used as a mixture of mushroom growing media.

3.3. Growth of Oyster Mushroom Harvesting.
After passing the pinhead stage, gradually, the pinhead will enlarge and form a mushroom fruiting body that is ready to be harvested. The harvesting of oyster mushrooms is taken out when the fruiting bodies meet the criteria for ready to harvest with the characteristics of the mushroom hood being clean white, not dry, and the hood's side has not been broken. Therefore, optimal harvesting of oyster mushroom fruit bodies should be carried out approximately 2-4 days after the prospective fruiting bodies begin to grow. In addition, the method of harvesting does by pulling carefully without leaving any part of the fungus in the baglog to avoid spoilage which can lead to pest attacks [14]. Therefore, calculating harvest time starts from the growth of the pinhead until the fruit body is released from the baglog.

3.3.1. Growth of Oyster Mushroom Harvesting. The results of the observations are presented in Figure 3, showing that the harvest time calculated from the inoculation process until the mushrooms release from baglog for baglog P2 was 46 days, P3 was 56 days, and P8 was 27 days. The ANOVA test shows an F-value (1215.8) 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 (α = 0.05) shows that baglog P2, P3, and P8 significantly differed from each other and significantly differed with all treatments. In this study, harvesting was carried out 3-6 days after pinhead emergence. Karmila [4] stated that the average harvesting time was two days after pinhead growth, and the total length of harvest time was calculated from the inoculation process until the mushrooms were released from the baglog using candlenut sawdust and a mixture of promi, which was 55 days.

Figure 3. Harvest time for oyster mushroom cultivation for each treatment calculated from the emergence of pinhead

3.3.2. Fresh Weight of Oyster Mushroom Fruit Body. The fruit bodies weigh to determine the fresh weight. The observations presented in Figure 4 show that the average fresh fruit weight for baglog P2 is 14 g, baglog P3 is 45 g, and baglog P8 is 68 g. The ANOVA test shows an F-value (39.3) 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 and P8 significantly different from each other and
also significantly different with all treatments. The growth process of the fruiting body determines by the nutrients and water contained in growth media. Water plays an essential role in the growth process of the oyster mushroom fruiting body because if the water contained in the baglog reduces, there will be a drought which results in fruiting the body becomes bigger [6].

![Figure 4](image1.png) Figure 4. The wet weight of oyster mushrooms

![Figure 5](image2.png) Figure 5. Number of oyster mushroom fruit bodies

3.3.3. The Number of Oyster Mushroom Fruit Body. The weight of the fruiting body also cannot be separated from the number of fruiting bodies because the more the number of fruiting bodies, the heavier the yield of oyster mushrooms that can produce. For example, the number of harvests of fruiting bodies presented in Figure 5 shows that the fruit bodies are in baglog P8, which is five pieces, then baglog P3...
is four pieces, and the least is in baglog P2 is one fruit. Therefore, it is directly proportional to the weight of the fruiting body presented in Figure 4. The ANOVA test shows an F-value (50.57) more significant than the F-critical value for the selected alpha level 5%. That means that immersion treatment in the media affects the number of mushroom fruiting bodies, so further tests need to be carried out. Duncan's Multiple Range Test (α = 0.05) showed that the baglog P2, P3 and P8 significantly different from each other and also significantly different with all treatments.

4. Conclusion
Pine fruit that received pretreatment with hot water soaking for 2 hours (P2) and 3 hours (P3) had the potential to become a medium for growing oyster mushrooms. Treatments P2 and P3 took 78 days and 105 days, respectively, to grow oyster mushrooms.

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