Growth of F0 seedlings of oysters mushroom (Pleurotus ostreatus) with different ages of explants

Ferial, S A Syaiful, and A Dachlan

Department of Agrotechnology, Faculty of Agriculture, Universitas Hasanuddin, Jl. Perintis Kemerdekaan KM 10, Makassar, 90245, Indonesia.

E-mail: ferialsp15@gmail.com

Abstract. The success of oyster mushroom cultivation is determined by the quality of seed and the planting media used and environmental factors. This research aimed to obtain quality oyster mushrooms F0 seed based on the type of mushrooms and ages of different explants. The experiment was conducted at the National Agriculture Training Center in Batangkaluku from January to April 2018. The trial was set using factorial randomized block design method with two factors. The first factor was the type of oysters mushroom, namely white oysters mushroom and brown oysters mushroom. The second factor was the difference in the age after the growth of pinhead, consisting of 3 levels, namely 1 day, 3 days and 5 days. There were 6 combination treatments repeated 4 times, resulted in 24 treatment units, and each treatment unit used 3 units as observation object, bringing the total to 72 treatment units. The results show that the interaction between the type of white oysters mushroom and age 5 days after the growth of pinhead provide the best response to the diameter of mycelium F0 (2.84 cm) and the density level of mycelium F0 that categorized as thick density (score 4.17). The largest mycelium thickness, with a score of 3.42, ie. mycelium grows thinly evenly. The treatment of oyster mushrooms gave the best response to white oysters mushroom, while the treatment of age after pinhead growth gave the best response at the age of 3 days.

1. Introduction
Mushrooms are one of commodities in the horticultural sectors that has many benefits, besides being used as a nutritious food ingredient or as traditional medicine. Oyster mushrooms are an excellent source of protein, vitamins and minerals for human health. Oyster mushrooms contain protein (25%-50%), fat (2%-5%), sugar (17%-47%), mycocellulose (7%-38%) and some minerals, such as potassium, phosphorus, calcium and sodium (8%-12%). In addition, oyster mushrooms are also rich in vitamins, such as niacin, riboflavin, vitamin D, C, B1, B5 and B6 [1].

Currently, the huge market demand of the oyster mushrooms sometimes is not followed by sufficient stock and quality product. Oyster mushroom products are often found in non-standard quality, such as a fruit hood that is too thin and small, the shape of the hood is abnormal and the color of the fruit hood is uneven. The success of oyster mushroom cultivation is determined by the quality of planting media, environmental factors and the quality of the seeds used. The quality of oyster mushroom seeds is a decisive factor as well as seeds for other plants, because the superior seeds will produce high-quality fruit body and allows it can adapt to the wider environment [2].
One effort that can be done to get good quality seeds is to use disease-free seed sources, such as the use of seeds produced through plant tissue isolation method. The type and source of explants obtained is a requirement of the successful establishment of seedlings of tissue culture techniques.

F0 seedling production or pure plant tissue isolation method is the beginning of all seedling production. The process of making F0 seedlings often fail due to the selection of oyster mushroom parent that is not inconsistent with the requirements for explant plant material such as seedling age, seedling planting media, and seed storage. A study conducted by Ibrahim et al. [3] showed that the best explants to be used as a source of explants in the culture of the large white ginger meristem were old harvested rhizomes (aged 8 months). Based on the description above, this research aims to obtain quality F0 oyster mushroom seeds based on different types of oyster mushrooms and explant age.

2. Research methods

2.1. Methodology
This research was conducted based on an experimental method arranged in a factorial Randomized Block Design (RBD). The first factor was the type of oyster mushroom (J) consisted of white oyster mushroom (j1) and brown oyster mushroom (j2). The second factor was the age of explants (U) which consisted of three levels of treatment, namely: 1 day after the growth of pinheads (u1), 3 days after the growth of pinheads (u2), and 5 days after the growth of pinheads (u3). Each treatment was replicated four times. Each treatment used 3 units as observation objects so that the total number was 72 units.

2.2. Materials and tools
Materials used were two types of oyster mushroom, namely white oyster mushroom and brown oyster mushroom, Potato Dextrose Agar (PDA) solution, shelled corn, agricultural lime, aquades, clorompenicol, 70 % alcohol, bleach solution, aluminum foil, and wrapping plastic. While the tools used were analytical scales, measuring cups, spoons, knives, basins, saucepans, tweezers, stoves, filters, breaker glass, magnetic stirrers with hotplates, stirring rods, glass bottles, cotton, aluminum foil, rubber bands, pH indicators, Laminar Air Flow Cabinet (LAFC), petri dish, scalpel, hand sprayer, bunsen, autoclave, and stationery.

2.3. Sterilization of the tools
The tools were sterilized before use starting with washing all the tools that used firstly, namely, Erlenmeyer, measuring cup, spoon, breaker glass, stirring rod, petri dish, scalpel and tweezers. The tools that have been washed subsequently were sterilized using an autoclave at a pressure of 17.5 psi at a temperature of 121°C for 30 minutes. Before putting in the autoclave, these tools wrapped in aluminum foil.

2.4. Preparation of F0 media
The media used are pure Potatoes Dextrose Agar (PDA) media. PDA material was weighed as much as 39 grams and dissolved in aquades up to 1000 ml. Then it is heated and stirred until it boils, then the media was sterilized using an autoclave for 15 minutes at 121°C and a pressure of 1-2 Atm. After that, the media was poured into 72 Petri dishes and frozen at room temperature.

2.5. Sterilization of material surface
The mushroom isolation process began with surface sterilization of the sample material, i.e. the mushroom broodstock was cleaned first by using water to remove surface impurities. After that, the samples were drained and cut to a size of 0.5 cm x 0.5 cm. The sample pieces were then soaked in 70 % alcohol for 2 minutes, and rinsed with sterile water once. Then proceed with soaking in a 20% bleach
solution for 10 minutes, then rinsed again with sterile water 3 times for 5 minutes. After the sterilization process was complete, the surface of the sample was dried with sterile filter paper for 3 minutes.

2.6. Cultivation
After surface sterilization process was completed, the sample pieces placed on PDA media. Inoculation of samples carried out in the Laminar Air Flow Cabinet (LAFC) and in each petri dish placed one sample piece per the treatment. Then the grafting process was carried out for 2-21 days, depending on the growth rate at 25°C -29°C (room temperature).

2.7. Observation

2.7.1. Mushroom mycelium diameter (cm). Observations were made 4 days after inoculation by measuring mycelium diameter. Measurements are made from the surface of the media to a certain depth on the longest growth of mycelium.

2.7.2. The density of mycelium. Measurements were made using a Likert scale with score ratings as shown in Table 1.

| Category                      | Score |
|-------------------------------|-------|
| Mycelium has not grown        | 1     |
| Mycelium grows tightly thin   | 2     |
| Mycelium grows tightly        | 3     |
| Mycelium grows thick          | 4     |
| Mycelium grows very thick     | 5     |

2.7.3. The thickness of mycelium. Measurements were made using a Likert scale with score ratings as shown in Table 2.

| Category                     | Score |
|-------------------------------|-------|
| Mycelium does not grow        | 1     |
| Mycelium grows thin unevenly  | 2     |
| Mycelium grows thin evenly    | 3     |
| Mycelium grows moderately     | 4     |
| Mycelium grew thick           | 5     |

2.8. Data analysis
The data analysis method used is the analysis of variance. If there is a significantly different treatment effect, then the Duncan New Multiple Range Test (DNMRT) will be conducted at a 5% chance level.

3. Results and Discussion

3.1. Results
The comparison of F0 seedling growth on two types of oyster mushrooms with different explants age at 16 days after inoculation showed that the oyster mushroom with explant age 5 days showed the best results on the diameter of the mycelium, density, and thickness of the growing mycelium evenly covered the petri dish.
3.1.1. Diameter of mycelium. The treatment of the interaction between the type of oyster mushroom with explant age 5 days after the growth pinhead significantly different from the diameter of the mycelium. White oyster mushrooms with explant age of 5 days after the growth of pinhead give the results of the diameter of F0 white oyster mushroom mycelium seedlings by 2.84 cm. White oyster mushrooms can grow well at explants at 5 days after pinhead growth, while brown oyster mushrooms at explants 1, 3 and 5 days after pinhead growth are not optimal (Table 3).

Table 3. The average diameter of mycelium F0 (cm) in the interaction type of oyster mushrooms and aged explants, 16 days after inoculation.

| Type of oyster mushrooms (J) | Age of explants (U) | Average |
|-----------------------------|--------------------|---------|
|                             | 1 day (u1) | 3 days (u2) | 5 days (u3) |
| White oyster mushrooms (j1) | 0.00       | 7.20       | 7.61       | 4.94 |
| (0.71)                      | c          | (2.77)     | ab         | (2.11) |
| Brown oyster mushrooms (j2) | 0.00       | 6.17       | 6.35       | 4.17 |
| (0.71)                      | c          | (2.58)     | b          | (1.97) |
| Average                     | 0.00       | 6.69       | 6.98       |
| (0.71)                      |            | (2.68)     | (2.73)     |

The numbers that followed by the same letters mean that they are not significantly different at the DNMRT test level α = 0.01 (7.61; 7.20; 6.35; 6.17; 0.00 for mycelium diameter). The numbers inside the brackets represent data transformation results.

3.1.2. The density of mycelium. The interaction between treatments of white oyster mushroom and explant age 5 days after pinhead growth was significantly different from mycelium density with a score of 4.17, which means thick dense mycelium, and significantly different from the type of brown oyster mushroom with explant age 5 days after pinhead growth (Table 4).

Table 4. The average density of mycelium in the interaction type of oyster mushrooms and age after growing pinhead, 16 days after inoculation.

| Type of oyster mushrooms (J) | Age of explants (U) | Average |
|-----------------------------|--------------------|---------|
|                             | 1 day (u1) | 3 days (u2) | 5 days (u3) |
| White oyster mushrooms (j1) | 1.00       | 3.67       | a          | 4.17 |
| (1.00)                      | c          | (3.75)     | a          | (2.94) |
| Brown oyster mushrooms (j2) | 1.00       | 3.57       | a          | 3.00 |
| (1.00)                      | c          | (3.67)     | a          | (2.58) |
| Average                     | 1.00       | 3.71       | 3.58       |

The numbers that are still followed by the same letter mean that they are not significantly different at the DNMRT test level α = 0.01 (3.17; 2.75; 2.67; 2.00; 0.00 for mycelium density).

3.1.3. The thickness of mycelium. The treatment of explant age 3 days after the growth of pinhead was significantly different from the thickness of mycelium with a score of 3.42 which means that the mycelium grew thick (Table 5).
Table 5. The average thickness of mycelium F0 on the treatment of age after growing pinhead, 16 days after inoculation

| Type of oyster mushrooms (J) | Age of explants (u) | Average |
|-----------------------------|---------------------|---------|
|                             | 1 day (u1) | 3 days (u2) | 5 days (u3) |         |
| White oyster mushrooms (j1) | 1.00       | 3.42       | 3.58       | 2.67   |
| Brown oyster mushrooms (j2) | 1.00       | 3.42       | 3.00       | 2.47   |
| Average                     | 1.00       | 3.71       | p          | 3.58   |

The numbers that are still followed by the same letter mean that they are not significantly different at the DNMRT test level $\alpha = 0.01$ (3.17; 2.75; 2.67; 2.00; 0.00 for mycelium density).

3.2. Discussion

Based on observations in the laboratory, it was found that the interaction of the treatment between the types of oyster mushrooms and the age after the growth of pinhead was significantly different from the mycelium diameter and mycelium density in the F0 oyster mushroom seeds.

The interaction of treatment between types of white oyster mushrooms with age 5 days after the growth of pinhead on mycelium diameter F0 and mycelium density F0 gave the best results compared to other treatments. This is because the type of white oyster mushroom can adapt to the environment where it is grown. The ambient temperature in the laboratory (25°C) makes optimal growth of oyster mushrooms. This is consistent with the opinion of Suryani and Carolina [4] that the suitable temperature for the cultivation of oyster mushrooms is 22°C – 28°C with 60% -90% humidity, in addition to the nutrients and explant conditions used. According to Djariah [5], the growth of white oyster mushroom mycelium requires nutrients that contain carbohydrates, proteins, minerals, and vitamins, as well as the conditions of explants, were healthy and free of pests and diseases.

Nutrients used in this research is PDA (Potato Dextrose Agar). The high content of dextrose and carbohydrate in PDA media plays an important role in the process of fungal metabolism. According to Suparti and Karimawati [6], in the growth of oyster mushrooms, there is a need for media that contain nutrients and energy sources as well as certain environmental conditions to support the growth of oyster mushrooms. PDA is the most commonly used media because it has nutritional content in the form of carbohydrates, water, and protein derived from potato extracts and glucose.

Carbohydrates in PDAs come from potatoes, used as a source of carbon used by the fungus as structural compilers of cells and energy sources. Protein in the PDA is used as a source of amino acids containing nitrogen which serves to assist the process of metabolism in mushrooms. While the water content in the media is needed to help the smooth transportation or flow of chemical particles between cells that guarantee the growth of mushrooms mycelium [7]. The function of the mycelium is to absorb water, nutrients and organic matter from the growing media for use in the growth and development of white oyster mushrooms [8].

Explants aged 3 days after the growth of pinhead used are young tissue that is actively growing, the cells are still actively dividing and fungal broodstock tissue has a higher regeneration power. The physiological condition of explants has an important role in the success of tissue culture techniques. According to Ehsanour [9], genotype factors and explant sources are very influential in the growth and morphogenesis of tissue culture. All parts of plants that are still young (juveniles) whose cells are still actively dividing are the best plant parts for sources of explants.

In addition to the source and age of explants, the explant size also influences the success of tissue culture techniques. The age of oyster mushroom explants of 3 days after the growth of pinhead has a larger spore size so that adequate food supply and growth regulators are available to start new cell
growth and the pace of life getting bigger to grow. Zulkarnaen [10] stated that the smaller the explant size, the smaller the possibility of contamination, both internally and externally, but the pace of life will be even lower. But on the contrary, the larger the explant size, the greater the possibility for proliferation, but the possibility for microorganism contamination will be even greater.

The treatment of white oyster mushroom at explant age 1 day after pinhead growth and brown oyster mushroom type at explant age 1 day after pinhead growth gave the lowest yield on $F_0$ mycelium diameter of 0.71 cm, $F_0$ mycelium density with a score of 1.00 (mycelium has not been grown) and $F_0$ mycelium thickness with a score of 1.00 (mycelium does not grow). This happens because both explants of white oyster mushrooms and brown oyster mushrooms that are used at the age of 1 day after the discharge of the pinhead experience stagnation, since planted until a certain period in a non-dead state also does not grow. And also because the spores are still very small and not perfect, so the cells have not been able to divide properly. The condition of explants with different ages results in explants having a different texture, water content, fiber, and starch. These conditions will affect the physiological nature and ability to grow [3].

The results of observations on the parameters of $F_0$ mycelium density and $F_0$ mycelium thickness indicate that the age variable after the growth of pinhead gives a very significant effect. This is due to the physiological condition of explants that are still young and explant conditions that are fresh and healthy, and do not contain pests and diseases. The physiological state of explants will encourage the growth and development of explants, especially in the formation of hyphae which subsequently forms the mycelium. According to Rosita et al. [11], the age of the parent plant, physiological age of explants, the right size of explants and the stage of explant development can influence the success of tissue culture methods.

4. Conclusions
The interaction between white oyster mushrooms and explant age 5 days after pinhead growth gave the best results on the diameter of the $F_0$ mycelium (2.84 cm) and the density of the $F_0$ mycelium at tight and thick levels (score 4.17). As well, high $F_0$ seeds from white oyster mushrooms can be obtained at the age of 3 days after pinhead growth.

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