

The Effectiveness of Different Types of PDA Culture Media on the Mycelial Growth of Shimeji Mushroom

Araya Musika*

¹*Department of Agriculture, Faculty of Agricultural Technology, Buriram Rajabhat University; E-mail: araya.mu@bru.ac.th*

Abstracts: This experiment aims to compare different types of PDA culture media recipes, including potato, sweet potato, pumpkin, and sugar banana, for their effects on the growth of Shimeji mushroom mycelium. It also aims to provide alternative options for formulating culture media for Shimeji mushroom spawn cultivation. A completely randomized design (CRD) was employed for the experiment, with four treatments and five replicates each, as follows: (T1) potato-based media; (T2) sweet potato-based media; (T3) pumpkin-based media; (T4) sugar banana-based media. Data collection involved daily measurements of the length (in centimeters) and width (in centimeters) for a period of seven days. After the experiment concluded, data analysis was conducted to determine the variation and compare the mean values of the treatments using Duncan's Multiple Range Test (DMRT) and package software. The experimental results showed that over a period of 3, 5, and 7 days, the potato-based media (T1) exhibited the highest growth of Shimeji mushroom mycelium and the highest mycelium density values, measuring 6.2000, 3.2000, and 0.0000a (density scale +++). The pumpkin-based media (T3) had lower mean values of 0.9000, 3.1600, and 0.0000a (density scale +) for mycelium growth and density. Therefore, it can be concluded that among the four PDA media recipes tested over a period of 3, 5, and 7 days, the potato-based media (T1) demonstrated the highest mycelium growth and density values of approximately 6.2000, 3.2000, and 0.0000a (density scale +++).

Keywords: PDA Mother Mycelium, Mushroom Mycelium, Shimeji Mushroom.

1. INTRODUCTION

Mushrooms, or fungi, are classified as living organisms in the microbial kingdom. They are non-photosynthetic, meaning they cannot produce their food due to the absence of chlorophyll. However, mushrooms consist of hyphae and reproduce sexually through spores, while in some cases they can reproduce asexually. Therefore, mushrooms sustain their lives by depending on other living organisms and can be considered heterotrophic microorganisms or self-supporting microorganisms (Pongpal, 2004).

Mushrooms are a rich source of high-quality protein and contain high levels of potassium and vitamin B, which aid in the proper functioning of the digestive system. They are low in sodium and sugar content, making them suitable for health-conscious individuals, especially those looking for meat alternatives. Mushrooms have a delicious taste, high nutritional value, and certain types of mushrooms have medicinal properties that can prevent and treat diseases. Specifically, Shimeji mushrooms are considered economically valuable mushrooms and are popularly cultivated for commercial purposes. They are expensive and have distinctive features, with small caps that grow in clusters and range in color from white to brown; their caps have patterns resembling marble or water spots, and their colors can range from light brown to dark brown or even grayish black. The long white stems range from 5 to 10 cm in length, and their price is approximately 180 to 250 baht per kilogram (Thongtiang, 2019). They thrive in cool and highly humid environments (Pakkatang & Krachongram, 2020). They offer benefits such as inhibiting cancer cell growth, reducing blood sugar and cholesterol levels, cleansing and detoxifying the liver, providing antioxidants for a strong body, preventing anemia, and aiding in weight control due to their low-fat and low-sodium content (Herbal Organic, 2017). In Thailand, Shimeji mushroom cultivation is not widely practiced, and farmers typically cultivate them in open-system greenhouses without proper control of temperature and humidity. This often leads to low yields and small-sized mushrooms that are insufficient for the market. Besides issues related to cultivation conditions, the quality of Shimeji mushroom spawn is another important factor affecting the quantity and quality of mushroom yields. Spawn of poor quality directly affects the yield and quantity of mushrooms.

Therefore, the researchers are interested in studying and comparing different PDA culture media recipes to promote the growth of Shimeji mushroom mycelium. These recipes include agar recipes using potato, pumpkin, sweet potato, and sugar banana. These recipes can be considered alternative options for selecting raw materials for culturing PDA spawns to enhance the yield of Shimeji mushrooms, increase the quantity of mushroom production, and improve the quality and efficiency of mushroom caps. Furthermore, the knowledge gained from these studies can be applied to future mushroom cultivation practices and can also be used as a primary or additional source of income in mushroom spawn production.

1.1. Research Objectives

1. To study and compare different types of PDA culture media recipes, including potato, sweet potato, pumpkin, and sugar banana, for their effects on the growth of Shimeji mushroom mycelium.

2. To provide alternative options for formulating culture media for Shimeji mushroom spawn cultivation.

1.2. Expected Outcomes

1. To acquire knowledge about the four different PDA culture media recipes, namely potato, sweet potato, pumpkin, and sugar banana, and their impact on the growth of Shimeji mushroom mycelium, aiming to determine the recipe that promotes the best growth results.

2. To disseminate the acquired knowledge to farmers and enthusiasts, enabling them to apply it in their profession and potentially generate income.

1.3. Hypotheses

1. H_0 : There is no significant difference in the types of potato, sweet potato, pumpkin, and sugar banana used as PDA culture media.

2. H_1 : There is a significant difference in the types of potato, sweet potato, pumpkin, and sugar banana used as PDA culture media.

1.4. Scope of the Research

To investigate the effects of four PDA culture media recipes, potato, sweet potato, pumpkin, and sugar banana, on the growth of Shimeji mushroom mycelium.

2. Research Methodology

For cultivating pure culture or cultivating mother mycelium on different types of PDA culture media, such as potato, sweet potato, pumpkin, and sugar banana, the process includes the following steps:

2.1. Preparation of PDA Culture Media

PDA P = Potato

 D = Dextrose

 A = Agar

The recipe for preparing PDA culture media consists of the following ingredients:

1. Potato, sweet potato, pumpkin, or sugar banana, each 200 grams

2. Dextrose	20 grams
3. Agar	15 grams
4. Water	1000 cc

The proportions of all ingredients are the same, with the only variation being the main ingredients, which are pumpkin, sweet potato, pumpkin, and sugar banana.

2.2. Preparation of Nutrient Agar Media

1. Wash potatoes thoroughly to clean off any dirt. Peel the skin and cut them into small pieces approximately 1x1x1 cm or similar to the size of small dice, then weigh the pieces to obtain around 200 grams.

2. Boil the diced potatoes in 1000 cc of water. Cook them over low heat for about 15 minutes, starting from the boiling point. Be careful not to overcook the potatoes, as it may result in cloudy agar, which may hinder the observation of mushroom mycelium.

3. Strain the potato residue and continue boiling the filtered water. After that, add approximately 15 grams of agar. To avoid agar clumping, it is best to add the agar gradually. Keep stirring until the agar is completely dissolved.

4. Add about 20 grams of prepared dextrose into the mixture by adding it gradually, using a ladle to maintain continuous stirring until the glass rod is completely dissolved.

5. Once the nutrient agar media is completely dissolved, pour the mixture into flat bottles, being careful not to contaminate the bottle's rim. Fill the bottle with approximately 20–30 cc of the mixture. Seal the bottle tightly with clean cotton and secure it with rubber bands (Repeat this process for each treatment following steps 1–5).

6. Place the nutrient agar media bottles in a pressure cooker for sterilization, using a pressure level of 15 pounds per square inch at a temperature of 121 degrees Celsius for approximately 40 minutes.

7. Once the 40-minute sterilization time is complete, turn off the heat and allow the temperature and pressure to decrease gradually until they reach zero. Then, remove the nutrient agar media bottles from the pressure cooker. Set aside to warm up to about 50 degrees Celsius.

8. Place the nutrient agar bottles at an inclined angle of approximately 30 degrees to increase the surface area of the agar.

9. Let the agar solidify by allowing it to cool. Afterward, the agar can be used for mushroom mycelium cultivation or stored in a clean room for future inoculation.

3. MUSHROOM PURE CULTURE SEPARATION OR MUSHROOM INOCULATION ON AGAR MEDIA

After preparing four different recipes of PDA media, the next step is as follows:

3.1 Mushroom Selection for Propagation

When selecting mushrooms for propagation, consider choosing mature, strong, and firm caps that are free from diseases and pest infestations. Ensure that the mushrooms are fresh and harvested from the field or new mycelium blocks. Avoid using mushrooms that have been exposed to excessive moisture.

3.2 Mushroom Inoculation or Isolation Process

Once the equipment for inoculating is prepared, follow these steps:

1. Place the equipment used for inoculation, including nutrient agar media bottles, an alcohol burner, mushroom caps, and sterilized inoculation needles, into the laminar airflow.
2. Insert hands into the glove ports located at the bottom of the flow and ignite the alcohol burner.
3. Dip the inoculation needle into alcohol and then flame the tip of the needle until it turns red-hot. Leave it in the air of the refrigerator for about 10 seconds.
4. Inject the mushrooms along the longitudinal axis, separating them into two halves. Take care not to let your hand or any other object touch the stem or central part of the mushroom that has just been injected.
5. Use the inoculation needle to cut out small pieces of tissue precisely at the area between the stem and the cap, which is the part with the most intact and healthy tissue.
6. Once you have obtained the tissue, place the mushroom with your hand holding it down. Switch to holding the PDA media bottle in your other hand, keeping the bottle in your palm. Move the agar media bottle towards the hand holding the needle, avoiding any movement of the hand holding the needle. Then, using your little finger on the hand holding the needle, grip the cotton swab at the bottle's rim and gently pull it out. Bring the bottle's rim close to an alcohol burner flame to sterilize it, then insert the mushroom tissue onto the surface in the middle of the nutrient agar media. Flame the bottle's rim again and close the cotton swab tightly as before.



Figure 1. The characteristics of healthy Shimeji mushrooms



Figure 2. The agar media for each treatment is left to solidify for one day

4. CARE AND HANDLING OF AGAR BOTTLES FOR MUSHROOM CULTURE

1. It is important to prevent ants or insects from biting the paper covering the bottle's cap and crawling into the nutrient agar media.

2. Observe the nutrient agar media to check for any signs of contamination at each stage of mycelial growth. It may be difficult to detect contamination once the mycelium has fully colonized the surface of the agar.

3. If you want the mycelium to grow rapidly, store the nutrient agar bottles used for mushroom cultivation in a dark area. Once the mycelium has fully colonized the nutrient agar, promptly transfer it to the next phase of growth on cereal medium bottles.

4. Measure the width and density of Shimeji mushroom mycelium in all four recipes at 3, 5, and 7 days.

5. DATA RECORDING AND ANALYSIS

Record the width and density of Shimeji mushroom mycelium in all four recipes at 3, 5, and 7 days. Subsequently, analyze and compare the differences between treatments using package software.

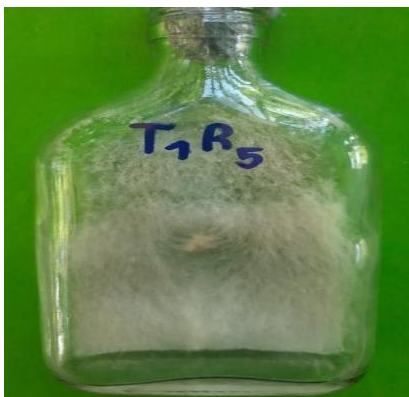


Figure 3. Mycelial Growth in the Potato Recipe



Figure 4. Mycelial Growth in the Sweet Potato Recipe

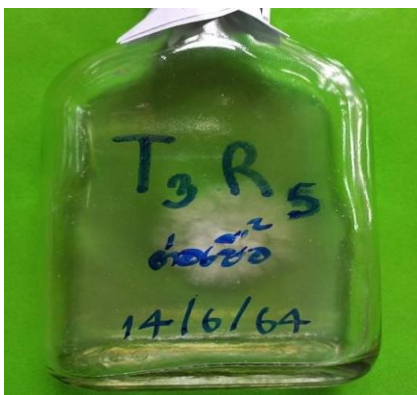


Figure 5. Mycelial Growth in the Pumpkin Recipe

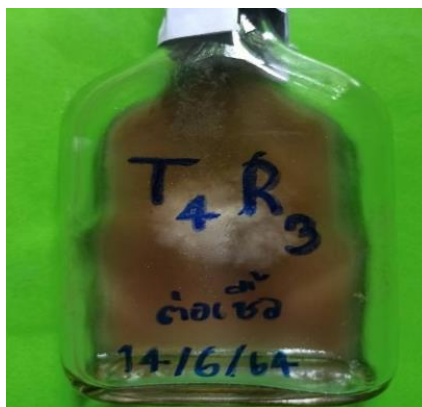


Figure 6. Mycelial Growth in the Sugar Banana Recipe

6. RESULTS

The growth of Shimeji mushroom mycelium in four different culture media over 3, 5, and 7 days is as follows:

Table 1. Different Culture Media Types at 3, 5, and 7 Days

Types of culture media	Diameter size (cm)			The density of Shimeji mushroom mycelium
	3 Days	5 Days	7 Days	
T ₁ : Potato	6.2000	3.2000	0.0000 ^a	+++
T ₂ : Sweet potato	0.3600	0.0000	0.0000 ^a	+
T ₃ : Pumpkin	0.9000	3.1600	0.0000 ^a	+
T ₄ : Sugar banana	0.6800	0.0000	0.0800 ^b	+
F-test	ns	ns	**	
ns	52.60	53.14	40.38	

Note: ** = Statistically significant difference (P<0.01)

* = Statistically significant difference (P>0.01)

ns = No statistically significant difference (P>0.0)

+++ means high

++ means moderate

+ means low

From Table 1, different types of culture media were observed over a period of 3, 5, and 7 days, and it was found that

On day 3, the highest average diameter (cm) was found to be T₁: potato with an average of 6.2000, and the second highest was T₃: pumpkin with an average of 0.9000.

On day 5, the highest average diameter (cm) was found to be T₁: potato with an average of 3.2000, and the second highest was T₃: pumpkin with an average of 3.1600.

On day 7, the highest average diameter (cm) was found to be T₄: sugar banana, with an average of 0.0800^b.

DISCUSSION

Based on the study of using potato, sweet potato, pumpkin, and sugar banana recipes for culturing Shimeji mushroom mycelium on PDA for 7 days, it was found that the mycelial growth in all four recipes on days 3 and 5 had the largest diameter (cm) in T₁: potato (6.2000, 3.2000 cm), followed by T₃: pumpkin (0.9000, 3.1600 cm), respectively. This is because the growth of mushroom mycelium is often associated with sugar. Hoa & Wang (2015) reported that the concentration of sugar used in the culture media, including the sugar content in each plant species, affects mycelial growth and colony diameter. However, if the sugar concentration is too high (more than 3% (w/v)), it can affect the metabolism process inside the mushroom cell. Nevertheless, in general, mushroom mycelium can grow on almost all types of agricultural plant-based culture media (Wongjirathiti & Yottakot, 2018). This finding is consistent with the research of Phanthavong et al. (2016), who found that the mycelial diameter of Yanagi mushrooms grown on 12 nutrient agar media recipes remained unchanged for 10 days. It is also consistent with the research of Jamnong (2012), who studied the influence of different culture media on the mycelial growth and beta-glucan content of reishi mushrooms. The mycelium was cultured using six different solid culture media, including ready-to-use agar, potato, sweet potato, ripe sugar banana, jackfruit seeds, and pumpkin. The results showed that the Linzhi mushroom mycelium exhibited significantly better growth ($P < 0.05$) on culture media containing jackfruit seeds, sweet potato, and potato as raw materials. The mycelium had a mean diameter of 8.88 ± 0.16 cm, 8.75 ± 0.48 cm, and 8.44 ± 0.44 cm, respectively.

Research Summary

From the study comparing different types of PDA culture media for the growth of Shimeji mushroom mycelium over a period of 3, 5, and 7 days, it was found that T₁: potato gave the highest average diameter (6.2000, 3.2000 cm) and the highest density (+++). The next in line was T₃: pumpkin (0.9000, 3.1600 cm) with a low density (+).

Suggestions

1. Recommendation for practical application: The recipe using potato can be utilized in the production of PDA culture media for Shimeji mushroom spawn to promote the highest mycelial growth.

2. Recommendation for future research: It is suggested to explore and experiment with recipes using pumpkin and other agricultural crops to study the effectiveness of developing mycelial growth for various types of mushrooms.

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