

THE QUALITY OF GROWTH AND YIELD OF *Pleurotus florida* ON
WILD WATER SPINACH *Ipomea aquatica* AND SAWDUST AS
SUBSTRATES

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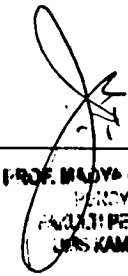
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ABSTRACT

A field experiment was conducted in the mushroom house of Faculty of Sustainable Agriculture, University Malaysia Sabah, Sandakan, Sabah (5.929665'N, 118.010928'E) to do the comparison between the wild water spinach (*Ipomea aquatica*) and sawdust as substrates for the growth and yield of white oyster mushroom (*Pleurotus florida*). The objectives of the study were to compare the growth and the yield of white oyster mushroom (*Pleurotus florida*) which will grow on sawdust and wild water spinach (*Ipomea aquatica*). The experimental design of this study was Completely Randomized Design (CRD). There were total five treatments which were 100% of sawdust (Controlled), mixture of 75% sawdust and 25% wild water spinach (*Ipomea aquatica*), mixture of 50% sawdust and 50% wild water spinach (*Ipomea aquatica*), mixture of 25% sawdust and 75% wild water spinach (*Ipomea aquatica*), and 100% of wild water spinach (*Ipomea aquatica*) each of them was replicated 3 times. Each replicate weighs 500 gram. Results were analysed by Statistical Analysis System (SAS), using One-way ANOVA procedure to test the effect between the wild water spinach (*Ipomea aquatica*) and sawdust as substrates for white oyster mushroom (*Pleurotus florida*) on the growth and production of the fruitbody. The results showed that there were no significant difference found in the length of the stem ($p>0.05$). In contrast, there were significant differences ($p<0.05$) in duration for complete mycelium run, duration for primordia formation, duration for fruitbody development, number of fruitbody, fresh weight, dry weight, moisture content, diameter of the cap, and biological efficiency. From this study, T₁ (100% Sawdust) required the least number of days (16.67 ± 0.58 days) in completion of mycelium run but shortest duration for the primordia to initiate belongs to T₂ (75% Sawdust +25% *Ipomea aquatica*) which only required 3.33 ± 0.58 days. For duration of fruitbody development, T₁ (100% Sawdust) required the shortest duration for the fruitbody to develop which was 2.00 days. T₁ (100% Sawdust) was the treatment with the significant highest number of fruitbody which was 10.00 ± 2.00 . The highest fresh weight of fruitbody was harvested from T₁ (100% Sawdust) which was 31.23 ± 1.63 g while the highest dry weight of fruitbody was obtained from T₂ (75% Sawdust +25% *Ipomea aquatica*) which had 4.64 ± 1.33 g in weight and it was statistically similar with T₁ (100% Sawdust) and T₃ (50% Sawdust +50% *Ipomea aquatica*). The maximum moisture content of fruitbody from T₁ (Control) was $86.00\pm 0.96\%$, while T₃ was the treatment with the minimum moisture content ($54.83\pm 3.30\%$) compared to other treatments. For the length of the stem, all treatments were statistically similar but numerically different to each of the treatments. Based on the numerical reading, it was indicated that T₂ (75% Sawdust +25% *Ipomea aquatica*) produced the longest stem in average which was 2.91 ± 0.88 cm in length. Diameter of the cap obtained from T₁ (100% Sawdust) had the largest diameter (4.65 ± 0.32 cm) among the treatments. T₁ (100% Sawdust) was the treatment with the highest biological efficiency ($18.37\pm 0.96\%$) among the treatments whereas T₅ (100% *Ipomea aquatica*) had the lowest biological efficiency which were $2.29\pm 1.02\%$. Hence, this study showed that the sawdust is the recommended substrate for *Pleurotus florida* and *Ipomea aquatica* failed to perform well with 100% composition in substrates but it might be recommended as a supplemental material to use with sawdust in *Pleurotus florida* cultivation so that the usage of sawdust could be minimised.



KUALITI PERTUMBUHAN DAN HASIL *Pleurotus florida* DENGAN KEGUNAAN KANGKUNG LIAR *Ipomea aquatica* DAN HABUK PAPAN SEBAGAI SUBSTRAT

ABSTRAK

Satu kajian telah dijalankan di rumah cendawan dalam Fakulti Pertanian Lestari, Universiti Malaysia Sabah, Sandakan, Sabah (5.929665'N, 118.010928'E) untuk membuat perbandingan antara kangkung liar *Ipomea aquatica* dan habuk papan sebagai substrat berkenaan kualiti pertumbuhan dan hasil untuk cendawan tiram putih (*Pleurotus florida*). Objektif kajian ini adalah untuk membandingkan kualiti pertumbuhan dan hasil cendawan tiram putih (*Pleurotus florida*) yang telah berkembang pada habuk papan dan kangkung liar (*Ipomea aquatica*). Rekabentuk eksperimen yang digunakan adalah Completely Randomized Design (CRD). Terdapat sejumlah lima rawatan dan rawatan adalah seperti berikut: 100% habuk papan (Kawalan), campuran 75% habuk papan dan 25% kangkung liar (*Ipomea aquatica*), campuran 50% habuk papan dan 50% kangkung liar (*Ipomea aquatica*), campuran 25% habuk papan dan 75% kangkung liar (*Ipomea aquatica*), dan 100% daripada kangkung liar (*Ipomea aquatica*) dan setiap gabungan telah diulangkan tiga kali. Keputusan telah dianalisis oleh Statistical Analysis System (SAS) menggunakan One-way ANOVA untuk menguji kesan di antara kangkung liar (*Ipomea aquatica*) dan habuk papan sebagai substrat untuk pertumbuhan dan pengeluaran cendawan tiram putih (*Pleurotus florida*). Keputusan menunjukkan bahawa tidak ada perbezaan yang signifikan didapati dari panjang tangkai ($p > 0.05$). Sebaliknya, terdapat perbezaan yang signifikan ($p < 0.05$) dalam jangka masa untuk kelengkapan miselium, jangka masa untuk pembentukan primordia, tempoh untuk pembesaran jasad buah, bilangan jasad buah, berat basah, berat kering, kandungan kelembapan, diameter topi, dan kecekapan biologi. Dari kajian ini, T₁ (100% habuk papan) perlukan jangka masa yang paling pendek (16.67 ± 0.58 hari) dalam menyiapkan miselium tetapi jangka masa yang paling pendek untuk pembentukan primordia tergolong T₂ (75% Habuk papan + 25% *Ipomea aquatica*) yang hanya perlukan 3.33 ± 0.58 hari. Untuk tempoh pembesaran jasad buah, T₁ (100% Habuk papan) memerlukan jangka masa yang paling singkat untuk membesarkan jasad buah iaitu 2.00 hari. T₁ (100% Habuk papan) adalah rawatan yang dapat bilangan jasad buah yang paling banyak iaitu 10.00 ± 2.00 . Berat basah tertinggi jasad buah yang telah dituai adalah dari T₁ (100% Habuk papan) sebanyak 31.23 ± 1.63 g manakala berat kering tertinggi jasad buah diperolehi daripada T₂ (75% Habuk papan + 25% *Ipomea aquatica*) sebanyak 4.64 ± 1.33 g dan ia sama dengan T₁ (100% Habuk papan) dan T₃ (50% Habuk papan + 50% *Ipomea aquatica*) secara statistik. Maksimum kandungan lembapan jasad buah dari T₁ (Kawalan) adalah $86.00 \pm 0.96\%$, manakala T₃ adalah rawatan dengan kandungan kelembapan yang minimum ($54.83 \pm 3.30\%$) berbanding dengan rawatan lain. Untuk panjang tangkai, semua rawatan adalah sama secara statistik tetapi berbeza dalam berangka antara rawatan. Berdasarkan bacaan berangka, ia telah menunjukkan bahawa T₂ (75% Habuk papan + 25% *Ipomea aquatica*) telah menghasilkan tangkai yang paling panjang iaitu 2.91 ± 0.88 cm dalam purata. Diameter topi diperolehi daripada T₁ (100% Habuk papan) mempunyai diameter tertinggi (4.65 ± 0.32 cm) di kalangan rawatan. T₁ (100% Habuk papan) adalah rawatan yang terdapat kecekapan biologi yang paling tinggi ($18.37 \pm 0.96\%$) antara rawatan manakala T₅ (100% *Ipomea aquatica*) mempunyai kecekapan biologi yang paling rendah hanya $2.29 \pm 1.02\%$. Oleh itu, kajian ini menunjukkan bahawa habuk papan adalah substrat yang disyorkan untuk *Pleurotus florida* dan *Ipomea aquatica* gagal menunjukkan prestasi yang baik dengan 100% komposisi substrat tetapi ia mungkin akan disyorkan sebagai bahan tambahan untuk digunakan dengan habuk papan dalam penanaman *Pleurotus florida* supaya penggunaan habuk papan dapat dikurangkan.



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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

$(\text{NH}_4)_2\text{SO}_4$	Ammonium sulphate
ANOVA	Analysis of Variance
BE	Biological Efficiency
C	Carbon
CaCO_3	Calcium carbonate
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	Calcium sulphate dihydrate
CO_2	Carbon dioxide
CRD	Completely Randomized Design
CaSO_4	Calcium Sulphate
FSA	Faculty of Sustainable Agriculture
HCl	Hydrochloric Acid
MC	Moisture Content
N	Nitrogen
NaOH	Sodium Hydroxide
PDA	Potato Dextrose Agar
PVC	Polyvinyl chloride
R	Replicate
SAS	Statistical Analysis Software
spp.	Species
T	Treatment
UMS	University Malaysia Sabah



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CHAPTER 1

INTRODUCTION

1.1 Background

Mushrooms are fungi which are classified into the Kingdom fungi due to its special fungal characteristics that are unique to animals or plants. Chang and Miles (2004) had defined mushroom in their book, *Mushroom Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact*, as follows: 'A mushroom is a macrofungus with a distinctive fruiting body which can be either epigeous (above ground) or hypogeous (underground) and large enough to be seen with the naked eye and to be picked by hand.' Mushrooms do not obtain nutrient by photosynthesis (Cho, 2004). They are heterotrophs which obtain nutrients from other sources. There are five main genera which are widely cultivated and they accounted for 85% of the edible mushroom production such as *Agaricus* species (30%), *Pleurotus* species (27%), *Lentinula* species (17%), *Auricularia* species (6%), and *Flammulina* species (5%) (Royse, 2014).

Mushroom cultivation is increasing tremendously, most probably due to their flavour, economic and ecological values, and medicinal properties (Aguilar-Rivera *et al.*, 2010). Based on Mishra (2014), stated in her book, *Techniques of Mushroom Cultivation*, the largest producer of edible mushroom in the world is China. Although only 5% of their production is exported, they are accounted for almost half of the cultivated mushrooms in the world. The consumption rate in China is also high, about 2.7 kg of mushrooms consumed by each person per year in a population of more than one billion people (Mishra, 2014).

Although the mushroom industry is still new and small in Malaysia, it is growing as one of the important food sources and extra income for farmers. Due to its high value, Malaysia government has listed mushroom into one of the seven high-valued crops that



will be grown commercially. Unfortunately, the current production in our country does not reach the demand of local market. Based on Haimid *et al.* (2013), the demand for fresh grey oyster mushroom was estimated to be around 50,000 kg per day but the growers are only able to produce 24,000 kg per day and this has induced the problem of increased price. Although there are many growers involved in mushroom cultivation, but 80% of them are small scale growers, 17% are medium scale and only 3% are large scale growers (Haimid *et al.*, 2013). Most of the small scale growers do not have high budget for advanced technology in mushroom cultivation to produce large amount of mushrooms. Therefore, mushroom cultivation technique should be exploited and improved before being introduced to mushroom growers in Malaysia so that the growers have an alternative choice to increase the yield and to meet the demand of local market.

Pleurotus species is the second most cultivated edible mushroom species after *Agaricus* species. It is found that the *Pleurotus* species can be grown on many different types of substrates, therefore it become a widely grown edible mushroom (Fernandes *et al.*, 2015). *Pleurotus* species is commonly known as oyster mushroom. There are approximately 40 species under genera *Pleurotus* and 25 species are commercially cultivated (Menaga *et al.*, 2013). *Pleurotus florida* is one of the mushrooms that belongs to *Pleurotus* species. It is the subspecies of *Pleurotus ostreatus*. Therefore, the appearance, odour, and taste of both mushrooms are similar. *Pleurotus florida* grows widely in temperate, subtropical and tropical zones. The range of fruiting temperature of *Pleurotus florida* is wider than other *Pleurotus* species therefore the yield of the *Pleurotus florida* is the highest among the *Pleurotus* species (Kong, 2004).

Ipomea aquatica commonly known as water spinach is one of the perennial or annual weeds. It can behave as aquatic, semi-aquatic or terrestrial plant based on its current habitat. *Ipomea aquatica* grow well in warm temperate zone, therefore it is commonly distributed in the South and South-East Asia, tropical Africa, South and Central America and Oceania. *Ipomea aquatica* is one of the important leafy vegetable for areas like South China and Southeast Asia but most of the consumers prefer the cultivated *Ipomea aquatica* instead of wild *Ipomea aquatica*. *Ipomea aquatica* also a troublesome weed in some aspect.

Plant growth hormone that exists in the shoot tips of *Ipomea aquatica* promotes the stem of the plant to regenerate and grow vigorously. Therefore, it is able to

regenerate around every three weeks once the fragment exists. Besides, the long floating stems may form a dense network and spread over the surface of lake, irrigation or drainage system that interfere the human activities and interrupt the water flow. It is also a major broad-leaved aquatic weed of dry-seeded wetland rice. *Ipomea aquatica* grows rapidly and this may acquire an exceptionally high cost to control it. Utilisation of the wild *Ipomea aquatica* in mushroom cultivation does not only solve the problem of over-spreading of weeds but also helps to reduce the cost in weed control.

Mushroom cultivation is getting important in Malaysia. Alternatives materials use as substrates in mushroom cultivation is one of the concern topic in the world including Malaysia to minimise the cultivation cost without degrading the quality and quantity of the mushroom production. This research is conducted to compare the growth quality and fruitbody production of *Pleurotus florida* using wild *Ipomea aquatica* and sawdust.

1.2 Justification

Sawdust is the most commonly used substrates in mushroom cultivation. Since the usage of sawdust is constantly being improved and it has been quite established, the demand of sawdust may increase. Besides, the issue of deforestation is getting focus from the government and society, therefore most of the wood-based products are being replaced by other materials. This will indirectly affect the sources of sawdust for mushroom cultivation. Shortage of sawdust sources will increase the purchasing cost of sawdust.

Ipomea aquatica is one of the common weeds that can be found easily in rural area including the Faculty of Sustainable Agriculture, Sandakan (Appendix C: Figure 1.0) (Appendix C: Figure 2.0). High cost in weed control has always been problem as the regeneration time of *Ipomea aquatica* is fast. *Ipomea aquatica* is able to regenerate every three weeks through its rhizomes. The plant growth hormone at the tip of the shoots induced the fast growing ability. Therefore, *Ipomea aquatica* is a renewable source and can be obtained easily. These criteria are important to prevent the problem of supply shortage. Utilisation of the abandoned and unwanted *Ipomea aquatica* in mushroom cultivation can solve the weeds problem significantly and reduce the usage of chemical that increase the weeding cost.

An alternative substrates material which has no source constraint will help in expansion and improvement of mushroom industry, especially in mushroom cultivation. Many growers will feel interested in growing mushroom due to its reduced cost. Since the production cost may reduce after utilising *Ipomea aquatica* as substrates, mushroom growers can obtain more profit consequently. Meanwhile, the market demand of white oyster mushroom (*Pleurotus florida*) is increasing tremendously. The mushrooms can be grown in any place as long as there is an ample ventilation and shade. Many agro-waste and residue can be reused to grow mushroom. In promoting health, mushroom as a source of non-starchy carbohydrate and protein can be promoted as partial substitution of animal protein. Therefore, improvement in the production yield of oyster mushroom is crucial.

1.3 Significance of the Study

This research study could provide more information about the change of yield and growing condition of *Pleurotus florida* by using *Ipomea aquatica* as substrates. Although sawdust is the recommended substrates for mushroom cultivation, the previous study showed that *Ipomea aquatica* substrates also gave a good result in *Pleurotus sajor-caju* production. It produced the highest mushroom yield among the aquatic weeds. Comparison between sawdust substrates and *Ipomea aquatica* substrates for *Pleurotus florida* production is needed to prove that *Ipomea aquatica* is a good alternative material of substrates. Utilisation of *Ipomea aquatica* as substrates will be able to solve the serious weed problem and reduce the input cost of mushroom cultivation. As a result, growers have another choice for substrates material and reduce the input cost in purchasing the sawdust.

1.4 Objectives

- a) To evaluate the effect of both wild water spinach (*Ipomea aquatica*) and sawdust as substrates on the yield of white oyster mushroom (*Pleurotus florida*).
- b) To evaluate the effect of both wild water spinach (*Ipomea aquatica*) and sawdust as substrates on the growth of white oyster mushroom (*Pleurotus florida*).

1.5 Hypotheses

- a) H_0 : There is no significant difference in yield of white oyster mushroom (*Pleurotus florida*) on the wild water spinach (*Ipomea aquatica*) and sawdust as substrates.
 H_a : There is significant difference in yield of white oyster mushroom (*Pleurotus florida*) on the wild water spinach (*Ipomea aquatica*) and sawdust as substrates.
- b) H_0 : There is no significant difference in growth of the white oyster mushroom (*Pleurotus florida*) on the wild water spinach (*Ipomea aquatica*) and sawdust as substrates.
 H_a : There is significant difference in growth of the white oyster mushroom (*Pleurotus florida*) on the wild water spinach (*Ipomea aquatica*) and sawdust as substrates.

CHAPTER 2

LITERATURE REVIEW

2.1 Mushroom

2.1.1 Description

Mushrooms are fleshy, spore bearing fruit bodies, which are classified into the Kingdom fungi. Mushrooms are not able to generate nutrients by photosynthesis as it do not contain chlorophyll. Mushrooms are heterotrophs which obtain nutrients from other sources. Special fungal characteristics of mushrooms had made them different from animals and plants. Chang and Miles (2004) had defined mushroom in their book, *Mushroom Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact*, as follows: 'A mushroom is a macrofungus with a distinctive fruiting body which can be either epigeous (above ground) or hypogeous (underground) and large enough to be seen with the naked eye and to be picked by hand.' Fruitbody of mushroom is formed by cap and stalk.

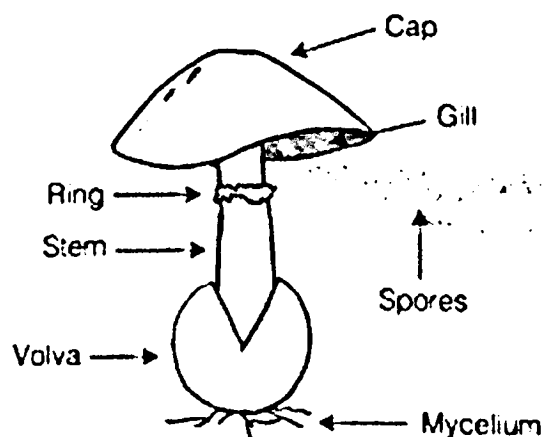


Figure 2.1 Morphology of mushroom
Source: Cho, 2004



The mushroom industry is getting concern from worldwide and growing rapidly. Mushroom cultivation increases tremendously, most probably due to their flavour, economic and ecological values, and medicinal properties (Aguilar-Rivera and de Jesús-Merales, 2010). China is the major producer of edible mushroom followed by United America, Netherland, Poland, and Spain (Mat-Amin *et al.*, 2014). China has produced 25.7 million tons of edible mushroom in year 2011. Although only 5% of the production is for exportation but this accounts for almost 40% of the total world mushroom exportation (Zhang *et al.*, 2014). There are five main genera that are widely cultivated and account for 85% of the edible mushroom production such as *Agaricus* species (30%), *Pleurotus* species (27%), *Lentinula* species (17%), *Auricularia* species (6%), and *Flammulina* species (5%) (Royse, 2014) (Appendix C: Figure 3.0).

Although mushroom industry in Malaysia is still new and small but it is growing steadily these few years. Mushroom industry is growing as one of the food sources and extra income for farmers. Due to its high value, Malaysia government has listed mushroom into one of the seven high-valued crops that will be grown commercially. There are around 1,000 tons of mushroom produced by Malaysia annually for exportation and domestic consumption (Mat-Amin *et al.*, 2014). The demand of mushroom in local market is increasing nowadays because people have increased concern about the nutritional value of foods they consume. However, the production of the mushroom in Malaysia does not meet the high demand of mushroom in local market. The high demand of mushroom showed that it is a high potential industry in Malaysia although it is new growing industry.

The climate of Malaysia is suitable for 17 varieties of mushroom to grow but there are only eight varieties being cultivated commercially. The commercial varieties in Malaysia are abalone (*Pleurotus cystidiosus*), rice straw (*Volvariella volvacea*), telinga kera (*Auricularia polytricha*), grey oyster (*Pleurotus sajor-caju*), white oyster (*Pleurotus florida*), red oyster (*Pleurotus flabellatus*), ganoderma (*Ganoderma applanatum*) and shiitake (*Lentinus edodes*) (Haimid *et al.*, 2013).

2.2 *Pleurotus* Species

2.2.1 Description

Pleurotus species commonly known as oyster mushroom due to its oyster-shaped cap. There are approximately 40 species under the *Pleurotus* species but only 25 species are commercially cultivated (Menaga *et al.*, 2013). It is the second most cultivated edible mushroom species after *Agaricus* species. There are some commercial species under *Pleurotus* species which are suitable to be grown in various climatic zone such as *Pleurotus eryngii* (King oyster mushroom), *Pleurotus citrinopileatus* (Golden oyster mushroom), *Pleurotus djamor* (Pink oyster mushroom), *Pleurotus ostreatus* (Tree oyster mushroom), *Pleurotus cystidiosus* (Abalone oyster mushroom), *Pleurotus sajor-caju* (Grey oyster mushroom) and *Pleurotus florida* (White oyster mushroom).

2.2.2 Specification of *Pleurotus* Species

Oyster mushroom became popular in the mushroom industry because it is easy to cultivate and with high nutritional value. It is found to be able to grow in temperate to tropical zones with the temperature range 12°C to 32°C (Patel *et al.*, 2012). *Pleurotus* species contain enzymes complex that are able to breakdown the polysaccharides such as lignin, cellulose, and hemicellulose that commonly found in the biomass and crop residue (Chang and Miles 2004). This process of converting the unwanted waste into another food source with high protein content can be an alternative source of protein to replace animal's protein.

Many researches have proved that *Pleurotus* species is able to grow under wide varieties of substrates that do not require special composting such as banana leaves, corn stalk, and sugarcane bagasse (Poppe, 2004). Substrates of *Pleurotus* species is easy to obtain and prepare compared to other mushroom species. This can reduce the input cost such as labour and raw materials cost which can maximise the profit from mushroom cultivation. Cultivation of *Pleurotus* species is environmental friendly yet profit returnable which can bring extra income to growers.

2.2.3 Life Cycle

There are two phases in mushroom life cycle, which are vegetative growth and reproductive growth (Appendix C: Figure 4.0). In vegetative growth, mycelium will establish and produce enzyme to digest the substrate components for food absorption. Visible mushroom formed under optimum condition from vegetative growth to reproductive growth phase. Optimum condition includes adequate light intensity, low temperature, high humidity and oxygen. Visible mushroom also known as fruit body formed from the aggregation of mycelium (Cho, 2004).

2.3 *Pleurotus florida*

2.3.1 Morphology

Pleurotus florida is commonly known as white oyster mushroom because of its white colour oyster shaped cap. 'Pleurotus' originated from Greek word 'pleura', which means formed from the lateral position of the stem while 'florida' refers to the origin location (Stamets and Chilton, 1983). *Pleurotus florida* is considered the subspecies of *Pleurotus ostreatus*. Therefore, the appearance, odour, and taste of both mushroom are almost the same.

Pleurotus florida grows in a large numbers on dead wood naturally and acts as primary decomposer to obtain nutrient by decomposition process (Har-peled and Lee, 2012). It is growing widely in temperate, subtropical, and tropical zone. Therefore, the range of fruiting temperature for *Pleurotus florida* is wider than other *Pleurotus* species. Fruiting induction like cold shock is not required for *Pleurotus florida* and it showed the highest productivity among the *Pleurotus* species (Kong, 2004).

Based on the description about the morphology of *Pleurotus florida* by Stamets and Chilton (1983) in the book 'The Mushroom Cultivator: A Practical Guide To Growing Mushrooms At Home', the cap is tongue-shaped, maturing to a shell-shaped form. The diameter of the cap is around 1.5 to 8.5 cm and the stem is around 0.1 to 0.5cm. The colour of the cap will change under different temperature, it is light brown when cultivated under low temperature (10 to 15°C), and it turns white pale to yellowish colour when the temperature is increased from 20°C to 25°C (Har-peled and Lee, 2012).

The flesh of *Pleurotus florida* is thin and white. The edge is flat and occasionally wavy. Its gills are white, extend downward and space broadly. The stem is short when it first appeared and attached in the center, but it will absent as it ages. The colour of spore print is creamy white (Stamets and Chilton, 1983).

2.3.2 Nutritional and Chemical Composition of *Pleurotus florida*

Nutritional and chemical composition greatly influence the medicinal value of mushroom. Nutritional value is also one of the reasons that contributes to the popularity of *Pleurotus florida* (Har-peled and Lee, 2012). Therefore, there are many researches regarding the nutritional and chemical composition of the *Pleurotus florida*. Har-peled and Lee (2012) have mentioned that there are higher amount of protein, lipid, phosphorus, iron, thiamine, and riboflavin found on *Pleurotus florida* compared to other edible mushrooms. Besides, this edible mushroom also contains 18 essential amino acids such as isoleucine, lysine, methionine, tyrosine, phenylalanine, cysteine, tryptophan, tyrosine, arginine, valine, histidine, aspartic acid, alanine, glycine, serine, proline, and glutamic acid. Nutrient contents and mineral contents found in 100 g dry matter *Pleurotus florida* from the study of Sánchez (2010) are as shown in Table 2.1 and Table 2.2.

Table 2.1 Nutrient contents in 100g of dry matter *Pleurotus florida*

Nutrient contents	Weight (mg)
Lipids	1.05
Sugars	8.95
Protein	15.10
Fiber	4.40
Ash	10.60

Source: Sánchez, 2010

Table 2.2 Mineral contents in 100g of dry matter *Pleurotus florida*

Mineral contents	Weight (mg)
Calcium	0.40
Magnesium	1.50
Potassium	14.75
Sodium	0.25
Phosphorus	13.35
Manganese	0.80
Iron	0.08
Copper	0.05
Zinc	0.05

Source: Sánchez, 2010

Nutritional composition analysis for mushroom varies due to many factors such as the types of growing substrates, cultivation method, harvesting stage and others (Alam *et al.*, 2007).

2.4 Spawn Production

Spawn is derived from an old French verb 'expendre' and Latin 'expandere' with the meaning of spread and expand. In the book '*Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact*', Chang and Miles (2004) have mentioned the definition of spawn as 'the mycelium of fungi, especially of mushrooms grown to be eaten, used for propagation' which describe the characteristic of spawn. The role of spawn in mushroom cultivation is similar to the role of seed in crop production where both are propagation materials (Gogoi *et al.*, 2006). Spawn is used to inoculate the substrates for mushroom grow. Spawn production includes three steps which are tissues culturing, sub-culturing, and grain culturing. All the steps above have to carry out under aseptic condition to prevent contamination which may affect the mycelium growth.

2.4.1 Tissues Culturing

Tissues culture is considered the best way to obtain the new culture because this method is able obtain the new culture that possess the exact genetic characteristics as the living mushroom. Tissues culture must be taken from the fresh mushroom, which have been harvested within 24 to 48 hours to prevent over-dry and mature that make it difficult to obtain the tissues from it. The parts of the fruit body to obtain the tissues as recommended by Stamets and Chilton (1983) are the cap, the upper region of the stem, and the area where the gill plate joins the underside of the cap. Mushroom tissues will be cultured on agar-agar medium, so that the mycelium can grow on a flat and solid surface. Agar-agar medium does not contain any nutrient, therefore, some supplementary nutrient will be added into the agar-agar medium formulation for nutrient supplement such as barley malt extract (Ogden and Prowse, 2004). Incubation temperature should be controlled for better mycelium growth.

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