Faculty of Resource Science and Technology

Mycelial Growth Performance of the *Pleurotus sajor-caju* (Fr.) Singer Strains on Sawdust Substrate

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Bachelor of Science with Honours

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Supervisor: Dr. Mohamad Hasnul Bin Bolhassan

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ABSTRACT

Pleurotus sajor-caju, mostly known as the grey oyster mushroom, is a popular delicacy in Malaysian dishes. Currently, the production of mushrooms is relatively low compared to the demand by the consumer. Therefore, cultivating the best mushroom strain with the best growth performance will alleviate mushroom production. This study evaluated the growth performance of two *P. sajor-caju* strains by measuring the mycelium linear growth and days for the mycelium to fill up the bag. The strains were cultivated in sawdust substrate supplemented with rice bran and calcium carbonate at a dry matter basis in the ratio of 100:10:1. The linear mycelia growth measurement was recorded every three days at intervals for three weeks. The result shows no significant difference (P>0.05) in mycelia growth and days needed to fill the substrate bag, as both strains took 22 days to fully colonise 12 cm substrate bags. However, the texture and density of mycelia exhibited by strain TK48 were more abundant and denser than strain TK60. Thus, the *P. sajor-caju* strains cultivated on sawdust produced similar mycelial growth performance.

Key words: *Pleurotus sajor-caju*, mycelia growth, sawdust

ABSTRAK

Pleurotus sajor-caju atau lebih dikenali sebagai cendawan tiram kelabu merupakan makanan yang terkenal dalam masyarakat di Malaysia. Akan tetapi, tahap pengeluaran semasa cendawan adalah lebih rendah berbanding dengan tahap permintaan pembeli. Oleh itu, penanaman cendawan dengan menggunakan strain yang terbaik dapat meningkatkan lagi tahap pengeluaran cendawan. Kajian ini telah dijalankan untuk menilai prestasi pertumbuhan P. sajor-caju dengan melakukan pemerhatian terhadap pertumbuhan miselia setiap strain. Dua jenis strain P. sajor-caju telah ditanam dalam substrat habuk kayu bersama dedak padi dan kalsium karbonat sebagai suplemen dengan nisbah 100:10:1. Ukuran pertumbuhan miselia telah direkod dalam selang waktu tiga hari selama tiga minggu. Keputusan yang diperolehi menunjukkan tiada sebarang perbezaan yang signifikan (P>0.05) dalam pertumbuhan miselia dan jumlah hari yang diperlukan untuk memenuhkan beg substrat. Hal ini kerana kedua-dua strain tersebut memerlukan 22 hari untuk memenuhkan beg substrat yang berukuran 12 cm. Akan tetapi, tekstur dan kepadatan miselia strain TK48 adalah lebih tebal dan lebih padat berbanding dengan strain TK60. Oleh itu, strain P. sajor-caju yang ditanam menggunakan habuk kayu mempunyai prestasi pertumbuhan miselia yang sama.

***Kata kunci**: Pleurotus sajor-caju, pertumbuhan miselia, habuk kayu*

Table of Contents

List of Abbreviations

%	percentage
$^{\circ}C$	degree Celsius
BE	biological efficiency
cm	centimetre
$CaCO_3$	Calcium carbonate
g	gram
kg	kilogram
mL	milliliter
mm	millimeter
PDA	Potato Dextrose Agar
PVC	polyvinyl chloride
SPSS	Statistical Package for Social Sciences

List of Figures

List of Tables

List of Appendices

CHAPTER 1

1.0 Introduction

Mushrooms, puffballs, hoofed fungi, coral fungi, and other large types of fungi belong to the Basidiomycetes and Ascomycetes groups (Torres et al., 2020). Mushrooms prefer moist environments and feed on decomposing lignocellulosic materials like leaves and stems, animal dung, logs, twigs, damp soils, and so on. They have a variety of roles in the ecosystem. For instance, most of them are decomposers and saprophytes, while others are parasites or form mutualistic relationships with other organisms (Reyes et al., 2009).

Mushroom is one of the popular food and delicacy in Malaysia. It is also selected as one of the seven agriculture commodities that have the potential to be cultivated commercially to generate income for farmers and the country due to their contribution to the gross national income (GNI) (Amin et al., 2014). Nowadays, the demand for mushrooms is skyrocketing, but insufficient supply is produced to meet the demand. It is expected that the mushroom industry and market in Malaysia will grow more in the future because the number of mushroom growers keeps increasing.

Pleurotus is one of the most commercialised mushroom genera in the world, and it is closely related to the *Agaricus* and *Lentinula* genera in terms of commercial importance (Rosado et al., 2003). *Pleurotus sajor-caju* (Fr.) Singer, or grey oyster mushroom, is one of the most cultivated mushrooms in Malaysia (Amin et al., 2014). Thus, their high ability to colonise and degrade substrates makes it easy for local farmers to cultivate the oyster mushroom using agriculture waste as substrates. Their taste and medicinal properties influence farmers and growers to cultivate the mushroom on a bigger scale.

The grey oyster mushroom is the world's second most significant fungus in terms of production, accounting for 25% of all cultivated mushroom production (Amin et al., 2014).

It has grown in popularity due to its ease of cultivation, great production potential, and nutritional value. Not only that, their colonising and degrading behaviour make it more popular among the farmers because of their wide range of lignocellulosic substrates, which provide another economical option for the cultivation substrates by using agricultural waste as their substrates.

1.1 Problem Statement

The diversity of *P. sajor-caju* strains in the market provides many options for the grower to select based on their preference. It is preferable for a strain to produce a high yield. Therefore, many strains of *P. sajor-caju* mushroom have been cultivated, but their overall performance is not yet determined. Thus, a study on different *P. sajor-caju* strains is necessary to identify the best *P. sajor-caju* strain based on their growth performance on sawdust substrate.

1.2 Objective

The aim of this study is:

- To evaluate the mycelial growth performance of *P. sajor-caju* strains on sawdust substrate.

CHAPTER 2

2.0 Literature Review

2.1 *Pleurotus sajor-caju* (Fr.) Singer

2.1.1 Taxonomy and Distribution

Pleurotus sajor-caju is a mushroom species from the genus *Pleurotus* from the Pleurotaceae family. *Pleurotus sajor-caju* belongs to the white-rot fungi group and the basidiomycetes class because of its white mycelium production (Tsujiyama & Ueno, 2013). They are in the Agaricomycetes class and grouped into the gilled mushroom group under the Agaricales order.

Apart from *P. sajor-caju,* there are other species in this genus that is hugely cultivate in tropical region such as *Pleurotus ostreatus*, *Pleurotus pulmonarius*, *Pleurotus eryngii* (king oyster), *Pleurotus florida* (white oyster), *Pleurotus cystididiosus* (maple oyster), *Pleurotus flabellatus* (red oyster) and *Pleurotus citrionipileatus* (yellow oyster) (Wan Mahari et al., 2020). *Pleurotus sajor-caju,* also widely known as a grey oyster mushroom because of its appearance, resembles an oyster shell. It also can be easily recognised due to its white spore print, gills attachment, and eccentric stipe.

Pleurotus sajor-caju grows naturally in hilly forests and is cultivated worldwide in temperate and subtropical climates as in Malaysia (Singh et al., 2011). Temperatures of 20-35 °C and humidity levels of 65–70 % are ideal for *P. sajor-caju* for 6 to 8 months of the year (Wan Mahari et al., 2020). They typically grow on waste materials and colonise dead organic materials. It grew on dead cottonwood, oak, or maple and acted as major decomposers, converting dead organic tissues into the necessary nutrients for growth (Wan Mahari et al., 2020). *Pleurotus sajor-caju* is marketed in fresh, dried, and powder form. It is usually used in a variety of food and medicine.

2.1.2 Nutritional and Medical Properties

Pleurotus sajor-caju is cultivated not only because of its taste but also because of its nutritional value. It is considered a pool of nutrients because they contain many nutrients such as fibre, minerals, vitamins, protein and chitin (Pokhrel, 2016). In addition, the mushroom has a low amount of calories and fat and significant amounts of dietary fibre, beta-glucan, vitamin B, and other beneficial minerals (Wan Rosli & Solihah, 2014).

According to Jantaramanant (2014), this mushroom contains many biologically active compounds, including polysaccharides, pleuran and proteoglycans. Pleuran helps to stimulate immunity and reduce blood cholesterol, and proteoglycans help with immune-modulatory and antitumor activities (Assis et al., 2013). Therefore, this mushroom also is consumed because of its medicinal properties. In addition, they have historically been used to cure and prevent cancer, diabetes, hypertension, constipation, and other diseases (Agrawal et al., 2010).

2.1.3 Life Cycle

The grey oyster mushroom has two growth phases. They are the vegetative phase and reproductive phase. Mycelia or mushroom roots develop underneath and inside the substrate during the vegetative phase and play an essential role in the nutrition synthesis process from the substrate material (Wan Mahari et al., 2020). They release enzymes to break down the substrate and absorb nutrients to develop. The mushroom will then reproduce under ideal conditions of moderate temperature (20-25 °C), relative humidity (65-70 %), oxygen flow and light intensity (Bellettini et al., 2019). The mushroom fruiting bodies can be mass-produced under these conditions by repeating these two growth phases, the vegetative and reproductive phases (Choi, 2004).

Wan Mahari et al. (2020) stated that *P. sajor-caju* life cycle began with the germination of basidiospores found beneath their cap. After the mushroom reproduces, the spores are released into the air. The spores will be carried by the wind and landed on suitable substrates where they will mate with other spores. *Pleurotus* spp. has four mating forms. Two different mating types of spores generate a monokaryotic mycelium, which grows into a dikaryotic mycelium. The dikaryotic mycelium evolves into fruit bodies with specialised features called basidia. Primordial emergence denotes fruit body creation. After meiosis, the new basidiospores continue to create spores, released when mature, and the reproduction cycle begins again.

2.2 Cultivation of *Pleurotus sajor-caju*

2.2.1 Basic Substrate

Many materials, such as agricultural and industrial waste, can be utilised as mushroom substrates. These wastes are cheap, easy to obtain, recyclable and reusable. In addition, these organic components include lignin, cellulose, and other compounds that oyster mushrooms can degrade using their enzyme system (Wan Mahari et al., 2020).

Sawdust is one of the commercial substrates used in the production of mushrooms in Malaysia (Saidu et al., 2012). The usage of sawdust as a substrate for mushroom cultivation is often supplemented with other additional substrates to improve nutrient content in sawdust. This is because the lignocellulosic components in sawdust are often poor in protein and insufficient for mushroom culture, necessitating nitrogen, phosphate, and potassium (Oseni et al., 2012). In addition, to release the needed elements for mushroom mycelium establishment, the sawdust substrate for mushroom culture should be composted for a length of time to break down the cellulose and lignin components of the wood (Obodai & Vowotor, 2002). Although sawdust has low protein content, it is still selected as one of the main substrates for mushroom cultivation. According to Islam (2009), sawdust is one of the most promising substrates for oyster mushroom cultivation.

There are four essential nutrient components that are needed by mushrooms to grow. The nutrients needed for mushroom growth are carbon, nitrogen, minerals and vitamins (Razak, 2013). Hence, to guarantee the success of mushroom cultivation, all these four components must be present in sufficient amounts in the basic substrate, focusing on carbon and nitrogen balance because carbon and nitrogen play a larger part in the overall mushroom growth process (Razak, 2013). For example, if the nitrogen content is high in a substrate, the excessive nitrogen will degrade lignin, and mycelial development will be hindered (Bellettini et al., 2019). At the same time, carbon is also essential for mushroom growth since it provides energy and helps build organic compounds such as membrane lipids, proteins, and polysaccharides, which make up the cell wall (Miles & Chang, 2014).

Monosaccharides, polysaccharides, and oligosaccharides are examples of various forms of carbon sources required for mycelium growth, particularly polysaccharides like cellulose and hemicelluloses (Razak, 2013). The majority of polysaccharides are degraded to generate sugar. On the other hand, mushroom requires nitrogen sources such as acid amino, urea, ammonium, and nitrate to synthesise proteins, purines, and pyrimidines, as well as to aid in the production of chitin (Hoa et al., 2015).

Most of the time, extra minerals are required to boost the growth of Basidiomycetes mushrooms. Mushrooms require more vitamins during the primordia and fruiting body production phases than during the vegetative or mycelia growth phases. Besides nutrients, substrate structure is also important for the growth of mushrooms because the design of the mycelium network projected is influenced by the structure of the fruiting substrate (Jambaro et al., 2014)

2.2.2 Supplement of Substrate

A substrate with low nutrients must be supplemented with additional nutrition. Supplements are used to improve nutritional value, speed up growth, and increase mushroom production (Philippoussis, 2009). A wide range of supplements can be utilised for mushroom cultivation. For example, rice bran, wheat bran, spent grain, spent yeast, molasses, cotton and coffee wastes, and a range of other protein-rich materials. Many studies have found that adding supplements to the basic spawn and fruiting substrates increases mushroom output, depending on the species of mushroom cultivated, supplement types, and supplement concentrations (Razak, 2013). On the other hand, the density of mycelia will be low if the substrate is deficient in nutritional content, which results in the growth of green mould (Wan Mahari et al., 2020).

Rice bran is used as a supplement to balance the ratio of carbon and nitrogen in the substrate mixture (Saidu et al., 2012). Substrate supplementation is a technique used in *Pleurotus* sp. production to increase yield and development (Carvalho et al., 2010). This is supported by Royse (2002), mushroom production can be sped up, and yields can be increased by adding nitrogen and rapidly degradable carbohydrates supplied by the supplements or additives. A study by Ibrahim et al. (2015) discovered that rice bran contains various nutrients, including carbohydrates, amino acids, and minerals, that can stimulate the growth of *P. sajor-caju* when added to sawdust (Ibrahim et al., 2015).

Calcium carbonate ($CaCO_3$) is another supplement that is usually added to provide additional calcium during the formation of mushroom fruiting bodies. During mushroom fruiting body formation, calcium concentrations are higher in the apical regions of developing hyphae than in the distal areas of hyphae (Royse & Sanchez-Vazquez, 2003). Therefore, the availability of calcium in substrates during mushroom cultivation stimulate the mushroom's hyphal tips to grow and develop more. According to Wang (2010), oyster mushroom yields were significantly increased when the fruiting substrate was supplemented (Wang, 2010). However, excessive supplements can also cause adverse mushroom effects. Excessive supplementation can cause the substrate's effects on mushroom production to be reduced (Razak, 2013). The standard ratio for sawdust, rice bran and calcium carbonate is 100:10:1 (Ibrahim et al., 2015).

2.2.3 Cultivation Method

Pleurotus sajor-caju is one of Malaysia's most popular mushrooms due to its short cultivation period and relatively low cost. Local farmers prefer them since they are easy to cultivate and cheap to sterilise the substrate. Moreover, the material needed for oyster mushroom cultivation is readily available. *Pleurotus sajor-caju* can be grown naturally using cut logs perforated with holes or synthetically utilising agriculture waste as a nutrient source (Wan Mahari et al., 2020). Nowadays, Malaysian mushroom growers prefer to cultivate this species in plastic bags, commonly known as cylindrical baglogs. The cultivation is commonly done in a cylindrical plastic bag with rice bran or sawdust substrate for growing fruiting body whilst using sawdust and grains for spawning.

Cylindrical baglogs cultivation method is easy to implement by growers. A 15 cm x 30 cm cylindrical plastic bag substrate container is used for growth (Mamiro et al., 2014). A hole is pierced into one end of the cylindrical baglog to facilitate inoculation and harvesting. Baglogs are frequently made from rice bran and sawdust (Utami & Susilawati, 2017). Before further processing, the opening at the baglog's end is sealed with a PVC cover when the mushroom growing media weighs around 1 kg (Wan Mahari et al., 2020). The cylindrical baglogs will then be autoclaved to kill bacteria and avoid contamination. After cooling the substrate to room temperature, *P. sajor-caju* spawn is put into it for inoculation. The baglog will be sprayed twice daily to keep the mushroom moist. The mushroom takes 30-35 days to fully colonise the substrate. In the meantime, the inoculated baglogs will be stored in an open air or well-ventilated air room. This is due to the fact that the presence of oxygen is critical throughout the fructification or the formation of mushroom bodies process (Wan Mahari et al., 2020).

2.2.4 Cultivation Conditions

2.2.4.1 Intrinsic Factors

Apart from the type of substrate and nutrient, other internal factors influence the growth of *P. sajor-caju.* The pH of the substrate plays an important role in the colonisation and the growth of mycelia on the cultivation substrate. Choi (2004) reported that 5.0-6.5 pH is the optimum pH range for the growth of mycelia in the substrate (Choi, 2004), but on the contrary, Bellettini et al. (2019) reported that the optimum range for the growth of mycelium and the development of their fruiting body is between 6.5-7.0 pH (Bellettini et al., 2019). Based on both studies conducted by Choi (2004) and Bellettini et al. (2019), it can be concluded that when the pH of the substrate falls below 5, mycelia growth is disrupted. When it falls below 4, mycelia growth is totally stopped. On the other hand, mycelia develop quicker with an odd structure when the pH exceeds the ideal range. Hence why *P. sajor-caju* need to be cultivated within its optimum pH range. At the correct pH, mycelia or mushrooms may effectively take nutrients from the substrate (Khan et al., 2013).

Moisture content is another internal factor that contributes to the growth of *P. sajor-caju.* The mushroom fruiting body can absorb nutrients transported by mycelia through sufficient moisture in the substrate (Oei & Nieuwenhuijzen, 2005). The moisture level in the substrate should not be too low and should not too high. Low moisture levels will cause the death of the mushroom fruiting body, while high moisture levels will disturb the mushroom growth rate (Bellettini et al., 2019). Therefore, the optimum moisture content level for *P. sajor-caju* is between 65%-80% (Islam et al., 2009).

2.2.4.2 Extrinsic Factors

Extrinsic factors are the external factors that influence the development of mushroom mycelia and the growth of the mushroom fruiting body. One of the important extrinsic factors is temperature. Temperature is important because the cultivated mushroom is usually placed in a mushroom room. Therefore, an optimum temperature in the room must be maintained.

Oyster mushroom is widely cultivated in our country because it is suitable for cultivation in the tropical climate and can easily adapt to a wide range of temperatures between 15^{o}C-30^{o}C (Ogundele et al., 2014). The optimum temperature for *P. sajor-caju* mycelia growth is 25^{o}C (Choi, 2004), while the optimum temperature for *P. sajor-caju* fruiting body formation is 18^{o}C-30^{o}C (Kibar & Peksen, 2008). At lower temperatures, *P. sajor-caju* growth will decrease because lower temperature with the combination of dry conditions causes mushrooms to be formed with shorter stalks and smaller cap sizes than other mushrooms cultivated at an optimum temperature (Sher et al., 2010). Apart from that, if *P. sajor-caju* is cultivated at a higher temperature, the mushroom will die due to the competition with green mould. At a temperature higher than 30^{o}C, *Trichoderma longibrachiatum*, mostly known as green mould, will grow on cultivation substrate and disrupt *P. sajor-caju* mycelia formation and lead to the death of *P. sajor-caju* in the long term (Shaiesta & Sahera, 2011).

Another external factor that plays an important role in developing a mushroom fruiting body is optimum relative humidity. An optimum relative humidity will produce a high yield of mushrooms. The perfect environment for *P. sajor-caju* spawn running and mycelia stimulation is 60%–80% humidity (Bellettini et al., 2019) or 80%-85% (Samuel & Eugene, 2012). In addition, a higher relative humidity of about 90%-95% is needed during the fruiting stage of *P. sajor-caju* (Choi, 2004). Although keeping the substrate mixture moist is critical, too much humidity may reduce mushroom yields. Overly humid conditions stimulate water vapour condenses on the mushroom's exterior and promote microbial growth and discolouration, negatively affecting mushroom growth and yield (Oei & Nieuwenhuijzen, 2005). Therefore, the relative humidity during the cultivation of *P. sajor-caju* must be maintained to ensure high yield production.

Last but not least, light is another factor that plays an important roles during the formation of *P. sajor-caju* fruiting body, especially its colour. In a dark room, *P. sajor-caju* can only produce stipe with no cap (Wan Mahari et al., 2020). Due to the lack of light, the mushroom would only be capable of growing long stems and small-cap (Oei & Nieuwenhuijzen, 2005). On the other hand, excessive light is also detrimental to *P. sajor-caju* growth as the production of mushrooms in excessive light may produce a short and dark colour fruiting body (Choi, 2004). Therefore, an optimum range of light that *P. sajor-caju* should receive is between 80-201 flux of light (Choi, 2004).

2.3 Growth Performance in Different *Pleurotus sajor-caju* Strains

Although they come from the same species, different *P. sajor-caju* strains' growth performance may differ slightly. This is because different *P. sajor-caju* strains have a variation of phenotype and genotype, differentiating their performance on the cultivation substrates such as their spawn running period, time for the first appearance of fruiting body, number of flushes produced and biological efficiency (BE) (Obodai & Vowotor, 2002). Each strain will express different traits that influence their potential biotechnological or ecological performance, as well as the intrinsic heterogeneity in their stress reactions, including their response towards xerotolerance, development of primordia freely under diverse conditions, and high growth rate (Kashangura et al., 2006). A study by Obodai and Vowotor (2002) reported that the varying mycelial density and growth rates displayed by all eight strains of *P. sajor-caju* used in the study indicate that the different mushroom strains utilise the substrates at different rates (Obodai & Vowotor, 2002). This is supported by a study conducted by Moonmoon et al. (2010), where the study reported that different *Pleurotus eryngii* strains and substrates used during cultivation produced different biological efficiency results in terms of their yield production due to the genotypes of strains used and the biological structure of substrates.

Apart from that, there may be a link between the phenotypic traits of strains and their geographical origin. This finding is supported by a study conducted by Kashangura et al. (2006), where *P. sajor-caju* strains from Thailand and Mauritius proves the relationship between biogeography, phylogeny and incompatibility in reproduction, implying ancient speciation among the *Pleurotus* genus (Vilgalys & Sun, 1994). In addition, a finding in Bangladesh in 2010 found that the native *Pleurotus* spp. strains have the highest production compared to other strains originally cultivated outside Bangladesh because of their compatibility with the environment. The study found that a strain endemic to Bangladesh produced the most yield compared to other strains of *Pleurotus eryngii* from another region due to its environmental adaptability (Moonmoon et al., 2010). Hence, providing evidence for the link between the phenotypic traits and their geographical origin.

Many studies have been conducted on the cultivation and performance of *Pleurotus* species on different substrates. There have been fewer attempts to study the growth performance, biological efficiency, yield production, spawn running period, and the