Grey Oyster Mushroom (*Pleurotus sajor caju)* Spawn Production Using Different Grains

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DECLARATION

I hereby declare that work embodied in this final year project thesis is the result of my work except for the excerpt that was cited in the references.

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Penghasilan Benih Cendawan Tiram Kelabu (*Pleurotus sajor caju*) Menggunakan Pelbagai Bijirin Berbeza

ABSTRAK

Penanaman cendawan tiram kelabu di Malaysia telah terkenal selama beberapa tahun kerana mempunyai faeudah yang unik dari segi gastronomi dan perubatan. Walaupun sektor industri ini berkembang pesat dikalangan ramai orang, masih terdapat masalah yang timbul di dalam industri ini. Penghasilan benih cendawan tiram kelabu telah menjadi satu isu yang kian berkembang dan berterusan di dalam industri ini di serata Malaysia. Pencarian penanam tempatan yang menghasilkan benih cendawan tiram kelabu secara komersial mungkin menjadi sedikit masalah untuk penanam cendawan tiram kelabu. Berikutan kerana kekurangan ilmu dan cara untuk menghasilkan benin cendawan tiram kelabu, penghasilan benih cendawan ini masish di skala yang rendah walaupun bijiran untuk menghasilkan benih boleh ditemui secara lokal. Tujuan projek ini adalah untuk menemui alternatif bijiran lokal untuk pembenihan cendawan tiram kelabu. Terdapat pelbagai bijiran lokal yang terdiri dari pelbagai saiz seperti jagung, sambau, bertih jagung, kacang soya dan barli akan digunakan. 200 g jagung dan bijirin millet dimasukkan ke dalam balang besar manakala 150 g kacang soya, bertih jagung dan barli akan dimasukkan ke dalam balang kecil. Setiap balang akan mempunyai dua ulangan. Untuk menghasilkan benih cendawan ini, agar PDA akan digunakan untuk menghasilkan spora cendawan tiram kelabu. Kemudian, media itu akan digunakan sebagai benih yang diletakkan di dalam bekas yang berisi pelbagai bijirin untuk menjalar. Matlamat utama penyelidikan ini adalah untuk mencari bijiran apakah yang sesuai digunakan untuk penghasilan benih cendawan tiram kelabu. Bijirin jagung telah dikenalpasti sebagai bijirin yang akan memberikan perkembangan miselia dan penghasilan benih cendawan yang baik. Daripada eksperimen ini, bijirin jagung menunjukkan berat ramifikasi miselia dan produktiviti benih yang lebih baik untuk cendawan tiram kelabu dengan kedua-dua replikasi min dua pembolehubah ialah 9.83 dan 4.95 manakala kedua-dua replikasi millet menunjukkan 8.00 dan 3.96 min. Untuk balang kecil, kedua-dua replikasi barli menunjukkan 18.9 dan 12,7 untuk kedua-dua pembolehubah. Kedua-dua replikasi popcorn menunjukkan 14.1 dan 9.4 min manakala kedua-dua replikasi kacang soya menunjukkan 11.2 dan 7.5 min. Di antara semua jenis bijirin, hanya biji jagung menunjukkan perbezaan yang ketara dengan $(p<0.05)$.

Kata kunci: Cendawan Tiram Kelabu, Bijirin, Perkembangan Miselia, Penghasilan Benih

Grey Oyster Mushroom (*Pleurotus sajor caju*) Spawn Production

Using Different Grains

ABSTRACT

Grey oyster mushroom cultivation in Malaysia have been well known for years due to its beneficial properties in both gastronomy and medicinal sectors. Although this economic sector is popular among all sort of people, there's still problem that risen from this industry. Spawn production have been a growing continuous problem in grey oyster mushroom cultivation across Malaysia. Finding local growers that are able to produce grey mushroom spawn in a big scale might may be a slight problem for oyster mushroom producers as not many oyster spawn producers can be found. Due to lack of specific ways to produce spawn, spawn production activity is still at the low rate although grains need can be find locally. This research project mainly will be done to identify alternative grains that can be find locally for spawn production. There will be various local found grains which varies in size that will be used such as corn, millet, popcorn, soy bean and barley. 200 g of corn and millet grain was put into the big jar while 150 g of soybean, popcorn and barley grain will be put into the small jar. Each jar will have two replications. For the grey oyster mushroom spawn production, potato dextrose agar (PDA) will be use as media to culture the spore of the grey oyster mushroom. Then, the media that full of spawn will be put into jars containing various grains to ramify. Key of this experiment is the find which grains work excellent for grey oyster mushroom spawn growth. From this experiment, corn grain shows better weight of mycelia ramification and spawn productivity for grey oyster mushroom with both replications of mean of two variables were 9.83 and 4.95 while both replication of millet shows 8.00 and 3.96 mean. For small jar, both replication of barley shows 18.9 and 12,7 for both variable. Both replication of popcorn shows 14.1 and 9.4 mean while both replication of soybean shows 11.2 and 7.5 means. Between all types of grains, only corn grain shows significant difference with ($p<0.05$).

Keywords: Grey Oyster Mushroom, Grains, Mycelia Development, Spawn Production

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LIST OF ABBREVIATION AND SYMBOL

PDA	Potato Dextrose Agar
$CaCO_3$	Calcium Carbonate
UMK	Universiti Malaysia Kelantan
WMR	Weight of Mycelia Ramification
ANOVA	Analysis of Variance
SPSS	Statistical Package for the Social Sciences
C/N	Carbon Nitrogen Ratio

LIST OF ABBREVIATION AND SYMBOL

Min.	Minimum
g	Gram
%	Percentage
>	More than
<	Less than
cm	Centimeter
˚C	Degree of Celsius

CHAPTER 1

INTRODUCTION

1.1 Research Background

Mushroom is known as nutritional food as it contains 25% - 50% of protein and has a high content of multiple vitamins such as vitamin D, B2 or niacin. *Pleurotus sajor caju* or grey oyster mushroom, is the most cultivated species in Malaysia's mushroom market with 90.86% (Rosmiza et al., 2016). This mushroom does not require complex cultivation conditions as it can be grown on various agricultural remnants and a wide range of temperatures (Roshita et. al, 2015). According to Elly (2011), these agricultural remnants have a high potassium content, phosphorus, and nitrogen ratio, which can be used as mushroom substrate as grey oyster mushrooms need a balanced carbon/nitrogen ratio in substrate composition.

Besides optimum mushroom substrate for oyster mushroom cultivation, spawn production also plays a significant initial role in determining the mushroom production outcome. According to Stanley and Herbert (2010), the essential part of mushroom cultivation is spawn production. Mushroom spawn is mycelium that growth on its suitable substrates, which usually come from grains. Grains are commonly used to produce spawn as it can ramify the substrate faster, which will ease the next cultivation stage (Stanley and Herbert, 2010). The mushroom spawn will impregnate the grains with mycelium and will be put in the mushroom substrate for the inoculating process. In Malaysia, spawn production activity is still low. Most of the time, the spawn needs to import from Thailand, which usually grows on wheat grain. According to Chinda and Chinda (2007), spawn production is a technical process that requires specialized expertise and broad knowledge, and specific care. Thus, this study also wants to focus on spawn production using Malaysian local grain of corn.

1.1 Problem Statement

Spawn production has always been a crucial step for most producer grey oyster mushroom cultivation. Each grains produced a different final product such as mycelia development and spawn production quality. At the current time, most grey oyster mushrooms spawn producers in various countries including Malaysia mainly rely on wheat grains. As the demand keep increasing and limited supply of wheat, the base price increase from time to time. Other than that, oyster mushrooms need a lot of carbon supplies and less nitrogen source. The spawn cultivation activity also does not get significant attention by oyster mushroom producers. It is due to lack of scientific knowledge and techniques which are needed for spawn cultivation. There are no specific and permanent steps in producing oyster mushroom cultivation which make the activity of oyster mushroom spawn cultivation low. Moreover, the spawn cultivation of various grains mostly based on grains that are found at the outside country. Besides, Malaysia mushroom production in Malaysia lack of technology and facilities to produce high quality spawn. (Rosmiza et al., 2016). Due to this issue, spawn will need to be imported from neighboring country such as Thailand. So, this research is focusing on both grains and substrates that can be found locally for the mushroom cultivation process.

1.3 Hypothesis

a) **H_0:** There is no effect on types of grains to weight of spawn mycelia rate and spawn productivity.

b) **H_1** There is an effect on types of grains to weight of spawn mycelia rate and spawn productivity.

1.4 Significance of the Study

This study offers significant contributions on many terms. The oyster mushroom business is one of the fields that interest various types of people, from entrepreneur to researcher. It is because of the creation of wealth, reduced poverty, and job creation. By doing this research, the mushroom industry can be nourished with new ways to cultivate substrate without relying on a single source. This study primarily attempts to seek greater utilization of local found grain such corn, millet, soybean, barley, popcorn and soybean for the oyster mushroom spawn cultivation. It not only can increase spawn productivity but can provide an alternative grain in the oyster mushroom industry. This research serves tremendous benefits not only for spawn cultivation. Doing this research can provide permanent and effective ways for mushroom producers to utilize grain found locally to produce spawn. Therefore, this study could improve mushroom productions locally.

1.5 Research Objectives

1. To identify the difference of growth and productivity between different grains.
2. To evaluate the performance of different spawn grains in terms of various parameter.
3. To find the most effective grains for grey oyster mushroom cultivation.

CHAPTER 2

LITERATURE REVIEW

2.1 Grey Oyster Mushroom (*Pleurotus sajor caju*)

Pleurotus sajor caju species of mushroom usually identified by its common name which is grey oyster mushroom. This mushroom obtains this name from its physical appearance of its cap that look like an oyster shell. Grey oyster mushroom is one of the mushroom species that were commercially cultivated around the world due to its excellent properties. This type of mushroom strain was firstly isolated from the Indian region. Due to this mushroom's unique strain properties, this mushroom has been widely cultivated in tropical and subtropical region (Rashidi & Yang, 2016). Rout et al. (2012) stated that grey oyster mushroom served as an important edible commercialize mushroom which were highly regarded for its purpose in medicine and nutrient. This mushroom contains rich amount of protein, various vitamins and mineral salts which make them signuficant for human consumption.

Oyster grey mushroom contained approximately 30 – 40% of protein when dried (Srisa-Saard, 2013). Because of this, they were called as vegetarian meat that can act as a replacement for animal meat. This mushroom does not contain any hazardous cholesterol that can harm the blood vessel which make them proper incentives for patients that have kidneys, liver, heart and high blood pressure disease. (Srisa-art, 2013). Other than that, Rashidi and Yang (2016) also stated that grey oyster mushrooms has an antioxidant that protect the human body from oxidative damage. Randive (2012) also stated that this mushroom also preserved high amount of niacin, vitamin B complex and C that comparatively more than other plants. According to Rashidi & Yang (2016), among three types of mushrooms which are grey oyster mushroom, button mushroom and straw mushroom that are commercially cultivated in India, grey oyster mushroom have more delicate aroma, taste and multiple value from medicinal and nutritional site.

2.2 Taxonomy of *Pleurotus sajor caju* (Grey Oyster Mushroom)

Taxonomy of *Pleurotus sajor caju* can be seen as below: Kingdom: Fungi Phylum: Basidiomycota Class: Agaricomycetes Order: Polyporales

Figure 2.0.1: Taxonomy of *Pleurotus sajor caju* (Source: National Centre for Biotechnology Information)

2.3 Morphology of *Pleurotus sajor caju*

According to Priyadarshini (2018), morphology of grey oyster mushroom can be seen as follow,

2.3.1 Pileus

The cap size of grey oyster mushroom usually ranged from 3 – 8 cm and have broad length of 4 – 16 cm. The shape of its cap usually in fan form with a broad convex shape, curled into the inside of cap and roll towards cap top. Cap of the young mushroom will have a flat form but when it gradually grows and mature, the cap will fold inside towards fruiting body. It will have a smooth surface with a little bit of greasiness at the top part of the cap. Once it matures, the cap will show an exposed fleshy gill in white to yellowish colors alongside some odor release.

2.3.2 Lamellae

Gills decurrent that are white in color that run down to the stipe part continuously. It will have white to creamy color when it was fresh and will change to yellow once it gets over the time.

2.3.3 Stipe

This part can be seen in the mature fruiting body. It can be identified as the oldest and tall form in one cluster that usually ranging from 0.5 – 2.0 cm thick and 0.5 – 3.0 cm long. It also has white hair at the base.

2.3.4 Spore

Have a smooth texture and in form of cylindrical to kidney shape that are narrow and elliptical. It can be seen in white to yellow colors.

2.4 Grey Oyster Mushroom Cultivation

Cultivation of oyster mushroom can be described as lignocellulosic waste conversion into edible protein (Padmavathi et al, 2019). Cultivation of this type of mushroom work by growing them on various types of lignocellulosic substance. Based on research made by Garg et al. (2017), it is stated that this mushroom easily grown on multiple lignocellulose substance rather than in their natural habitats. As a saprophytic fungus, it degraded the lignocellulosic substance using multiple enzymes (Siddhant et al, 2019). By using this method to produce food that rich in protein such as mushroom, it can make protein rich food accumulation easier. According to Khan and Garcha (1984), cultivation of oyster mushroom got a lot of attention around the world due to their growing flexibility in cold and warm temperature as well utilizing multiple sources of lignocellulose. Lignocellulose mostly came from agricultural waste such as rice straw, wheat straw and oil palm frond. Based on Philippousis et al. (2001), a lot of agriculture waste contain rich amount of lignocellulosic compound.

Grey oyster mushroom was one of the widely cultivated mushrooms in Malaysia among other 17 mushroom species. Due to their growth properties that are suitable with local Asian climate, they can be grown easily with local found substrates with only a small cost. Cheaper production cost and suitable climate all year around make their cultivation last for a whole year. As the grey oyster mushroom cultivation become a natural part of mushroom growers, demands of this mushroom will continue to rise up. It is expected that grey oyster mushroom production in Malaysia will rise up from 23 000 tons per year in 2008 to 72 000 tons per year in 2020 (Rashidi & Yang, 2016).

2.5 Composition of Substrate

Oyster mushroom can be generally cultivated on various agriculture waste (Elattar et al., 2019). Agriculture waste have rich content of lignocellulosic components which can be effectively break down by mushroom to convert it into valuable product. According to Amal et al. (2008), substrate composition is one of the strongly reliable component for lignocellulosic enzyme production. Although every agriculture waste can be used as substrate, it is significant to identify substrate composition that can give effective result on growth and yield of oyster mushroom.

2.6 Spawn Production

Spawn can be known as vegetative mycelium which inoculated the fungus mushroom. According to Lee at al. (2014), spawn cultivation is an important stage to determine the success and productivity performance of mushroom. Production of the spawn will depend on spore culture as it will lead to many quality and quantity in oyster mushroom. Spore will transfer and trigger formation of mycelium. This method does not make significance changes in the characteristics and will produce oyster mushroom that similar to its pure culture. Grains have been a suitable media for mushroom growth as it has hard shell that contains protein and starch for consumption of mycelium. Grains also can retain a lot of water with the capability to swell. Advantages of grains spawn is mycelium will be evenly distributed on all grain surfaces (Behnam and Naser, 2008).

To obtain the selected mushroom spawn for spawn cultivation, the mushroom spore can be cloned from the basidiocarp tissue into the agar media plate. (Hsu et al., 2018). From the basidiocarp tissue, mycelia of the fungal can be obtained and used as a mother culture for spawn preparation. As the spawn growth and spread throughout the plate, it can be sub cultured periodically to a new media. During the culture process, careful storage of the culture is vital to make sure that the condition of the spawn is maintained and available according to its specific growth properties. Then, from the subculture, fully covered agar media will then transfer to grain for inoculation process of the spawn.

2.7 Spawn Production Using Grains

According to Elhami & Ansari (2008), mushroom producers during old day initiate spawn production using the soils which mushroom grow naturally. The substrates of them will be covered on top of the soil for spawn inoculation. Nowadays, commercial spawn producers use grains as carrier because it has a hard shell that contain high quality of starch and protein for the mycelia to consume easily. Other than that, grains also can easily attract water as the shell of the grains can consume a lot of water by swelling. There are various advantages of producing spawn using grains. First, grains can be kept for a long time as it offers good and fixed resistance for mycelia to growth. Next, grains also let the mycelia to distribute evenly across the surface of grains. Therefore, choosing a suitable type of grains is essential for maximize mycelia ramification and spawn production. According to Mottaghi (2006), types of spawn can influence mycelia growth and time taken for mycelia to cover the substrates. Types of spawn grain such as millet, corn and wheat grain can affect the carpophores production of the spawn (Elhami & Ansari, 2008). Addition of certain additives such as soybean powder also can affect the formation of mushroom fruiting part.

2.8 Properties of Corn Grains

Corn grains originated from the species of *Zea mays* is one of the third most essential cereal grain in the whole world. According to Zilic et al. (2011), choosing of corn hybrid in the use of daily will based on specific selected criteria such as disease resistance, drought tolerance, storage characteristics and production of grains. Corn grain usually used for human consumption in majority of the country but it also used as araw materials in agricultural industry. Due to its importance, corn grains have a lot of hybrids that have specific composition such as white and sugary and have high contain of lysine and oil. Corn grains can be classified according to its characteristics of endosperm. It can be characterized using endosperm pattern, quality and quantity of endosperm.

Common type of corn grains including sweet, popping, waxy, flint dent and flour (Zilic et al., 2011). Each of the corn grains type have its own nutritional value. Flint corn have high contents of beta carotene and protein while other corn types have high vitamin A and beta carotene. According to Elhami et. al (2008), corn grains have effect on carpophores production of oyster mushroom. Both of these grains have been known to trigger the appearance of mushroom fruiting body. Grains that have larger seed such as corn contain more nutrient for mycelium growth. Corn grains can help to improve the yield production of mushroom and increase the dry weight of mushroom fruiting body as larger grains provide larger storage.

2.9 Properties of Millet Grains

Millet have been known as part of the oldest foods among humans. It is one of the primary pioneers of grains use for commercial production. These grains belong to the grass family of Poaceae. Millet can be reffered to as s small seeded annual grass that belong to multiple genera such as *Panicum* and *Pennisetum*. Two major species that are widely used in food and feed production are *Pennisetum glaucum* and *Eleusine coracana*. Millet grains have been consumed as a staple food for people in Africa and semi-arid regions of Asia. According to Karuppasamy (2015), millet have great potential to be used as a processed grain and turn into high quality foods and raw materials. There are multiple types of millet that are commercially cultivated such as finger millet and pearl millet.

Millet has been known as nutri cereal grains as it consists of high content of carbohydrates, sugar and starch. Other than that, millet grain is also rich in vitamin B and offer high nutrient content such as calcium, potassium, iron and zinc.

Millet grains can withstand many challenges in its cultivation such as harsh climate make it a great choice for primary grains used for spawn production in arid region. Millet has a lower endospore load than wheat and sorghum. According to Hassan et al (2021), pearl millet covers 75% of its endosperm which make it endosperm proportion 4:5:1 than sorghum. Small endosperm means that millet have low exposure to contamination. Small size of millet grain also provides more inoculation points for mycelia ramification than other grains.

2.10 Properties of Barley Grain

Barley has become part of essential grain in the world today as it ranks in fourth place in quantity of production aspect and total cultivation area from whole grain production. Barley grain originated from grass family of Poaceae. There are three types of barley species from the same genus *Hordeum* that are commonly found in commercial production such as *Hordeum vulgare*, *Hordeum irregulare* and *Hordeum distichum.* Barley grain have been used commercially for malt production, animal feed, food production and seed cultivation. According to Zhou (2010), barley grain has majorly used commercially as it contains a major source of energy, fibre and protein. In several regions of Central Asia and North Africa, barley have become a staple food as it can be cultivated in extreme condition. According to Rukhsana et al. (2019), particular size of grains used in spawn cultivation affect the time taken for mycelia ramification. It is because grains retained moisture effectively to aid in mycelia production. Therefore, barley that are considered big in size among other grain may provide better ramification rate and inoculation point for better oxygen absorption.

2.11 Properties of Soybean Grain

Soybean is one of the major crops that is widely consumed across the world. Soybean grain can be scientifically known as *Glycine max* have been used widely in various agricultural scenario. According to Hauth et al. (2018), this grains is commonly cultivated in the commercial world due to its specific chemical composition that can be found in the soybean. Soybean have a composition of 20% oil and 40% protein. As soybean becomes a demanding product in both gastronomy and agronomy sectors, the quality of the bean need to be in a great condition. In order to assure soybean quality, the amount of water needed to be reduced when used as spawn grain because it can decrease the biological activity of the grains. According to Hauth et al. (2018), moisture content is one of the factors that play a significant role in grain deterioration prevention. During the soybean grain preparation for spawn production, soybean need to have low moisture content and low temperature to reduce microorganism spoilage and increase respiration rate of the grain.

2.12 Properties of Popcorn Grain

Popcorn usually known as snack that consume buy various consumers across the world. Popcorn is a type of product that originated the corn plant (*Zea mays*). Beside of commonly known as snack food, it also was known as nutritious food with various beneficial properties. According to Abdullah (2021), popcorn have high content of fibre and low in energy. Another nutritional properties of popcorn, it possessed various physicochemical such as protein, carbohydrate, fats, ash content and fiber. Various nutrient also can be found in popcorn such as vitamins, amino acid, fatty acid and minerals. Quality of popcorn can be measured according these aspects which are popcorn variety, kernel shape and size, kernel damage and density, thickness of pericarp, moisture content and temperature. From this aspect, popcorn grain can be inspected to provide a maximum ramification rate for spawn production.

CHAPTER 3

METHODOLOGY

3.1 Material and Apparatus

3.1.1 Spawn

Fresh oyster mushrooms were bought from UMK mushroom house as a sources for mushroom spore which was cut with razor. Grains specifically corn, millet, barley, popcorn and soybean was bought from both online shop and local shop. For the preparation of media, PDA agar was used which consist glucose, sliced potato, agar powder and distilled water. Spawn bottles were used as media for grains with addition of $CaCo_3$ and autoclave was used to reduce contamination. Grain bottle then was incubated in incubator for the inoculation process (Stanley and Herbert, 2010).

3.2 Methods

3.2.1 Spawn Production

3.2.1.1 Culture Media Preparation

Potato Dextrose Agar (PDA) was used as growth media for oyster mushroom spawn. 200 g slice and peeled potatoes, 20 g glucose, 20 g agar powder and one litre distilled water was used to make PDA agar (Stanley and Herbert, 2010).

3.2.1.2 Solid Culture

Fresh oyster mushroom was used to gain spores. Small part of the oyeter mushroom was cut using a razor that have been sterilized. Part of the mushroom then was put on top side of petri dish for the spore to be fall into the media. Agar media then was incubated for 10 days with a temperature of 27^{o}C. Sub culture was done to obtain pure cultures.

3.2.1.3 Spawn Preparation

200 g of millet and corn grain, 150 g of soybean, popcorn and barley grains was washed three times with clean water to remove dirt and residue. Both grain tem soaked for one days to maximize water absorption. After that duration, grains was washed again then will be put into the spawn bottle. Grains will be put 2/3 of the spawn bottle. 5 g of $CaCO_3$ was put into those spawn bottle. Spawn bottle was autoclaved with 120^oC for 2 hours. Next, each spawn bottle was inoculated with one quantities of mycelial discs. Two types of spawn bottles which vary in size was incubated in 27^oC in dark cool room. Spawn of mycelium ramification weight and productivity rate was recorded.

3.2.1.4 Experimental Design

There are two replications that was used for the preparation of spawn and spawn utilization for mushroom production. Each treatment had different type of grains used. Each treatment includes 5 g of $CaCo_3$. For each treatment, five different grains were used such as corn, millet, barley, soybean and popcorn. Each treatment served as different variables. List of replications that involve with substrate preparation was listed as follow:

Big jar

R1 = 5 g $CaCo_3$, 200 g corn grains

R2 = 5 g of $CaCo_3$, 200 g millet

Small jar

R1 = 5 g of $CaCo_3$, 150 g barley grains

R2 = 5 g of $CaCo_3$, 150 g popcorn grains

R3 = 5 g of $CaCo_3$, 150 g soybean grains

For each treatment, ten subcultures and one pure culture was prepared for the fungi culture and two bottles of spawn varies from big jars for R1 and R2 and small jars for each R1, R2 and R3. Treatments was prepared for spawn production.

3.2.1.5 Data Collections

Spawn growth characteristics result was collected according to properties shown in Table 2. These properties were calculated based on Equation (3.1) according to Adebayo et al. (2014) as follow.

Table 3.1: The spawn ramification rate and productivity on different grains (Source: Adebayo et al., 2014).

Treatments	Ramification rate (cm/d)	Ramification day (d)	Weight of mycelia ramification (g)	Spawn productivity (%)
T1				
T2				

Mycelia ramification rate = Average length of mycelia ramification / Average number of days

Weight of mycelia ramification (WMR) = Final weight (Grain + Bottle + Mycelia) – Initial weight (Grain + Bottle)

Ramification day = Average number of days for full ramification

Productivity rate = Total weight of mycelia × 100% Substrate weight **(3.1)**

3.2 Statistical Analysis

Spawn ramification we and productivity on different grains analysis will be used to identify oneway ANOVA mean, standard deviation, maximum and significant different value at 95% probability value by using SPSS Statistics 26.

CHAPTER 4

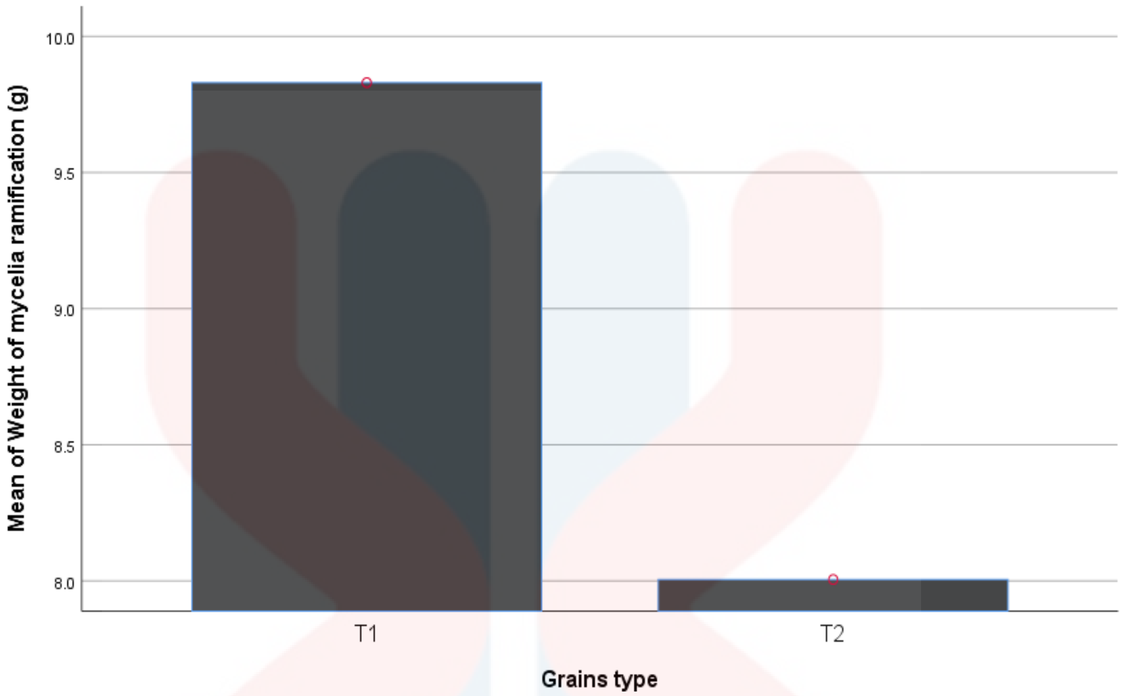
RESULT AND DISCUSSION

4.1 Big Jar

4.1.1 Weight of Mycelia Ramification of *Pleurotus sajor caju* Spawn

The result of Figure 4.1 showed the weight of mycelia ramification based on different types of grains used in the spawn cultivation which are corn grains and millet grains. Based on the figure below, it is found that there was significant difference between weight of spawn ramification of corn grains compared to millet grains ($p<0.05$) (Appendix A) based on the graph shown below. From the Table 4.1 presented, both replicates of corn grains weight a 9.85 g and 9.81 g of mycelia rate while millet grains weight of 7.59 g and 8.42 g. Mean for both replicates of corn grain are 9.83 and for both replicates of millet grains are 8.00.

A finding found by Elhami & Ansari 2008 has stated that the maximum growth of spawn of multiple Pleurotus sp. can be found in corn grains while the minimum growth of spawn can be found in millet grains. They found out that due to the size of corn grain size that are larger than millet, mycelia growth promoted faster in this grain. The larger surface of corn grain means that there's also a larger surface of pore. Larger surface of pore will promote better ventilation and increase in the oxygen concentration in corn grains which lead to the increase growth of mycelia development. However, findings by Siddhant, Yadav & Singh (2019) propose a different result. Spawn that are prepared from the small grains produce faster mycelia coverage as it allows a quick spread of mycelia from the small propagation centre as it prepares more points of inoculum per gram of the spawn.



*T1: Both replication of corn grain, T2: Both replication of millet grain

Figure 4.1: Mean weight of mycelia ramification of *Pleurotus sajor caju* spawn.

Table 4.1: Weight of mycelia ramification of *Pleurotus sajor caju* spawn.

Grains Type	**Weight of Mycelia Ramification (g)**	
	R1	**R2**
Corn	9.85	9.81
Millet	7.59	8.42

*R1: First replication of corn and millet grain, R2: Second replication of corn and millet grain

T1

T2

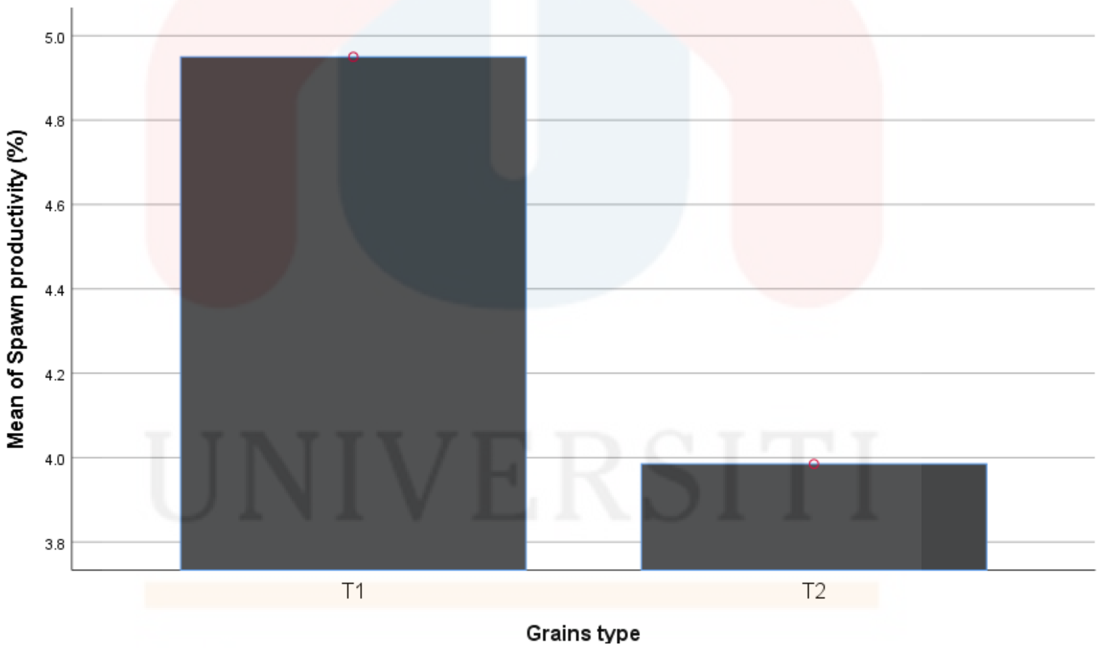
*T1: corn grain, T2: millet grain

Figure 4.2: Final weight of Pleurotus sajor caju spawn.

4.1.2 Spawn Productivity of *Pleurotus sajor caju*

Figure 4.2 have shown the result of spawn productivity of both different types of grains which are corn grain and millet grain. Based on the figure stated below, there are a slight significant difference between spawn productivity of corn grain and millet grain as ($p>0.05$) Appendix B. Mean result of spawn productivity for both replicates of corn grains are 4.95 and 3.96 for both replicates of millet grains can be seen from Figure 4.2. Based on the Table 4.2, both replicates of corn grain produce 4.98% and 4.91% of spawn productivity while both replicates of millet grains produce 3.76% and 4.21% of spawn productivity.

Based on the result it can be seen that corn grain produces a high ramification rate and spawn productivity than millet grain. A finding discovered by Mishra et al (2014) stated that corn grain is the best grain spawn for mushroom cultivation as it offers maximum spawn growth and millet is the least favourable grains for spawn growth. Mbogoh et al (2011) also find that larger grains have greater ability to withstand bad condition such as bad composting hence contributingto faster mycelia growth. However, finding by Jayachandran et al (2017) share contrast results they found out that smaller grains provide greater inoculation points per kg compare to larger ones.



*T1: Both replication for corn grains, T2: Both replication for millet grains

Figure 4.3: Mean spawn productivity of *Pleurotus sajor caju*.

Table 4.2: Spawn productivity of *Pleurotus sajur caju*.

Grains Type	Spawn Productivity (%)	
	R1	**R2**
Corn	4.98	4.91
Millet	3.76	4.21

*R1: First replication of corn and millet grain, R2: Second replication of corn and millet grain

T1

T2

*T1: corn grain, T2: millet grain

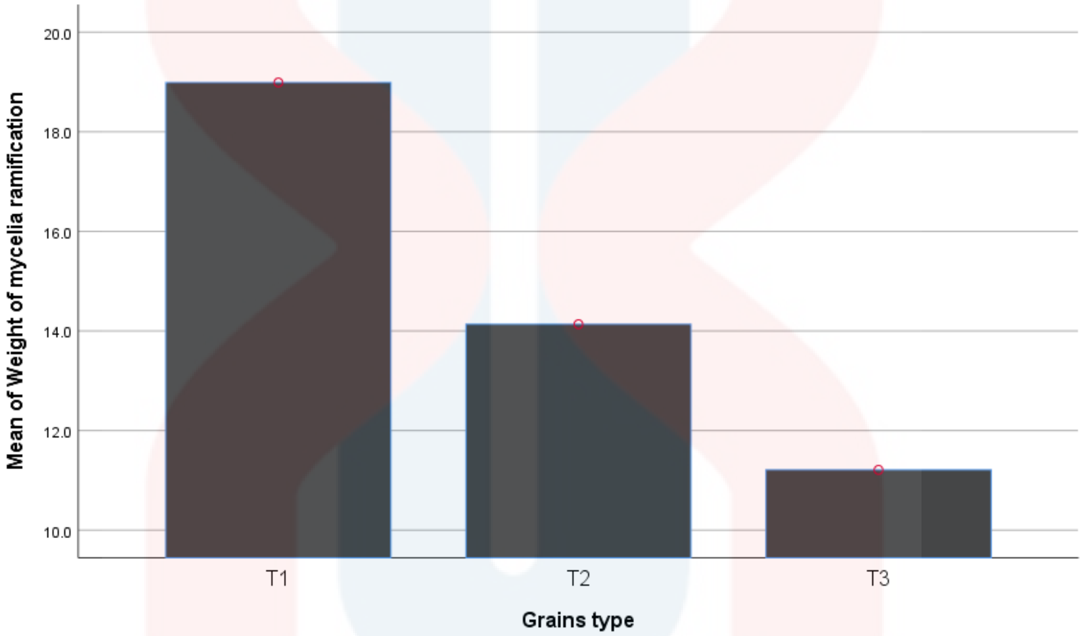
Figure 4.4: Final spawn productivity of *Pleurotus sajor caju*.

4.2 Small jar

4.2.1 Weight of Mycelia Ramification of *Pleurotus sajor caju* Spawn

The result in Figure 4.3 shown the both the weight of mycelia ramification of grey oyster mushroom cultivated in different types of grains which are soybean, popcorn and barley. From the figure, mean result of these grains were presented. Both replication of barley produces the highest mean which is 18.9, followed by both replication of popcorn grain which is 14.1 and the lowest mean from all the grains is both replication of soybean grains which is 11.2. The mean produce is non-significant as the ($p>0.05$) Appendix C. Result from Table 4.3 show the weight of mycelia ramification for these three grains. Weight of both replication of barley grains is 17.14 g and 20.84 g. For both popcorn grains replication is 14.32 g and 13.95 g while for both replication of soybean grains is 7.60 g and 14.82 g.

The finding by Siddhant et al in 2019 found out that barley give out significant yield and biological efficiency in spawn production. It happens because of less density of grain and seed coat presence of grain. Cereal grain such as popcorn tend to be as reservoir for carbohydrate content but due to its small surface area it can house the mycelia ramification inefficiently. However, finding from Zied et al in 2011 differ from the current finding as soybean was considered as a good source of nitrogen for spawn production to reduce concentration of carbon. It can be used to balance the C/N ratio as it contains high level of protein thus increase weight of mycelial ramification.



*T1: Both replication of soybean grain, T2: Both replication of popcorn grain, T3: Both replication of barley grain

Figure 4.5: Mean weight of mycelia ramification of *Pleurotus sajor caju* spawn.

Table 4.3: Weight of mycelia ramification of *Pleurotus sajor caju.*

Grains Type	**Weight of Mycelia Ramification (g)**	
	R1	**R2**
Barley	17.14	20.84
Popcorn	14.32	13.95
Soybean	7.60	14.82

*First replication of barley, popcorn and soybean grain R2: Second replication of barley, popcorn and soybean grain

T1

T2

T3

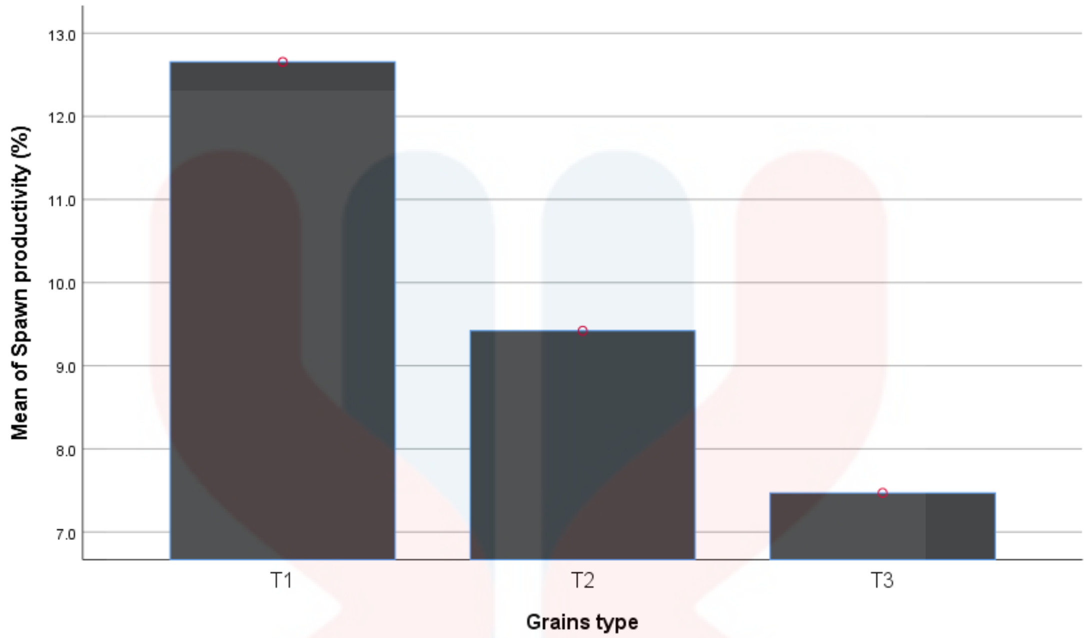
*T1: barley grain, T2: soybean grain, T3: popcorn grain

Figure 4.6: Final weight of *Pleurotus sajor caju* spawn.

4.2.2 Spawn Productivity of *Pleurotus sajor caju*

Figure 4.4 show the result of spawn productivity of three types of grains which are soybean grain, popcorn grain and barley grain. The mean result can be seen from the figure where barley have the highest mean from all of the three grains with both replications of 12.7. Next after is the mean of popcorn grain both replication which is 9.4 and the lowest out of all grains is soybean beans with a 7.5. The mean produce is non-significant as the ($p>0.05$) Appendix D. From the Table 4.4 showing spawn productivity percentage of the last day, it can be seen that barley produce the highest productivity rate. The productivity rate of barley for both replications are 11.42% and 13.80% while for both replication of popcorn grain is 9.54% and 9.30%. Lowest productivity rate will be both replication of soybean grains with 5.06% and 9.88%.

Finding from Konga, Khare and Jongman in 2013 have found out that barley was recommended to be use in production of high quality spawn in oyster mushroom cultivation. Barley can become a good carriers of mycelia when the condition of pre-treatment methods before spawning in favours with them. The conditions included lower soaking period as barley have soft coat on the outside shells which tend to increase moisture uptake. Spawn productivity using grains came with an advantage as grain it nutritious for fungi and let it form kernels easily and highly distributed in the substrate but it still came with a disadvantage. They also can provide optimum condition for other organism too, (Tesfaw et al, 2015)



*T1: Both replication of barley grain, T2: Both replication of popcorn grain, T3: Both replication of soybean grain

Figure 4.7: Mean spawn productivity of *Pleurotus sajor caju*.

Table 4.4: Spawn productivity of *Pleurotus sajur caju.*

Grains Type	**Spawn Productivity (%)**	
	R1	**R2**
Barley	11.42	13.89
Popcorn	9.54	9.30
Soybean	5.06	9.88

*First replication of barley, popcorn and soybean grain R2: Second replication of barley, popcorn and soybean grain

T1 T2

*T1: barley grain, T2: popcorn grain

Figure 4.8: Final spawn productivity of *Pleurotus sajor caju*.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Grey oyster mushroom cultivation in Malaysia has been well known among all sorts of people ranging from non-mushroom growers to common people who are interested in growing oyster mushrooms by themselves. The reason behind these due to easy cultivation method of it and its availability a long year harvest production. Other than that, grey oyster mushroom provides countless medicinal and gastronomy benefits towards humans as it can be consumed as ‘vegetarian meat’ due to high content of protein and its nutritional qualities that are similar to meat. Due to this, there’s an overgrowing demand on the grey oyster mushroom production. This condition leads to the shortage of raw material stocks that are significant in grey oyster mushroom cultivation which are its spawn availability. Most of the growers in Malaysia rely heavily on import production of spawn as there are shortage of spawn production. They prefer to buy the spawn from outside countries such as Thailand and China as their raw material.

This condition happens due to shortage of mushroom cultivators that involve in spawn production commercially which force other growers to import the spawn. This condition can be improved if there are standardized and complete ways to produce the spawn commercially. Most growers are involved more in grey oyster mushroom cultivation commercially as they do not have enough knowledge and skills to be involved in spawn production industry. From the aspect of mushroom spawn producers, they tend to use wheat grains that are usually found in urban area and mostly difficult to found in small village. Thus, this study was carried out to acknowledge the utilization of locally found grain to find another alternative for wheat grains and reduce the consumption of wheat grains thus increasing the chance for small growers to join the grey oyster mushroom spawn production.

The finding showed that big jar parameters are significant ($p>0.05$) while the small jar parameters shows insignificant between the data ($p<0.05$). The types of grains give different outcomes based on the weight of mycelia ramification on the final day and spawn productivity at the last day of production. For the big jars, corn grain shows a better trend in both mean ramification weight (9.83) and spawn productivity mean (4.95) than the millet grain. For the small jar, barley shows a great outcome in both ramification weight mean (18.9) and spawn productivity (12.7) than popcorn grain and soybean. The best grain that can be used as a replacement of common grain used in spawn cultivation will be corn grain. It is because the mycelia ramification weight is significantly different compared to barley grain. Other than that, its spawn productivity also shows a slight significance than barley grain parameters. Thus, this show that corn grain can be used as spawn grain for local mushroom growers as corn grain can be found easily in both small animal feed shops and big commercial supermarkets. It also can help easing for small farmers to catalyse spawn production in rural area yet increasing the total of farmers involved in grey oyster mushroom spawn production.

5.2 Recommendation

Further research of spawn production is needed to discover more experimental design that are suitable with small and local farmers to utilize a fully spawn production process. The study on more diverse local found grains can be initiated to improve the lack of this research study. Lastly, corn grains should be used as a common grain in spawn production to increase involvement from many mushroom growers to maximize the grey oyster mushroom cultivation and performance.

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APPENDIX A

TABLE A: One way ANOVA of mycelia ramification weight of corn and millet grains.

Weight of Mycelia Ramification (g)					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	3.331	1	3.331	19.294	.048
Within Groups	.345	2	.173		
Total	3.676	3			

APPENDIX B

TABLE B: One way ANOVA of spawn productivity of corn and millet grains.

Spawn productivity (%)					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	.931	1	.931	18.073	.051
Within Groups	.103	2	.052		
Total	1.034	3			

APPENDIX C

TABLE C: One way ANOVA of mycelia ramification weight of soybean, popcorn and barley grains.

Weight of mycelia ramification (g)					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	61.770	2	30.885	2.810	.205
Within Groups	32.978	3	10.993		
Total	94.748	5			

APPENDIX D

TABLE D: One way ANOVA of spawn productivity of soybean, popcorn and barley grains.

Spawn productivity (%)					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	27.435	2	13.717	2.800	.206
Within Groups	14.695	3	4.898		
Total	42.130	5			

APPENDIX E

Figure E.1: PDA agar media preparation.

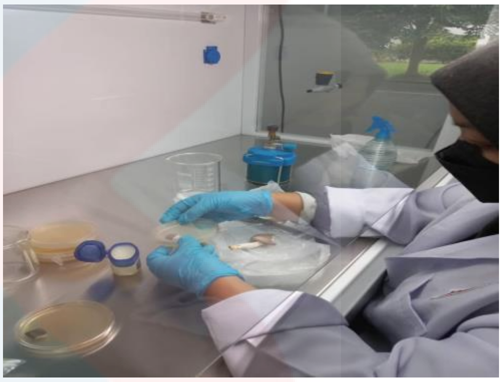

Figure E.2: Mushroom spore collection.

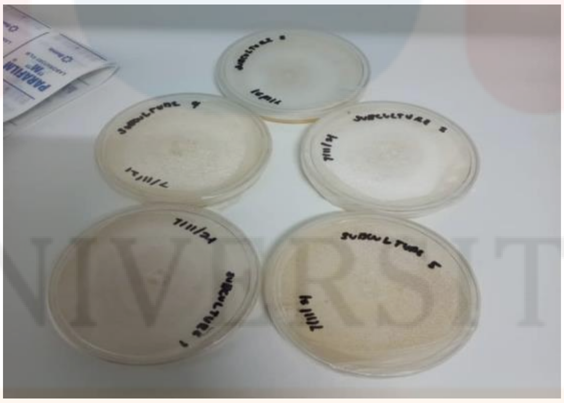

Figure E.3: Utilization of fully ramified plate.

Figure E.4: Brief grains washing.

Figure E.5: Grains boiling process.

Figure E.6: Grains rinsing.

Figure E.7: Putting grains into jar.