Evaluation of the Rice Straw substrate for Grey Oyster Mushroom Cultivation (*Pleurotus Sajor-Cajo)* and Compost for Biofertilizer

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DECLARATION

I hereby declare that the work embodied in here is the result of my own research except for the excerpt as cited in the references.

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Penilaian substrat Jerami Padi untuk Penanaman Cendawan Tiram Kelabu (Pleurotus Sajor-Cajo) dan Kompos untuk Baja Bio

ABSTRAK

Permintaan dalam industri makanan terhadap cendawan tiram semakin meningkat dari semasa ke semasa. Habuk papan getah, dan dedak padi adalah dua bahan substrat komersial yang paling biasa digunakan untuk penanam cendawan tiram kelabu. Bahan mentah yang digunakan dalam substrat untuk penanaman cendawan tiram kelabu. Bagaimanapun, kebergantungan kepada pembuatan habuk papan getah meningkatkan harga habuk papan yang menjejaskan penanam cendawan. Oleh itu, dalam kajian ini menyiasat jerami padi sebagai alternatif substrat pertumbuhan cendawan serta menggunakan substrat cendawan bekas sebagai kompos organik (SMS dicampur tahi kambing) terhadap pertumbuhan bayam merah (Amaranthus gangeticus) di bawah keadaan semaian di Universit. Malaysia Kelantan, kampus Jeli. Dalam kajian ini menggunakan rawatan sebagai T1 (pemalar) habuk kayu substrat, dan T2, Jerami padi substrat. Hasil daripada kajian ini menunjukkan terdapat perbezaan yang signifikan dalam prestasi pertumbuhan antara rawatan dalam penanaman Pleurotus sajor caju. Ia menunjukkan bahawa T2 mempunyai prestasi pertumbuhan Pleurotus sajor caju yang lebih baik berbanding T1 (kawalan) dari segi tempoh pendek kolonisasi penuh miselium (32.67±2.67 hari), tempoh tuaian pertama (43±1.77 hari), berat hasil yang lebih tinggi (26g). ±1.31g), panjang stipe (3.56±0.81 cm) dan diameter pilus (6.72±0.94 cm). Berdasarkan keputusan keseluruhan kajian ini, menunjukkan bahawa (T2) sesuai digunakan sebagai alternatif atau substrat baru untuk menanam Pleurotus sajor caju. Untuk hasil kompos, T2 mempunyai pertumbuhan yang lebih baik berbanding T1 dari segi ketinggian tumbuhan (2.3±2.04cm) dan bilangan daun (4±3hari).

Kata kunci: substrat komersial, baja bio, jerami padi, cendawan tiram, kompos organik

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ABSTRACT

The demand in the food industry for oyster mushrooms is increasing from time to time. Rubber sawdust, an rice bran are the two most common commercial substrate materials utilized for the grey oyster mushroom grower. The raw materials used in the substrate for the grey oyster mushrooms cultivation are sawdust, rice bran and calcium carbonat. However, dependency on rubber sawdust making increased the price of sawdust which affect the mushroom grower. Therefore, this study investigates rice straw as an alternative substrate for mushroom growth as well as to use the spent mushroom substrate (SMS) as organic compost mixed with goat dung on the growth of red spinach (*Amaranthus gangeticus*) under nursery conditions at the Universit Malaysia Kelantan, Jeli campus. In this study used treatment as T1 (control) sawdust substrate, and T2, rice straw substrate.. The result from this study conveyed there were significant differences in growth performance between the treatments in the cultivation of *Pleurotus sajor caju*. It showed that T2 has better growth performance of P. sajor caju compared to T1 (control) in terms of the short duration of mycelium full colonization (32.67±2.67 days), first harvest period (43±1.77 days), higher yield weight (26g±1.31g), stipe length (3.56±0.81 cm) and pileus diameter (6.72±0.94 cm). Based on the overall result in this study, it showed that (T2) is suitable to be used as an alternative or new substrate to grow P. sajor caju. For compost result, T2 has better growth compared T1 in terms of the height of the plant (2.3±2.04cm) and the number of leaves (4±3days).

Keywords: commercial substrate, bio-fertilizer, paddy straw, oyster mushroom, organic compost

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NO REFERENCES		PAGES
RS	Paddy straw	Iv
SD	Sawdust	Iv
SMS	Spent Mushroom Substrate	Iv
FAMA	Federal Agricultural Marketing Authority	3
WTO	World Trade Organization	27
RCBD	Randomized Complete Block Design	

CHAPTER 1

INTRODUCTION

1.1 Background Research

Oyster mushrooms are rich in a nutritious and dynamic equilibrium diet, including protein, and it has a dry weight protein concentration ranging from 18% to 37%. Fat is found in modest concentrations ranging from 1 to 8%. Fat The high content of linoleic acids in mushrooms is one of the reasons they are regarded as a healthful diet. Vitamins and minerals Mushrooms are high in vitamin B, riboflavin (vitamin B2), ascorbic acid (vitamin C), as well as folic acid. Mushrooms are also high in vitamin D. They are high-nutritional-value products that may be produced from lignocellulose waste. The oyster mushroom is the world's third most farmed edible fungus. Oyster mushrooms are the simplest to cultivate since they can grow on a variety of substrates. (Randive,2012).

The oyster mushroom is a nutritionally dense food fungus that includes adequate amounts of phosphorus, iron, protein, fat, riboflavin, and thiamine. The calorific value of mushrooms is almost 345 kg per 100 g dry weight. Mushrooms are considered vegetarian meals due to their high mineral and protein content. It is well known that mushroom protein serves as an intermediary between vegetables and cattle. Oyster mushrooms were also discovered to contain amino acids that are needed for a variety of biological activities in the human body. The medicinal or pharmacological effects of oyster mushrooms include antimicrobials, immunostimulants, antioxidants, and anti-tumoral properties. Oyster (Pleurotus sp.) is a lignin-degrading mushroom that can grow on a variety of lignocellulosic substrates (Singh, Maurya, Singh, 2019). Mushroom cultivation is a potential industry in Malaysia, with several enterprises sprouting up every year. China is Malaysia's major mushroom supplier, as the majority of the mushrooms available in the market are imported from China.

According to reports, mushroom importation in Malaysia has increased year after year. Datuk Seri Ismail Sabri Yaakob, Malaysia's Minister of Agriculture and Agro-Based Industry in 2013, declared that 84 percent of mushrooms are imported, while only 16 percent are cultivated in Malaysia (Siti Suraya MdTop, 2014; Nooraishah, 2014). Sawdust is commonly used in mushroom cultivation practices. As a result, a rise in sawdust demand leads to an increase in substrate preparation costs, which has an impact on small grower production. Thus, finding that Veve substrates are another option to reduce the dependency on sawdust substrate (Wu, Liang, and Liang,2020). In addition, spent mushroom substrates are very harmful if didn’t manage in the right way. If farmers are ignored it, it will hurt the mushroom industry and the environment. Spent mushroom substrates can recycle use as compost for plant growth.

According to the Federal Agricultural Marketing Authority, FAMA (2011), demand for local vegetables is estimated to rise by 4.5 percent per year, from 1.6 million tonnes in 2010 to 2.4 million tonnes in 2020. In keeping with the trend in emerging nations, per capita, vegetable consumption is predicted to rise by 2.6 percent each year, from 55 kilos to 70 kilograms during the same period. Vegetable output must be raised to fulfill client demands for quality and quantity. Fertilization is one method that may be used to improve outcomes. Organic compost is now strongly suggested for use in conservation systems. Organic compost contains substances that can improve the amount of organic matter in the soil, contribute to nutrient supply, improve the physical qualities of soil, prevent erosion, and boost the activity of soil microorganisms (Dewi, 2016).

Agriculture had grown dramatically in terms of enterprise production and yield around 10,000 years ago. Although agricultural systems such as irrigation, crop rotation, fertilizer usage, and pesticide use have been there for a long time, significant progress has only been realized in the recent century. New technologies and crops were implemented during the expansion period. Agriculture is a field that is inextricably linked both to human life and the environment. Hierarchically, ecosystems in various components of life create a chain that influences each other, and the elimination of one member in the chain affects the sustainability of other living species, thus the entire chain must be conserved.

Over the last four decades, agricultural activities have become much more reliant on chemical fertilizers and pesticides to achieve and maintain high yields that define current cultivation systems. In general, it is believed that synthetic nitrogen amounts to 2 to 5% of all materials used in fertilizer manufacture globally. (Dewi, 2016)

1.2 Problem Statements

According (Hamid, Rahim, and Dardak,2013), the cost of raw materials, especially sawdust is increasing in every year. For example, to buy sawdust from Johor and pay a higher cost for transportation. Competition to obtain sawdust is getting tougher. As a result, the price is increasing. Previously the price of sawdust was around RM300 for a 3 tones lorry, but now it has increased to around RM550 (personal communication from the grower). The price of input such as plastic bags sawdust and the salary of workers is increasing every year (Haimid et al., 2013). Therefore, small farmers are facing problems to grow oyster mushrooms using sawdust which influences to decline of production. On the other hand, demand is increasing day by day worldwide including in Malaysia. So increasing mushroom cultivation is a national policy in Malaysia. Every year there is a growing demand for mushrooms.

However, the demand cannot be met by domestic manufacturing. Since 2009, Malaysia, therefore, has been required to import more than five tonnes of fresh and dry mushrooms annually. Imports rise per year to more than 10 million tonnes, led by higher domestic demand in 2012. Malaysia imports more than USD 1,55 million tonnes of dry mushrooms, which was over USD3,0 million in 2012, and 2.71 million tonnes of fresh mushrooms. The imports in China of Chinese mushrooms gradually rise, for fresh, by 9.23% on an annual average and by 12.93% in 2009 (Amin and Harun, 2015).

Many farmers are still planting on land areas that have shown less favorable results in terms of farm productivity since the soil has lost its nutrients (Simon, 2012). The use of chemical fertilizer or mineral fertilizer, commonly referred to as inorganic fertilizer, can increase crop production but hurts a negative effect on sustainability over time. It can also degrade the structure and texture of the soil, which can lead to soil acidity and erosion as a reaction to nutrient leaching effects (Omidire, Shange, Khan, Bean & Jewel, 2015).

Furthermore, it is anticipated that the utilization of organic waste to decay will boost agricultural yields. Organic waste contains a lot of organic stuff that may be utilized as fertilizer (Simon, 2012). Goat dung and vegetable waste are two examples of organic waste that are readily available and can help to minimize pollution. The usage of prepared compost is a good solution for preserving production while also improving soil quality and partially substituting mineral fertilizer. As a result, it is one of the solutions for reducing reliance on chemical fertilizer through the use of compost made from combined vegetable waste and goat dung. As a consequence, compost made from vegetable waste and goat dung may be the greatest alternative as a natural fertilizer, as evidenced by several studies that yielded favorable results for the test plant species. As a result, the dependency on chemical fertilizers is greatly reduced, as is the environmental effect.

1.3 Hypothesis of the Study

Hoi: There is no significant difference in producing mushroom yield between the substrate; sawdust, and rice straw

Ho ii: There is no significant difference among all the substrates as biofertilizer compost for plant growth.

Ha i: There is a significant difference in producing mushroom yield between the substrate; sawdust, and rice straw.

Ha ii: There is a significant difference among all the substrates as biofertilizer compost for plant growth.

1.4 Objective of the study

1. To evaluate the effectiveness as substrate of paddy straw for grey oyster mushroom growth.

2. To evaluate the substrate as compost for growing plants, red spinach.

1.5 Scope of the study

This study focused on the use of agricultural waste as substrate for the oyster mushroom (*Pleurotus sp*) for the two largest Malaysian crops, paddy field. The research will then begin with paddy straw substrate preparation with the optimum required ratio. Development, mushroom yield, mushroom productivity, and harvest interval will define as the parameters. The substrate sawdust, and manipulated substrate. Furthermore, this research will undertake projects focused on the compost output challenge. The substrate with the paddy straw substrate may be a successful compost as a biofertilizer for plant-growth compost. For composting and substratum each substrate contains a differentiation between carbon and nitrogen. Parameters are which include plant height, how many plants leave, and how much gets compost nutrients.

1.6 Significant of the study

The significance of the study is to evaluate the substrate which consists of paddy straw as an alternative substrate beside the sawdust. The reduction of cost production could be achieved if the substrate shows a positive result on the mushroom growth. In addition, the environmental pollution caused by the burning of rice straw could be reduced, might help to manipulate the agriculture waste into beneficial use. This study might be value-added to encourage smallholder entrepreneurs to involve in the mushroom industry, to increase the mushroom industry in Malaysia.

1.7 Limitation of the study

The limitation of the study, to get a sample of rice straw as it is seasonal material. Another most important limitation of mushroom contamination by green mold fungus might be delayed work. Besides that, seasonable rain also disturbs for the process of planting and making compose. Monsoon east coast happens every year for east coast Malaysia. To get direct sunlight are difficult to dries spent mushroom substrate (SMS).

CHAPTER 2

LITERATURE REVIEW

2.1 Oyster Mushroom (*Pleurotus sajor cajo*)

2.1.1 Origin, taxonomy, and distribution of oyster mushroom (*Pleurotus sajor cajo*)

Mushrooms are cultivated in a controlled biological environment and it has been extensively used as food since ancient time, due to their nutritive and medicinal values. Oyster mushrooms, the common name for the species *Pleurotus sajor cajo*, are one of the most common types of cultivated mushrooms in the world. They're also known as pearl oyster mushrooms or tree oyster mushrooms. The Fungi grow naturally on and near trees in temperate and subtropic forests around the world, and they're grown commercially in many countries. Oyster mushrooms are eaten in a variety of cuisines and are especially popular in Chinese, Japanese, and Korean cooking. They can be dried and are typically eaten cooked. Oyster mushrooms are beloved the world over for their delicate texture and mild, savory flavor. The mushrooms typically have broad, thin, oyster- or fan-shaped caps and are white, gray, or tan, with gills lining the underside. The caps are sometimes frilly-edged and can be found in clusters of small mushrooms or individually as larger mushrooms. Oyster mushrooms are more expensive than white button mushrooms but less so than rarer mushrooms like morels and take little prep since they can be used whole or chopped. They are even used to make mycelium furniture and many other products.

Kingdom: Fungi

Division: Basidiomycota

Class: Agaricomycetes

Order: Agaricales

Family: Pleuritaceae

Genus: *Pleurotus*

Species: *P. ostreatus*

Binomial name: *Pleurotus ostreatus*

2.1.2 Origin, taxonomy, and distribution of red spinach (Amaranthus gangeticus)

In the *Amaranthaceae* family, the genus *Amaranthus* has slightly over 100 recognized plant species (The Plant List, 2013). A. Tricolor has several synonyms, including A. gangeticus, which was previously considered as a distinct species. Some of the common names reflect the place of origin of this species, while the name Joseph's coat refers to the variety of colors that the leaves can have and is derived from the Biblical character Joseph, who is reported to have worn a coat of many colors. *Amaranthus gangeticus* is native to Asia, spreading from India through China and Japan in the north to Indonesia in the south, while also New Guinea, the New Hebrides, and some other Pacific Island nations (Fiji, etc.). It would be the most developed of the vegetable amaranth species and has been widely planted (Advisory Committee on Technology Innovation, 1984). Is also said to be planted in Benin, Nigeria, Kenya, and Tanzania in Africa (Grubben, 2004). It is also grown in the Caribbean. It has been imported to Louisiana, Michigan, and Missouri in the United States, where it has escaped from cultivation (Flora of North America Editorial Committee, 2015).

Plants thrive at temperatures as high as 25°C during the day and as low as 15°C at night. Chilling injury may occur if temperatures fall below 10°C, causing the production of fibrous plants that do not grow straight. Plants prefer full sun and thrive in low to medium humidity. Elevations range from sea level to 1000 m. It also has a low day-length sensitivity to short days and requires 15 hours or less for direct sunlight to bloom. It grows best in loam or silty-loam soils with good water-holding capacity, although this may thrive in a variety of soils with pH levels from 4.5 to 8. (Grubben, 2004; Ebert et al., 2011; Ecocrop, 2018; Infonet Biovision, 2018). *Amaranthus gangeticus* has shown tolerance for soil with high amounts of aluminum (Foy and Campbell, ***1981).***

- Domain: Eukaryota
- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Order: Caryophyllales
- Family: Amaranthaceae
- Genus: Amaranthus
- Species: *Amaranthus gangeticus*

2.1.3 Health benefit

The grey oyster mushroom is one of the commercially grown mushroom varieties worldwide because of its outstanding properties. (Rout et al,.2012) reported that grey oyster mushrooms are a major edible marketplace mushroom that has been widely respected in medicine and nutrients for its purpose. The mushroom possessed a wealth of protein, vitamins, and mineral salts that made it essential for human use. When dried, Oyster Grey Mushroom possessed around 30-40% protein (Saard, 2013). As a result, they were considered vegetarian meat and could serve as a substitute for animal meat. There is no dangerous cholesterol in this fungus to harm the blood vessel and provides adequate incentives for people with kidney, heart, liver, and high blood pressure conditions (Saard,2013).

2.1.4 Medicinal value

Randive(2012) reported that a great amount of niacin and vitamin B complex and C, compared with other plant species, has been stored in this mushroom. It can help farmers from waiting for another round to harvest, can use compose from spent mushroom substrates as media planting. Rice straw showed potential for improvement of surface soil physical and hydraulic properties, and then its effectiveness was partly dependent on amendment types and application rates (Eusufzai, Maeda&Fujii,2007). Rice straw can replace sawdust as raw materials.

2.2 Mushroom cultivation requirement

2.2.1 Nutrition requirement for mushroom growth

In addition, block of oyster mushroom mostly farmer didn’t know that can use as compose planting material. Cultivation of oyster mushrooms is of most concern as its spores are allergic to some people, so related preventive measures should be done in the working facility. Besides this, oyster mushrooms have a short life span, so they are beneficial to those growers who can sell them fresh on market (Tables 1-3).

Table 1: Various types of agricultural waste used for mushroom cultivation

Substrates number	**Agricultural waste**	**Strains**
1	Rice straw Wheat straw Cotton straw Tea leaves Banana leaves	*Pleurotus sp.*
2	Wheat straw	*Agaricus bisporus*
3	Rice bran Coffee pulp	*Lentinula edodes*
4	Tea leaves	*Volvanella sp.*
5	Sawdust	*Ganoderma sp.*

Source: Kamthan and Tiwari, 2017

Table 2: Composition of various types of substrates.

Substrates number	Substrate	Composition
1	Wheat straw	1% Protein 13% lignin 39% hemicelluloses 40% cellulose
2	Rice straw	41% cellulose 14% lignin 0.8% Total nitrogen 0.25% P_2O_5 0.3% K_2O 6% SiO_2 pH 6.9
3	Sugarcane bagasse	Cellulose 35-40% Hemicellulose 20-25% Lignin 18-24% Ash 1-4% Waxes <1% Nitrogen 0.7%

Source : (Kamthan and Tiwari, 2017)

Table 3: Combination of substrate reported on various strains and their effect.

Substrates number	Substrate (in combination)	Strain	Effect
1	Barley straw + wheat bran and wood chips + soybean powder + rice bran treatments	*Pleurotus eryngii*	4.64% protein content
2	Wheat straw + wheat bran + soybean powder treatment	*Pleurotus eryngii*	13.66% protein content
3	Soybean straw + wheat straw	*Pleurotus sajorcaju*	87.3% biological efficiency
4	Soybean straw + sawdust	*Pleurotus sajorcaju*	43.8% biological efficiency
5	Corncob (CC) + Sugarcane bagasse	*Pleurotus ostreatus* *Pleurotus cystitis*	The high content of protein, ash, and mineral (Calcium, Potassium, Magnesium, Manganese, and Zinc

Source: (Kamthan and Tiwari, 2017)

2.2.2 Humidity and substrate moisture content

Substrate bags will be put in the mushroom shed with 25ºC – 27ºC temperature and 85% – 93% humidity. Mycelium coverage of the bed, days taken to cover the entire bag, and total yield will be observed In a spawn room at 25 C, the inoculated substrates will be maintained with relatively high humidity of 70% in dark circumstances. The spawning time will be recorded for the colonization of the substratum (the number of days from the inoculation to full mycelium colonization). In the colonized culture bag separated by the incubation period the mycelial growth rate was estimated as mycelial height (mm) (days) (Yang, Guo, and Wan, 2013).

2.2.3 Oyster Mushroom (*Pleurotus* spp)

Pleurotus mushrooms rank second in the worldwide mushroom market and are well-known in China. As a result, it is well recognized among higher fungi as an important economic genus (Aishah, 2013). *Pleurotus spp*. belongs to the basidiomycetes class and, as a result of the production of white mycelium, to the group known as "white-root fungus" (Tsujiyama and Ueno, 2013). The oyster mushroom has the shape of a trumpet or an oddly shaped ear on an erratic and short stem. Fresh oyster mushrooms range in color from white to light grey, while dried oyster mushrooms range in color from light brown to golden brown. These mushrooms might grow to be more than a half-foot in diameter. Each fruiting body grows in a cluster and is overlapped with the others. It grows excellently on decayed hardwood.

There are around 40 species of Pleurotus mushrooms that have been identified (Alananbeh, Bouqellah, & Al Kaff, 2014). Several commercial Pleurotus mushrooms have been widely farmed and have significant economic worth, such as *P. ostreatus (oyster mushroom), P. eryngii (cardoncello or king oyster), P. pulmonary, P. djamor (pink oyster mushroom), P. Sajor-caju (Indian oyster), P. Sajor-caju (Indian oyster), P. Sajor-caju (Indian oyster), P. Sajor-caju (Indian oyster), P. Sajor-caju (Indian oyster), P. cornucopiae (golden oyster mushroom), P. cystidiosus (abalone oyster), and P. citrinopieatus (abalone oyster)*. Pe'rez-Martnez et al. (2015); Knop et al. (2015); Zhang et al., 2016; Bellettini et al. (2016). Pleurotus species have a short growth period and great resistance to fruiting bodies. When compared to other species of mushrooms, it is more resistant to pests and disease. Furthermore, it is cultivable has a high yield, a wider substrate use, spinelessness, a wide temperature range, and chemical resistance. Tolerance, as well as easy and cost-effective environmental bioremediation 2016 (Bellettini et al.).

2.2.4 Watering

Generally, the humidity level required by the oyster mushroom is around 80-90%. So, to raise and maintain the temperature and humidity of mushroom houses, frequent watering need to be carried out to support the growth of the mushroom. It is advisable to water the mushroom in small amounts but with high frequency rather than twice a day with a large amount. Furthermore, the mushroom bags cannot be directly sprayed with water as the water droplet will harm the pinhead where there is a variety of bacteria and pathogens in the water which may lead to an infestation of disease to mushroom also the substrate. The effective method in rising the mushroom house humidity is by watering the floor in the mushroom house, spraying the water in mist form, and fixing the sprinkler on the rooftop of the mushroom house (Lin, 2004).

2.3 Compost

Composting is a solid waste management solution that may be utilized to transform organic materials into valuable objects. Furthermore, it may be utilized to reduce the growth in the trash. This process is known as the most efficient, environmentally safe, and agronomically efficient, since compost may be used as a soil conditioner as well as an organic fertilizer due to its high nutritional contents. Compost's microbial population, which includes bacteria, fungus, and worms, can also help to stabilize degradable organic matter. The composting process's efficiency will also be determined by the trash's qualities, as composting is only appropriate for biodegradable garbage. Compost can improve soil conditions that are in urgent need of rejuvenation by boosting the organic carbon content of the soil. Moreover, compost works as a soil infiltration rate, water retention capacity, and tilth (Suhas & Hemali, 2018). According to Mathur, Owen, Dinel, and Schnitzer (1993), compost stability or maturity is an important factor influencing the efficient use of composts in agriculture. This fact is corroborated by Keeling et al. (1994), who said that the usage of unstable composts might cause low plant growth and crop damage by competing for oxygen or producing phytotoxicity to plants owing to inadequate organic matter biodegradation.

2.3.1 Nutritional quality

Malaysia has to face the challenges of trade regulation and requirements from the World Trade Organization (WTO), where only the high quality of vegetables and fruits can penetrate the foreign markets such as Europe and developed countries. In the domestic agricultural food market, the demand for high-quality vegetables has increased as more people can afford high-quality foods. Thus, producing vegetables and fruits with good nutritional quality is a concern by agriculturists (Tey et al., 2009). Hassan et al., (2012) said that despite plant genetic makeup, maturity and climatic conditions are play a more important role in the composition of plant nutrients.

Next, Liu and Li (2003) have shown that organic vegetables produce high concentrations of vitamins A, B1 (thiamin), B2, B12 (cyanocobalamin), C, E, soluble proteins, carbohydrates, total sugar, and mineral compounds which are Ca, K, Mg, S, and Na when compared to inorganic fertilized vegetables (Liu and Li, 2003). Moreover, the crops that are organically managed usually have higher vitamin C than inorganic treatments. This is because when the plants are exposed to more nitrogen, the production of protein will increase and the synthesis of carbohydrates will decrease. Vitamin C levels are also minimized because it is synthesized from carbohydrates. Inorganically managed soils, the plants are exposed to lower amounts of nitrogen and organic crops would be expected to maintain a higher vitamin C as well (Shree, Singh & Kumar, 2014).

2.4 Substrate composition

The substrate of mushroom is a type of lignocellulosic medium that supports mushroom growth, development, and fruiting process (Chang & Miles, 1988; Mondal, Rehana, Noman & Adhikary, 2010). The substrate requirements are varying according to the type of mushroom species cultivated. According to Madelin (1956), the sources of carbon and nitrogen in the substrate need to be balanced for fruiting body induction (Nooraishah, 2014). Substrates used for mushroom cultivation will give impact on characteristics of chemical, functional and sensorial of the yield produced (Oyetayo & Ariyo, 2013; Bellettini et al., 2016)

2.4.1 Agricultural lignocellulose by-product in Malaysia

According to (Barmina et al., 2013), compose from sawdust commonly has been unused composed material. Now day, rice straws as a waste material at paddy fields after the harvesting period of paddy plants.

2.4.2 Rice straw

Rice straw is considered lignocellulose biomass that contains 38% cellulose, 25% hemicellulose, and 12% lignin (Kamthan&Tiwari,2002). Using rice straw as substrate cultivation and composing materials can decrease activity open burning after paddy is harvested. Compared to the biomass of other plants, such as softwood, rice straw is lower in cellulose and lignin and higher in hemicellulose content (Barmina et al. 2013). Biochemical decomposition o rice straw is a sequential process that initially involves the loss of less recalcitrant components (i.e. oligosaccharides, organic acids, hemicellulose, and cellulose) followed by the degradation of the remaining highly recalcitrant compounds (i.e. lignin or suberin). In addition, straw is a valuable addition to the compost pile, as it decomposes quickly and helps heat the mixture. You can add it directly from the bale or add it at the end of the season after you've used it as mulch along garden rows. Cleaning out straw used as animal bedding usually contains manure, which adds another layer of nutrients to the compost. Allow the manure-straw blend to cure at least several months before redistributing it in your garden, as fresh hot manure can damage plants (SF Gate Contributor, 2021).

2.4.3 Sawdust

Currently, lignocellulosic materials such as sawdust obtained from various tree species are widely used for commercial production of this mushroom (Yamanaka, 2011; Hwang et al., 2015). Currently, the commercial cultivation of mushroom, grey oyster mushrooms especially is using rubber tree sawdust as the base substrate. However, due to high demand and insufficient supply of sawdust, the price is getting higher each year especially in certain states such as Selangor, Terengganu, and Kelantan (Zaffrie & Azahar, 2015). In addition, a study showed that substrate composition which consists of the mixture of sawdust with other agricultural waste can improve the yield performance of P. ostreatus (Obodai & Johnson, 2002; Mohamed, Refaei, Abdalla & Abdelgalil, 2016).

CHAPTER 3

MATERIALS AND METHODS

3.1 Material and Equipment

3.1.1 Raw materials Collection

Fresh rice straw gathered from the local paddy field center at the Rantau Panjang district. The controlled substrate which is sawdust will be bought from a local mushroom supplier. 60 cm x 30 cm polybag with 100 gauge thickness used as substrate bag. For substrate preparation, a shredder and the manual cutter will be used for cutting both rice straw into small pieces. Then, for the storage of mushrooms, it placed specialized mushroom shed (Kavitha, Rajannan, and Jothimani, 2013). This cellulosic material can be used for a broad variety. It can easily and rapidly be grown in composted substrates like paddy and cotton waste or other organic cellulose waste (Biswas, 2014).

Figure 3.1: Raw Substrate material: Rice Straw (RS), Sawdust (SD)

3.2 Methods

3.2.1 Substrate preparation

Rice straw must be cut into small pieces. The size of the rice straw must be 2-3 cm in length. These can be factors in mycelia's growth. If too long, mycelia growth takes time and maybe cannot grow. After cutting the rice straw into small pieces, the rice straw must be soaked 2-3 hours before starts boiling. The functions of soaking are to wash the rice straw and also make sure no dirt on the surface area of the rice straw. Dirty or mud around the surface area can be affected to the substrate in case of the substrate is contaminated. Next, the rice straw will boil on the gas stove for around 1 hour. The boiling process is needed to make sure the rice straws are clean or sterile and kill the bacteria from contamination. Rice straw cannot be autoclave. Boiling is the otherways to kill the bacteria. After boiling, the rice straw must be dried under the sun. Water from boiling didn't require because the content of boiling water is unknown.

Following the composting process, the media were placed in plastic bags with a planted media overall weight of 1000 g and a plastic bag minimum thickness of 0.003 mm. Any bag log was mounted horizontally on wooden shelves and remained until the white grew (Yang, Gou, and Wan, 2013)

Figure 3.2 shows the processed of preparation substrate

3.2.2 Substrate Filling in Polypropylene Bags and Spawning Process

For treatment 2 polybags substrates, 650 g shredded straw that has been sterilized and 150 g of spawn put into each of them. At the bottom of the bags, straw bits placed uniformly to 5 cm in height.

Table 4: The percentage of substrate composition per bag

SUBSTRATE/TREATMENT	COMPOSITION OF SUBSTRATE
T1 (CONTROL)	**100% SD + 10%RB + 10% CaCo2**
T2	**100% RS + 8% RB + 8% CaCo2**

Notes; T: treatment, SD: Sawdust, RB: Rice bran, RS: Rice stra w:

3.2.3 Experimental conditions and induction

The inoculated mushroom bags were arranged vertically on the growth rack as Figure 3.2 to encourage mycelium growth. This kind of arrangement could promote the colonization of the mycelium in mushroom bags. The growth rack was put in small room (Figure 3.3) to control the light intensity as the spawn-run stage should be carried in dark conditions for the mycelium to grow. The mushroom bags were left in a dark room for 17 to 21 days to allow the spawn-run process. The relative humidity was maintained around 65-75% (spawn-run stage) and raised to 75-85% (after pinhead formation) by spraying water mist around the rack. The room temperature was maintained around 25-28o C. After the spawn-run stage was complete, the bags were sliced open carefully to allow pinhead formation and fruiting bodies maturation.

Figure 3.3 shows all substrate arranged vertically

3.3 Growth parameters of mushroom and red spinach

The growth and progress of common bean will be monitored following the parameters:

I. Mycelium run rate (cm/day)

The measurement of Mycelium will be in centimeters. The measurement will be taken once a week.

II. Number of primordial initiation

The number of primordial means the data collected based on the number of primordial that are present on the substrate. The measurement will be taken once per week.

III. Days required to first harvest

Days required to the first harvest means the data collected based on the days for the mushroom to mature and be ready to be harvested.

IV. Yield per mushroom bag

Yield per mushroom bag means the data collected based on the number of fruiting bodies that are present on each bag of mushrooms. The data will be collected during harvesting.

The observation and data of research for the growth performance of grey oyster mushrooms on different types of substrates were recorded based on seven parameters since the day after spawning. The parameters measured in this study were the duration of mycelium full colonization, pinhead formation days, time is taken for first harvesting after spawning, stipe length, pileus diameter, and biological efficiency. For the mycelium full colonization, the days were counted from the first day of spawning until the mycelium was fully covered in the substrate bags. Then continued with the formation of pinhead recorded from the first day until fruiting body developed and time taken for first harvesting were recorded after the spawning day until the fruiting body completely mature. For stipe length and pileus diameter were being measured by using tape measure while yield weight of fresh mushrooms was measured by using a weighing scale.

3.4 Preparation of substrate as compost

Composting mixes waste materials, followed by conditions that allow for a higher level of organic resources for biological decomposition. The speed and efficiency of composting are determined by the form, quality, chemical, and physical characteristics of the raw substances, conditions, and climate (Gummert et al., 2020). For composting preparation in this study used hot composting by using Berkeley Hot Composting Method by Atchley (2013).

3.4.1 Seedling growth test

The experiment was carried out in the nursery of University Malaysia Kelantan, Jeli Campus (5.7445oN, 101.8642o E). The seeds of red spinach (*Amaranthus gangeticus)* were allowed to germinate in the seedling tray that was filled with commercial soil and also compost. Four seeds were planted for each hole. After 1 week of germination, the seedlings were thinned out to one plant and transferred into the polybag with 16cm of diameter that formerly was filled with topsoil as a growing medium. 18 polybags were used to investigate the performances growth of red spinach. Both leafy vegetables were grown and watered twice a day or when necessary to maintain the soil moisture.

3.4.2 Measurement of growth parameters

All of the parameters were measured after 1 month of the treatment being applied to the vegetables. The vegetable's height was measured by using the ruler and number of leave. The shoot fresh weight was measured by using a weighing scale while the chlorophyll content of leaves was measured by using a spad meter. All measurements were done in three replicates and the mean was calculated.

3.5 Experimental design

In this experiment, the design of experiments is RCBD. Two treatments were used in this substrate preparation and mushroom cultivation stage also in biofertilizer observation.

T1= control as saw dust (100) :Rice burn (10) : CaCo2 (1)

T2= Rice straw (100): Rice burn (4): CaCo2(8)

Before starting, the experiment weight of the block substrate arises same, 800g. every single substrate will collect its data of the time of mycelium growth, high of mycelium growth, day of body mushroom merge, and day of mushroom can be harvested.

T1= control as commercial soil

T2= compost product

Before starting, each treatment is filled in a seedling tray before transfer in a polybag. Each treatment has 9 polybags. 1-month observation to see differentiate of growth by *Amaranthus gangeticus.*

3.6 Statistical analysis

The experiment was designed based on Randomized Complete Block Design (RCBD) with control and one different treatment. Each treatment had nine replicates. All data were analyzed by using a one-way to establish the significant difference between the means where $p<0.05$. the calculations were done using Microsoft excel

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Growth performance of Pleurotus sajor cajo on the duration of mycelium full colonization.

The result showed the duration of mycelium full colonization against the treatments applied on *Pleurotus sajor-cajo* cultivation. Substrate with used rice straw is more effect in duration of mycelium full colonization. It was found that there was a significant difference in T2 compared to the T1.

Moonmoon, Uddin, Ahmed, Shelly, and Khan's 2010 found new results after they used rice straw and sawdust as *Pleurotus eryngii* cultivation substrate. They found that sawdust substrate has a quicker mycelium run rate over rice straw. However, their study's results were not considered related to those of Khandakar et al. (2008), who investigated mycelial formation on numerous cultural mediums. The spawn running variation is affected by the change in biochemical activity during mycelial development where the lignocellulosic materials are converted or degraded by extracellular enzymes from the large component into low molecular weight compounds (Nurul et al., 2017).

Figure 4.1: colonization of mycelium started growth full the surface area of the substrate

4.2 Growth performance of Pleurotus sajor cajo on the duration for harvest after spawning

The result showed the duration for the first harvest after spawning against the treatments applied on P. sajor cajo cultivation. It was found that there was significant differences in treatment 1 compared to the Treatment 2 ($P<0.05$) as shown in the table above where treatment 2 took shortest days (43 days) to be harvested compared with Treatment 1 which 53 days.

The result of this study was by research done by Neupane, Thakur, Bhatta, Pathak, & Gautam, (2018) where the harvesting duration in rice straw substrate was faster (26.25 days) compare to sawdust (50.25 days)

Figure 4.2: this figure showed block form T2 with full mycelium ah pop up with many stipes in one bunch

4.3 Growth performance of Pleurotus sajor cajo on the yield weight

The result above showed the yield weight against the treatments applied on *Pleurotus sajor cajo* cultivation. It was found that there was a significant difference in treatment 2 compared to the treatment 1 ($P<0.05$) as shown in the graph above where T2 has higher yield weight 26 gram compared to T1 only 21 gram. Thus, among contaol and treatments, Treatment 2 was recorded as having the highest while Treatment 1 has the least weight of yield.

Survase D.M. (2012) discovered that using paddy straw yielded the highest yield among the various substrates used in oyster mushroom production, including wheat straw, soybean straw, pigeon pea straw, and green gram straw (Patel & Trivedi, 2015). Another study employed paddy straw, wheat straw, apple leaves, and Chinar leaf substrate by Shauket, Abdul, and Riyaz et al. (2012). He discovered that paddy straw yielded the most, followed by wheat straw, apple leaf, and chinar leaf substrate (Patel & Trivedi, 2015)

Figure 4.3: weight of mushroom from T2 is recorded for future data

4.4 Growth performance of Pleurotus sajor caju on the length of stipe

The result in the table showed, the stipe length of the mushroom against the treatments applied on Pleurotus sajor caju cultivation. The shortest stipe length showed in Treatment 1 (2.5cm) had a mean of 3cm. Treatment 2 (4.5cm) produced the longest stipe with a mean of 3.56cm and it was different significantly. A different study showed that rubber sawdust utilized resulted in the smallest stipe length when compared with other types of substrates employed (Nurul et al., 2017).

Another study, done by Mondal, Rehana, Noman, and Adhikary (2010), showed that the biggest stalk length of P. Florida was grown from the sawdust substrate. Unlike Neupane et al. (2018), the stipe length of oyster mushroom developed from rice straw was longer (6.02 cm) than that of sawdust substrate (4.6cm). As shown in research, some users prefer to consume mushrooms with short stipes, while the others prefer mushrooms with bigger cap widths and longer stipes (Yang, Guo, & Wan, 2013

Figure 4.4: Length of stipes are recorded for both treatment, T1 and T2.

4.5 Growth performance of Pleurotus sajor caju on the diameter of pileus

The result showed the pileus diameter against the treatments applied on Pleurotus sajor caju cultivation. It was found that there are significant differences in Treatment 2 compared to Treatment 1 ($P<0.05$) as shown in graph above where mean diameter of pileus in Treatment 2 was 6.72 cm meanwhile Treatment 1 was 4.5cm. Treatment 2 is shown to have a bigger cap diameter compared to Treatment 1. Used rice straw as substrate give a good performances for diameter pileus compare to sawdust substrate.

A similar result was found by Neupane, Thakur, Bhatta, Pathak, & Gautam, (2018) where the pileus diameter of oyster mushroom in the rice straw substrate was bigger (8.96cm) compared to pileus diameter mushroom produced from sawdust substrate (7.14 cm).

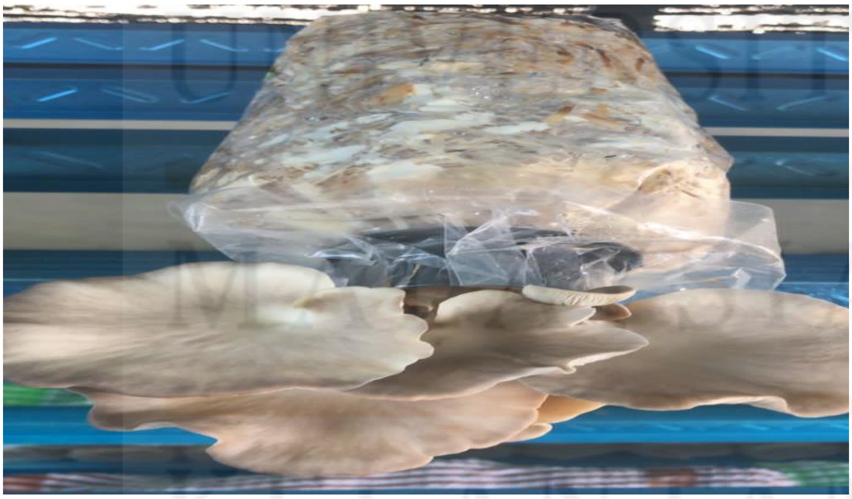

Figure 4.5: pileus diameter of treatment 2

4.6 Growth performance height of red spinach

The plant height against the treatments applied red spinach is having a similar trend. It was found significant differences between control and compost on the plant height red spinach. The application of compost treatment on red spinach showed the highest plant height which gives an increase of 37.6% and 47.2%, respectively.

Figure 4.6: high of plant are measured using ruler

4.7 Growth performance number leaf of red spinach

The result in the figure showed the number of leaves has a significant difference between T1 and T2. The application of compost treatment on red spinach showed the number of leaves T2 (3 leaves) more than T1 (2 leaves).

Treatment 2 Treatment 1

Figure 4.7: number of leaves each treatment are counted to see differentiate between two treatments

Table 4.8 Effect substrate on *P. sajor-caju* growth characteristics

T	DURATION OF MYCELIUM FULL COLONIZATION (DAYS)	TIME FOR FIRST HARVEST AFTER SPAWNING (DAYS)	YIELD WEIGHT (G)	STIPE LENGTH (CM)	CAP DIAMETER (CM)
T1	42.78±3.28	53±1.94	21±0.64	3±0.35	4.5±0.35
T2	32.67±2.67	43±1.77	26±1.31	3.56±0.81	6.72±0.94

Values in the table showed the mean±standard deviation of nine replicates. T indicated treatment

Table 4.9 Growth characteristics of red spinach

T	NUMBER OF LEAF	HEIGHT OF PLANT (CM)
T1	2.33±0.18	1.66±0.05
T2	3±0.35	2.04±0.09

Values in the table showed the mean±standard deviation of nine replicates. T indicated treatment

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Mushroom farming in Malaysia is regarded as one of the relatively modest biotechnology industries that have been growing year after year. This is owing to the industry's capacity to use agricultural byproducts through biotransformation of lignocellulosic organic materials into food for human consumption. Demand for mushroom supply is increasing as people become more aware of the nutritional and therapeutic benefits provided by mushrooms. However, the domestic output is still insufficient to fulfill local demand, necessitating imports from other countries, most notably China.

Sawdust is frequently used as the primary substrate material in commercial mushroom production in Malaysia. The availability of sawdust is restricted, and prices are rising, impeding the growth of mushroom cultivation in Malaysia. Aside from that, Malaysia produced a considerable number of agricultural leftovers, particularly from oil palm and paddy fields, which are the country's main commodities. Because oil palm and paddy wastes include lignocellulosic elements, they were chosen for this study to determine their potential as mushroom substrates to identify another alternative material to minimize sawdust consumption. Thus, the purpose of this research was to identify an alternate substrate for the growth of grey oyster mushroom (P. sajor caju), thereby reducing the need for sawdust usage in the substrate by utilizing agricultural by-product.

All 6 criteria were found to be significant in this investigation, according to the findings. The use of different materials in varied proportions for each treatment had a substantial influence on the development and features of the mushroom generated.

The treatment were compared to the control, which was a commercial substrate and commercial soil, and the overall results showed that they differed considerably from the control as well as among themselves. Some of the measures performed better in treatments 2 than in treatment 1. Treatment 2 had the lowest mean days need of parameters for mycelium full colonization, first harvest time, stipe length, and pileus diameter when compared to Treatment 1. Treatment 2 generated more fruiting bodies (26g±.31g) than Treatment 1 (21g±.64g) in terms of yield weight. In the meantime, Treatment 1 had the smallest stipe length when compared to Treatment 2. Due to a number of variables, Treatment 2 was good substrate to be chosen as an alternate substrate for mushroom culture. The first is that this treatment outperforms Treatment 1 (commercial substrate: SD+RB+CaCo3) in a few metrics that are significantly different. The time of mycelium complete colonization, first harvest period, yield weight, stipe length, and pileus diameter all differed substantially (p0.05).

The results of this investigation demonstrated the stimulating impact of organic compost treatment on red spinach. The plant height and number of leaves rose considerably (P0.05) for both T1 and T2. To summarize, the study's overall findings show that compost waste combined with goat faces may be applied and employed to promote the more healthy and robust development of mustard green plants.

5.2 Recommendation

Further investigation into the experiments on nutritional content and chemical composition in all of these substrates is required. A research on various sorts of mushroom species would be valuable as well, since it would aid in filling up the gaps in this study's material. Following that, rice straw should be sold to local mushroom cultivators, and mushroom research should be expanded to increase the productivity and performance of Malaysia's mushroom business. It is really advised that compost be applied for improved growth performances of red spinach, such as plant height and quantity of leaves. However, the precise method through which the compost improves plant growth and development remains unknown.

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APPENDIX

APPENDIX A

First, rice straw was cut into small size, 2-3cm before boil

Stove are prepared for boiling processes

Third, rice straw are boiled for 30 minutes

Final step, after 30 minutes boiled, rice straw dried for a while before processes mixing.