

## Palm Press Fibre and Rice Straw for Cultivation Grey Oyster Mushroom (*Pleurotus sajor-caju*)

(Sabut Kelapa Sawit Tertekan dan Jerami Padi untuk Penanaman Cendawan Tiram Kelabu (*Pleurotus sajor-caju*))

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### ABSTRACT

Sawdust (SD) usually sourced from rubber plant is commonly used as substrate to cultivate the grey oyster mushroom *Pleurotus sajor-caju* in Malaysia. However, the market price of SD is increasing because of its declining availability. Thus, this study aimed to discover an alternative substrate to cultivate grey oyster mushroom. The raw materials used in the substrate were oil palm pressed fiber (PPF), rice straw (RS) and SD, either alone or in combination with different ratios including T1: 90% SD + 10% rice bran (commercial substrate as control); T2: 30% RS + 20% SD + 50% PPF; T3: 50% RS + 50% PPF; T4: 100% RS; and T5: 100% PPF. The suitability of the substrates was determined based on growth, yield, nutrition and mineral content in the mushrooms. The growth and yield of the mushroom fruiting body on the different substrates showed significant differences. The shortest harvesting day was obtained in T4 (100% RS) after 29 days, whereas the longest harvesting day was obtained in T1 (90% SD + 10% rice bran) after 51 days. The highest yield was obtained in T4. Nutrition analysis showed significant difference among the treatments. The highest amounts of protein (26%) and ash (1.29%) were found in T5. Overall, the results showed that mushroom yield performance was good in RS but nutritional content was the highest in PPF. Therefore, RS and PPF have good impact for mushroom growers either in commercial production or in functional food industry to reduce SD dependency.

Keywords: Grey oyster mushroom cultivation; growth performance; nutrition; palm pressed fiber; rice straw

### ABSTRAK

Habuk kayu (SD) kebiasaannya daripada sumber kayu getah yang sering digunakan sebagai substrat untuk penanaman cendawan tiram kelabu *Pleurotus sajor-caju* di Malaysia. Walau bagaimanapun, harga pasaran bagi habuk kayu meningkat disebabkan oleh sumber sedia ada yang semakin berkurangan. Oleh itu, kajian ini bertujuan untuk mengenal pasti substrat alternatif untuk penanaman cendawan tiram kelabu. Bahan mentah yang digunakan dalam substrat adalah sabut kelapa sawit tertekan (PPF), jerami padi (RS) dan SD, sama ada bersendirian atau dalam gabungan nisbah yang berbeza termasuk T1: 90% SD + 10% dedak padi (substrat komersial sebagai kawalan); T2: 30% RS + 20% SD + 50% PPF; T3: 50% RS + 50% PPF; T4: 100% RS; dan T5: 100% PPF. Kesesuaian substrat ditentukan berdasarkan pertumbuhan, hasil, nutrisi dan kandungan mineral dalam cendawan. Pertumbuhan dan hasil badan buah cendawan pada substrat yang berbeza menunjukkan perbezaan yang ketara. Hari penuaian cendawan paling pendek diperolehi pada T4 (100% RS) selepas 29 hari, manakala hari penuaian paling panjang diperolehi pada T1 (90% SD + 10% dedak padi) selepas 51 hari. Hasil tertinggi diperolehi pada T4. Analisis nutrisi menunjukkan perbezaan ketara antara rawatan. Jumlah protein tertinggi (26%) dan abu tertinggi (1.29%) diperolehi pada T5. Secara keseluruhan, keputusan kajian menunjukkan bahawa prestasi hasil cendawan yang baik pada RS tetapi kandungan nutrisi paling tinggi pada PPF. Oleh itu, RS dan PPF mempunyai kesan yang baik kepada para pengusaha cendawan sama ada dalam pengeluaran komersial atau industri makanan berfungsi bagi mengurangkan kebergantungan pada SD.

Kata kunci: Jerami padi; pemakanan; penanaman cendawan tiram kelabu; prestasi pertumbuhan; sabut kelapa sawit tertekan

## INTRODUCTION

Mushrooms belong to the phylum Basidiomycota under the kingdom Fungi. Mushrooms are heterotrophs that obtain food from decaying materials, such as dead bodies of organisms or debris from plants. Mushrooms are classified as edible, non-edible, and medicinal depending on their functions. Edible mushrooms are highly consumed by humans because of their rich nutrition, such as high amount of protein and low amount of carbohydrate. Currently, 17 major types of mushroom, including grey oyster, white oyster, straw mushroom, split gill mushroom, shiitake, and button mushroom, are commercially cultivated in Malaysia (MOA 2011). Grey oyster is the most popular species cultivated and marketed for household consumption, followed by shiitake or button mushroom that widely used in hotels and catering services. Nevertheless, mushrooms are used in various sectors, including the pharmaceutical, cosmetic and functional food industries (Tarmizi et al. 2013). Mushroom cultivation could directly enhance livelihoods through economic, nutritional and medicinal contributions (Marshall & Nair 2009). Mushroom cultivation does not require much land, and the business scale requires modest-to-low capital investment and labor use. The cultivation can become a viable and attractive activity for side income and a part-time enterprise for rural farmers and peri-urban dwellers.

The crop can also be cultivated on agricultural waste, making it an environmentally friendly. In Malaysia, the current practice is to cultivate this mushroom using sawdust (SD) from either rubber plant or any other plants, but dependency on SD causes problems in supply shortage and cost for the new grower. Both oil palm and paddy or rice are the utmost cultivated plants in Malaysia. However, massive production of residues from crops and very limited utilization for further downstream operations from these residues create problems in agriculture. The approximate number of agricultural residues has been utilized either as fuel in the timber industry and manufacturing. After cultivation of paddy, only 1% of rice straw (RS) is used for livestock, fertilizer, paper making, and activated carbon material, and the remaining RS is disposed by open burning, which negatively affects the environment and human health (Rosmiza et al. 2014). Straw left in the field can also lead to environmental problems with the release of methane, which is the major contributor to greenhouse gas (GHG) emissions, sharing approximately 80% of the total GHG emissions from the cultivation stage (Rosmiza et al. 2014; Silalertruksa & Gheewala 2013; Weil & Brady 2017).

Nutrients in the straw include nitrogen, phosphorus, potassium and sulfur, and in addition the cell wall structural composition (e.g. cellulose and lignin) of straw enriches mushroom cultivation (Rosmiza et al. 2014).

On the other hand, the production of waste from the oil palm industry was estimated to be approximately 65.5 million tons per year. It exists in various forms, such as palm empty fruit bunches, palm kernel shell (PKS), palm pressed fibers (PPF), and palm oil mill effluents. PKS and PPF are usually utilized as fuels for the generation of steam and electricity for palm oil mills. Nevertheless, excess unutilized fiber and shell are still available, leading to an accumulation problem. This biomass contains cellulose, hemicelluloses and lignin, which have potentials as renewable raw material (Noorhalieza et al. 2013). The nutritional and mineral contents of the grey oyster mushroom *Pleurotus sajor-caju* might be influenced or induced by the substrate components, which need to be studied for commercial functional food production. Therefore, the suitability of locally available substrates of RS, PPF, and SD either alone or in combination for the cultivation of grey oyster mushroom was determined. The growth performance and nutritional and mineral contents of the cultivated mushrooms were also analyzed.

## MATERIALS AND METHODS

### RAW MATERIALS

The rice straws were taken from a paddy field in Tumpat, Kelantan, Malaysia area after the harvesting season in June 2019. Meanwhile, palm pressed fiber was obtained from oil palm mill from Kg. Jerimbong, Jeli. *Pleurotus sajor-caju* spawn, sawdust, and commercial mushroom block (control) were purchased from AGROGENE company, Terengganu.

### SUBSTRATE PREPARATION

For RS, it was cut into 2 to 3 cm in length and washed thoroughly with clean water to remove any dirt. The cut RS was boiled in a big wok for 1 h 30 min. After that, the water was drained and the boiled RS was spread evenly on newspaper and left overnight to remove any excess water. On the next day, the RS was put under the sun for a while as it was still wet and then autoclaved at 121 °C for 1 h. The PPF was shredded into smaller pieces and washed with tap water for several times until the water became clean and clear. The clean fiber was transferred into the autoclave bag. Next, the filled bags were autoclaved for

1 h at 121 °C. Meanwhile the SD was straight forward autoclaved for 1 h at 121 °C. After autoclaved, the materials were kept for cool down at room temperature for the preparation of substrate bag.

#### SUBSTRATE FILLING IN POLYPROPYLENE BAGS AND SPAWNING PROCESS

All of the autoclaved substrate materials were mixed thoroughly according to the ratio stated in Table 1. All materials were ensured to combine well except for T1 which was purchased from commercial grower. Then, autoclaved water was added considerably using Palm Test Method to provide enough moisture in the substrate for the mushroom growth. The moisture content should be at 70-75%. Each of the mixed substrates was filled into polyethylene (PE) bag (22.86 × 48.26 cm) and the medium was pressed manually by hand to make it compact as much as possible to make the final weight of substrate bag as 500 g. Then, the medium was closed with a PVC neck set and cotton wool. The function of wool used to prevent from contamination and insect disturbance. The experiment was conducted in three replications in each treatment.

#### INCUBATION

The inoculated mushroom bags were arranged vertically on the growth rack to encourage mycelium growth. This kind of arrangement can promote the colonization of the mycelium in the mushroom bags. The growth rack was covered with black net to control the light intensity as spawn-run stage was carried out in dark condition for the mycelium to grow. The mushroom bags were left in dark room for 17-21 days to allow spawn-run process. The relative humidity was maintained at 65-75% (spawn-run stage) and raised to 75-85% (after pinhead formation) by spraying water mist around the rack. Ventilation fan was turned on for 24 h to allow proper aeration. The room temperature was maintained at 25-28 °C. After the spawn-run stage completed, the bags were sliced open carefully to allow pinhead formation and fruiting bodies maturation.

#### GREY OYSTER GROWTH ASSESSMENTS

Five different substrates as T1: 90% SD + 10% rice bran (commercial substrate as control), T2: 30% RS + 20% SD + 50% PPF, T3: 50% RS + 50% PPF, T4: 100% RS and T5: 100% PPF were used to evaluate the growth performance and nutritional composition. The growth

performance recorded based on seven parameters as days required for mycelium full colonization, the formation of pinhead, first harvesting, stipe length, pileus diameter, total yield weight and biological efficiency.

The parameters were analyzed according to the formula from Adebayo et al. (2014):

- i. Mycelium full colonization = first day of spawning- day that the mycelium was fully covered
- ii. The formation of pinhead = first day of spawning- first day of appeared the fruiting body
- iii. First harvesting = first day of spawning- day that the fruiting body completely mature
- iv. Stipe length = ruler scale was used from head to bottom for stipe length
- v. Pileus diameter = tape measure was used to measure the radius of the fruiting body
- vi. Total yield weight = 1st batch + 2nd batch + 3rd batch of harvested yield
- vii. Biological efficiency, BE (%) =  $\frac{\text{Weight of fresh mushroom (g)}}{\text{Weight of dried substrate (g)}}$

#### PROXIMITY ANALYSIS

Proximaty analysis is a method to determine the macronutrient contents and nutritional value in food sample. Protein, fat, moisture, ash and carbohydrate are key elements known as proximates while the determination process of the proximates is called proximate analysis (Nielsen 2010). For the proximate analysis, the cultivated mushrooms were harvested for every flushing, preserved in polypropylene plastic packaging and stored in the freezer at -18 °C until proximity analysis.

#### FAT

For the analysis of fat, the FOSS total fat procedure was used according to the manufacture (Soxtec/ Hydrotec™ 8000, Denmark). For extraction of fat, 2 g of the fine grounded mushroom powder was poured in the cellulose thimble before being placed in the Foss Soxtec apparatus extraction tube to determine crude fats. The weight of the sample used for the extraction depends on its approximate fat contents. The cellulose thimble containing samples and the extractor cups were placed together in the extractor to begin the extraction. Three main steps or processes undergone in an automated manner in the extractor were boiling, rinsing

and evaporation. After finishing the extraction steps, the extraction cups were removed and placed in muffle furnace at 100 °C for 30 min. After the incubation, the cups were left to cool down in a desiccator for 20 min. The equation below was applied to determine the crude fat content in the *P. sajor-caju*.

$$\text{Crude fat (\%)} = \frac{\text{Weight of final cup} - \text{Weight of initial cup}}{\text{Total weight of sample (g)}} \times 100$$

#### CRUDE PROTEIN

For the analysis of crude protein, the Kjeldhal method of Association of Official Analytical Chemists (Nielsen 2010) was followed. Firstly, 1 g fine ground mushroom powder was placed into each of the digestion tubes along with Kjeldahl tablets. Then, 12 mL concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was added into the tube for the digestion of the mixture using Gerhardt distillation apparatus for 2-3 h. After completion of digestion, the distillation process was carried out using Foss Kjeltac Distillation Unit. After the distillation process, the solution was placed in a conical flask containing a mixture of boric acid, methyl red, and bromocresol green. Methyl red and bromocresol green act as the indicator to detect the presence of the protein inside the receiver. It will change the receiver solution from red to green when the protein elements are transferred to it. Finally, all the protein elements collected during the distillation were titrated with 0.1 M hydrochloric acid, and the titration reading was recorded for the calculation of total nitrogen as shown below. The total nitrogen was then used to determine the percentage of crude protein as shown in the formula below.

$$N (\%) = \frac{(\text{Blank} - \text{Titre value}) \times 0.1 \times 1.4007}{\text{Weight of sample (g)}}$$

$$\text{Crude protein (\%)} = N \% \times 6.25$$

#### ASH

For analysis of ash, 1 g grey oyster sample in each treatment was used for the determination the ash contents. The sample was dried and finely ground to avoid spattering during ashing. The initial weight of the empty porcelain crucibles was recorded before approximately putting 1 g of finely ground mushroom inside the crucible. Then, all the crucibles were heated inside the muffle furnace for 4 h the temperature 550 °C.

The percentage of ash content was determined by using the formula as below:

$$\text{Ash (\%)} = \frac{\text{Weight of crucible with ash} - \text{Weight of empty crucible}}{\text{Weight of sample (g)}} \times 100$$

#### MOISTURE

The moisture was recorded by the oven-drying method which is one of the thermogravimetric approaches. It is an approach of subtracting the initial and final weight of the samples to calculate the moisture loss by the formula as below:

$$\text{Moisture (\%)} = \frac{(\text{Initial sample weight} - \text{Final sample weight})}{\text{Weight of sample (g)}} \times 100$$

#### CARBOHYDRATE

Generally, the percentage of carbohydrate was obtained by subtracting the summation of fat, protein, ash, and moisture of the dry matter from 100. To calculate the percentage of the carbohydrate content of *P. sajor-caju*, the following equation was implemented:

$$\text{Total carbohydrate (\%)} = 100 - \frac{\text{Total moisture} + \text{Total fat}}{\text{Total protein} + \text{Total ash}}$$

#### MINERALS ANALYSIS

A total of 1 g finely grounded mushroom was placed into porcelain crucible and heated in Carbolite muffle furnace for 6 h at 500 °C until it completely turned into white-greyish ash. Next, the crucible was cooled off for 15 min and then followed by digestion using 5 mL 20% hydrochloric acid inside the crucible to form ash of the mushroom powder. Afterwards, the completely digested solution was filtered using Whatman filter paper no. 1 and subsequently for second filtration by 0.45 mm syringe filter into a 50 mL volumetric flask. The filtrated solution was diluted with distilled water up to 50 mL and served as the pure stock solution of samples. The 50 mL of the stock solution was prepared in serial dilutions of 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, and 10<sup>-4</sup> in 15 mL of Falcon tube for the analysis of minerals of zinc, sodium, potassium, iron, and calcium using Atomic Absorption Spectrometry (AAS). AAS is the method in which the electromagnetic radiation is absorbed by free gaseous atoms at specific and precise wavelengths to obtain a measurable signal depending on the elements that were been inspecting.



## RESULTS AND DISCUSSION

### GROWTH PERFORMANCE

The growth of grey oyster mushroom in different substrate combinations (T1= 90% SD + 10% RS, T2 = 30% RS + 20% SD + 50% PPF, T3 = 50% RS + 50% PPF, T4 = 100% RS and T5 = 100% PPF) was assessed using the following parameters: full mycelium colonization, pinhead formation, first harvesting, stipe length, pileus diameter, total yield weight and biological efficiency.

Full mycelium colonization was determined based on the shortest number of days required to cover the substrate bag. Significant differences were observed between T4 and T1 (Table 2). T4 took 22.6 days, whereas T1 took 41 days for the mycelium to completely cover the substrate (Table 2). However, T1 showed no significant differences with T3 (35.6 days), T2 (37 days), and T5 (40.6 days).

The pinhead of mushroom formed faster in T2, T3, T4, and T5 than T1 (Table 2; Figure 1). In this category, T4 took the shortest duration (26 days) for pinhead formation, whereas T1 took the longest duration. The RS-related substrates (T2, T3, and T4) showed faster pinhead formation than the lignocellulosic substrate of SD and PPF. This finding is in line with the report of K et al. (2010) that the days required for the pinhead or primordial formation of *Pleurotus eryngii* is shorter in RS than in SD.

The stipe of the mushroom serves as its support. The formation and length of stipe also depend on mushroom substrate and its bag position. The small stipe length in T5 had a mean value of 1.33 cm, followed by T4 with a mean value of 1.93 cm. T2 and T3 had average stipe lengths of 4.167 and 4.333 cm, respectively. T1 produced a long stipe with a mean value of 8.2 cm, and it was significantly different from the other treatments. The cap diameter was also varied among the different substrates (Table 2). The mean cap diameter of T4 (9.6 cm) was significantly different from that of T1 (6.5 cm) ( $P < 0.05$ ; Table 2). T2 and T3 had average pileus diameters of 6.27 and 6.23 cm, respectively, and both had almost similar size to the cap diameter in T1. The lowest mean of pileus diameter (4.63 cm) was recorded in T5.

### YIELD PERFORMANCE

The yield performance of *P. sajor-caju* mushroom was investigated based on the duration for the first harvest and total yield in each substrate. T4 and T3 significantly differed with T1 ( $P < 0.05$ ; Table 2). T4 took the shortest duration (29.7 days), followed by T3 (40.3 days) and

then T1 (51.7 days). T2 took approximately 44.3 days and T5 took 49.3 days, which showed no significant difference with T1. The result of this study agrees with the report of Neupane et al. (2018) where the harvesting duration in RS was faster (26.25 days) than that in SD (50.25 days). Bellettini et al. (2019) reported that the combination of nitrogen and carbon proportion accelerates mushroom growth. The nutritional content in SD and RS showed that the nutrition component of RS is higher than that of SD (Bakker et al. 2013). RS is composed of cellulose, which is the major part of the cell wall. Cellulose is a polysaccharide linear amylose starch rich in carbon and glucose, which are easy to decompose by microbes. SD and PPF are composed of woody lignin or lignocellulosic materials. Lignin is highly non-polymer of phenol sub-units which is very stable organic molecule, thus it takes time to decompose by microbes. Hence, mushroom fruiting body grown in 100% RS facilitated good growth. During composting, the nitrogen and pH combination are higher in RS than in SD, which also influence mushroom growth (Keltawi et al. 2012). RS is rich in cellulose, which contains carbon, providing it with a good ratio of carbon and nitrogen, and this combination makes mushroom in RS to grow faster than that in SD (Bakker et al. 2013) (Figure 2).

Total yield was higher in T4 (173.33 g) than in T1 (140.80 g) (Table 2). The lowest yields of mushroom were obtained in T2 (106.30 g) and T5 (102.93 g). T3 showed medium yield (133.03 g) with no significant difference to T1 but exhibited significant difference with T4, T2 and T5 (Table 2). The yield for T3 was not significantly different from those of T1 and T4. The yields in T2, T3 and T4 were greater than those in T1 and T5. Song et al. (2017) found that mushroom treated with corn straw or corn cob and RS substrate exhibits better growth and higher yield than mushroom grown in SD. In addition, cellulose-based substrates such as wheat or RS obtain higher yields than SD (Iqbal et al. 2016; Patel & Trividi 2013). The main goal of entrepreneurs is to obtain high yield with low cost. Thus, the high yield in RS alone or in combination with other substrates indicates that RS is a good alternative substrate with low cost.

### NUTRITION ANALYSIS

In recent years, mushrooms have been increasingly consumed because of their nutritional composition. However, substrate type also may affect the nutritional diversification in mushrooms (Siwulski et al. 2019). Thus, the present study also analyzed the nutritional contents in mushroom fruiting body treated with T1, T2, T3, T4 and T5.

*Pleurotus sajor-caju* mushrooms cultivated with various agricultural substrates were analyzed for nutritional contents of lipid, protein, moisture, ash, and carbohydrate (Table 3). The nutritional content in mushroom was influenced by the substrate used. In specific, the highest moisture content (80.90%) was obtained in T1, the highest lipid content (4.95%) in T2, the highest carbohydrate content (12.68%) in T4, and the highest protein content (26.44%) in T5 (Table 3). Protein content was the highest in T5 (26.44%), followed by T4 with 19.05%, which was not significantly different from that in T1 with 15.93%. The human body acquires an optimum amount of protein in their daily nutrient uptake to help repair, build, and maintain body tissue. Among all the treatments, T5 obtained the highest protein content of 26.44 mg/g. Oyster mushroom is a good source of protein because it provides essential amino acids for human health (Ahmed et al. 2013). Results of this study showed that 100% PPF (T5) can increase protein content in mushroom.

The results of the present study are comparable with those of Salamat et al. (2017) and Yang et al. (2001) that obtained protein content percentages of 15-26% and 16-23%, respectively. The significant difference in protein amount within each treatment varied because of the different nitrogen contents in the substrates itself. The high nitrogen content in PPF for T5 contributed to the high protein content in mushroom fruiting bodies.

Meanwhile, the low protein content could be caused by the less nitrogen contribution in substrates or lack of mushroom nitrogen utilization efficiency. Various studies focused on the protein content of mushrooms. Most researchers agreed that mushrooms have high protein content among vegetables and fruits, and that their protein content is incomparable with those of raw meat, fish, and eggs. The protein content in mushroom varies based on species, types, and environmental factors that influence its growth and maturity stages (Wang et al. 2014). Sources of protein from mushroom are classified as high quality and rich with diverse types of essential amino acids (Dunkwal et al. 2007).

The nutritional content of fat was the highest in T2 (4.95%), followed by T3 (3.6%). Meanwhile, those in T1, T4 and T5 were 1.37, 1.74, and 1.29%, respectively (Table 3). Yang et al. (2001) reported that crude fats in mushroom range from 2 to 9%, which supports the results obtained from this study. The present result is in line with the study of Ahmed et al. (2013) that reported the lipid amount was within 4.0%, and below in *P. sajor-*

*caju* mushroom, which was cultivated on a mixture of wheat bran and SD substrate. The mushroom fat contained 0.76% of unsaturated fatty acids made up of linoleic acids, and 90% of polar lipids as linoleic acids in omega-six fatty acids in mushrooms consider as healthy food (Wang et al. 2014).

Mushroom is known for its highly perishable characteristic and perishable food containing large amounts of water and moisture. Mushroom itself depends on humidity and moisture for the development of its fruiting body. In addition, moisture is the most favorable factor that influences microbial growth and promotes enzymatic activity. Moreover, the moisture content in mushroom indicates its freshness and shelf-life stability. All the treatments almost had the same moisture content. As shown in Table 3, T1 achieved the highest moisture content (80.90%), followed by T2 (74.13%). The lowest moisture content was recorded in T4 (66.13%). This result indicates that RS can provide space for mycelium penetration and facilitate gas exchange in the mushroom bag. However, it cannot retain optimum water and moisture as a substrate. T5 also had a low moisture content (68.29%), though it was still slightly low than that of T4. T2 and T3 had moisture contents of 74.13 and 72.66%, respectively, showing a moderate difference. The results acquired from this study almost correlated with those reported in the study by Reis et al. (2012), where the moisture content of commercially cultivated mushrooms can be in the range of 79-91%. By contrast, Hoa et al. (2015) recorded a slightly higher moisture amount of 80.0-92.5% when the mushroom was cultivated on SD, corn cob, and sugarcane bagasse. However, higher amount of moisture in commodity corresponds to faster deterioration. Thus, mushrooms treated with RS or PPF can be stored longer than those treated with SD.

T5 obtained the maximum mean percentage of ash content (1.89%) in this study. In addition, T2 had high ash content (0.73%), followed by T3 (0.52%), and T1 (0.41%). Meanwhile, the lowest ash content was recorded in T4 (0.38%). Ash in food refers to any inorganic material after burning fat, water, and protein. Inorganic materials can be essential minerals, such as potassium, calcium and sodium, or toxic material, such as mercury. In the present study, a few essential mineral contents were measured. Therefore, further studies should clarify the toxic inorganic materials in mushroom to obtain complete information.

The highest carbohydrate content was obtained in T4 (12.68%), followed by T3 (7.57%), and T2 (4.46%). T5 and T1 showed the lowest carbohydrate amounts of 2.12 and 1.39%, respectively. However, the results obtained from this study are not in line with those obtained by Salamat et al. (2017) and Liang et al. (2019), where the various types of mushroom had carbohydrate contents ranging from 65 to 82%, which are much higher than the results obtained from this study. The contents of carbohydrate are not in line with previous findings possibly because previous studies used 100 g mushroom while this study used 1 g only. The same matter was found in the study by Onyeka and Okechie (2018), where 0.4-0.5 g mushroom was used, and the proximate component contents were lower than this study.

#### MINERAL ANALYSIS

*Pleurotus sajor-caju* has undergone tremendous amount of research through biological, chemical and pharmacological studies in order to illustrate the essential nutrients and minerals present in the mushroom fruiting bodies. As reported by Falandysz (2011), the existence of beneficial electrolytes is present in *P. sajor-caju*, such as potassium, sodium, and calcium as well as other micronutrients which are zinc and iron. The

mineral contents of *P. sajor-caju* cultivated on various agricultural substrates are shown in Table 3. The mineral contents varied based on treatments (Table 4) that T4 showed the highest amount of zinc content (0.015 mg/kg), followed by T5 (0.012 mg/kg). The commercially cultivated mushroom T1 is the treatment that has the lowest zinc content (0.008 mg/kg). The zinc content of *P. sajor-caju* in this study is comparably higher than the results obtained by Salamat et al. (2017) where the lowest Zn content in commercial grey oyster mushroom stated was 0.003 mg/kg.

The sodium in grey oyster mushroom cultivated in different substrates showed almost the same amount in a range from 0.069 to 0.076 mg/g in T1, T2, T4, and T6. However, the amount in T3 was 0.030 mg/g which was lower than the others (Table 4). The commercially cultivated substrate (T1) showed the highest reading of potassium content (12.91 mg/kg), and the second highest amount of potassium (8.90 mg/kg) was in T5. The lowest amount of potassium (1.84 mg/kg) was obtained in T4.

The iron content in mushroom treated with 100% rice straw (T4) was 0.013 mg/kg, the highest amount of iron compared to other treatments (Table 4). T1 had the second-highest amount of iron (0.009 mg/kg), followed by T2 (0.008 mg/kg) and T5 (0.008 mg/kg). The study

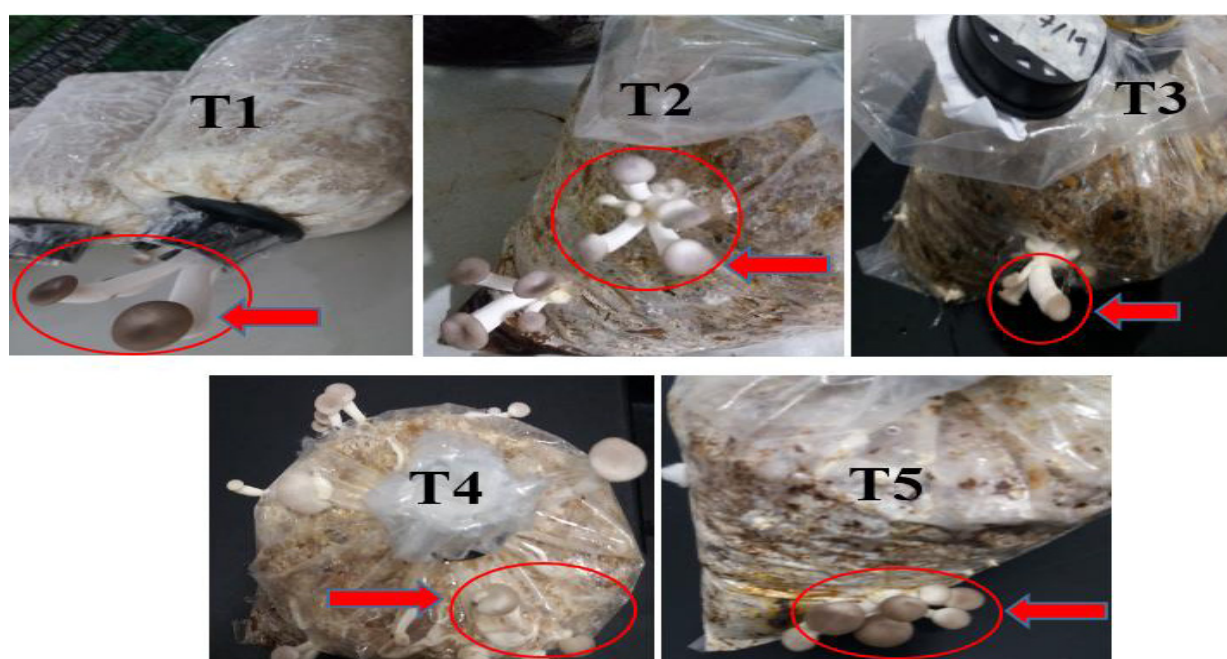


FIGURE 1. Pin head formation (indicated by arrow) of grey oyster mushroom in each treatment. T1: 90% SD + 10% RB, T2: 30% RS + 20% SD + 50% PPF, T3: 50% RS + 50% PPF, T4: 100% RS and T5: 100% PPF



of Muthu and Shanmugasundaram (2016) showed that iron content was higher in sawdust treated substrate. However, in this current study, rice straw treated substrate (T4) showed more iron in mushrooms. Furthermore, T3 had the lowest amount of iron (0.007 mg/kg), which is

in line with Salamat et al. (2017). For the calcium, the highest amount of calcium detected in this study was in T1, T2, and T3 ranging from 0.21 to 0.22 mg/g. Overall, it showed that nutrition content in the mushroom fruiting body was influenced by substrate combination.



FIGURE 2. The fruiting bodies (indicated by arrow) of grey oyster mushroom in each treatment. T1: 90% SD + 10% RB, T2: 30% RS + 20% SD + 50% PPF, T3: 50% RS + 50% PPF, T4: 100% RS and T5: 100% PPF

TABLE 1. Substrate composition in different ratios in each treatment

Treatments	Composition of substrate ratio
T1	90% SD + 10%RB
T2	30% RS + 20% SD + 50% PPF
T3	50% RS + 50% PPF
T4	100% RS
T5	100% PPF

SD: Sawdust; RB: Rice Bran; RS: Rice Straw and PPF: Palm Pressed Fiber



TABLE 2. Comparative analysis of grey oyster mushroom on growth performance and yield based on treated substrates

Treatment	Duration of mycelium full colonization (days)	Pinhead formation (days)	Time for first harvest after spawning (days)	Yield weight (g)	Stipe length (cm)	Cap diameter (cm)	Biological efficiency (%)
T1	41.00±1.00 <sup>a</sup>	48.33±1.53 <sup>a</sup>	51.67± 2.08 <sup>a</sup>	140.80±12.84 <sup>b</sup>	8.20±1.11 <sup>a</sup>	6.50±1.32 <sup>b</sup>	43.27±4.99 <sup>a</sup>
T2	37.00±2.65 <sup>a</sup>	39.67±3.06 <sup>ab</sup>	44.33± 2.52 <sup>bc</sup>	102.93±8.10 <sup>a</sup>	4.17±.15 <sup>b</sup>	6.27±.21 <sup>b</sup>	22.70±2.15 <sup>b</sup>
T3	35.67±2.08 <sup>a</sup>	36.67±6.11 <sup>b</sup>	40.33± 6.03 <sup>b</sup>	133.03±7.34 <sup>ab</sup>	4.33±.58 <sup>b</sup>	6.23±.06 <sup>b</sup>	31.60±2.02 <sup>b</sup>
T4	22.67±2.52 <sup>b</sup>	26.67±2.52 <sup>c</sup>	29.67± 1.53 <sup>c</sup>	173.23±4.29 <sup>a</sup>	1.93±.60 <sup>c</sup>	9.60±1.51 <sup>a</sup>	42.63±1.59 <sup>a</sup>
T5	40.67±3.51 <sup>a</sup>	45.00±3.61 <sup>ab</sup>	49.33± 4.04 <sup>bc</sup>	106.30±19.23 <sup>c</sup>	1.33±.09 <sup>c</sup>	4.63±.60 <sup>b</sup>	23.87±4.91 <sup>b</sup>

Values are mean ± indicates the standard deviation (SD) of triplicate samples. The different alphabets superscripted vary by ( $p < 0.05$ ) with 95% confidence

TABLE 3. Proximate composition of lipid, protein, moisture, ash, and carbohydrate in grey oyster mushroom cultivated on each treated substrate

Treatment	Lipid (%)	Protein (%)	Moisture (%)	Ash (%)	Carbohydrate (%)
T1	1.37±0.02 <sup>b</sup>	15.93±0.66 <sup>b</sup>	80.90±0.39 <sup>a</sup>	0.41±0.12 <sup>b</sup>	1.39±0.34 <sup>d</sup>
T2	4.95±0.11 <sup>a</sup>	15.75±0.78 <sup>b</sup>	74.13±0.97 <sup>ab</sup>	0.73±0.11 <sup>b</sup>	4.46±0.13 <sup>c</sup>
T3	3.60±1.18 <sup>a</sup>	15.65±0.45 <sup>b</sup>	72.66±2.10 <sup>bc</sup>	0.52±0.25 <sup>b</sup>	7.57±1.64 <sup>b</sup>
T4	1.74±0.72 <sup>b</sup>	19.06±2.7 <sup>b</sup>	66.13±1.93 <sup>c</sup>	0.38±0.30 <sup>b</sup>	12.68±0.94 <sup>a</sup>
T5	1.29±0.03 <sup>b</sup>	26.44±4.88 <sup>a</sup>	68.29±5.52 <sup>bc</sup>	1.89±0.51 <sup>a</sup>	2.12±0.80 <sup>cd</sup>

Values are mean ± standard deviation (SD) of triplicate samples. Different alphabets superscripted vary by ( $p < 0.05$ ) with 95% confidence

TABLE 4. Mineral profiling in grey oyster mushroom cultivated on each treated substrate

Treatment	Mineral elements (mg/g)				
	Zn	Na	K	Fe	Ca
T1	0.008±0.002 <sup>b</sup>	0.070±0.02 <sup>a</sup>	12.91±4.98 <sup>a</sup>	0.009±0.002 <sup>ab</sup>	0.021±0.005 <sup>a</sup>
T2	0.010±0.002 <sup>ab</sup>	0.069±0.01 <sup>a</sup>	5.31±2.08 <sup>ab</sup>	0.008±0.002 <sup>ab</sup>	0.021±0.004 <sup>a</sup>
T3	0.012±0.001 <sup>ab</sup>	0.030±0.01 <sup>b</sup>	6.01±3.48 <sup>ab</sup>	0.007±0.002 <sup>b</sup>	0.022±0.003 <sup>a</sup>
T4	0.015±0.001 <sup>a</sup>	0.058±0.00 <sup>ab</sup>	1.84±0.68 <sup>b</sup>	0.013±0.002 <sup>a</sup>	0.016±0.002 <sup>ab</sup>
T5	0.012±0.003 <sup>ab</sup>	0.076±0.01 <sup>a</sup>	8.90±3.03 <sup>ab</sup>	0.008±0.003 <sup>ab</sup>	0.015±0.003 <sup>ab</sup>

Values are mean ± standard deviation (SD) of triplicate samples. Different alphabets superscripted vary by ( $p < 0.05$ ) with 95% confidence

## CONCLUSION

The demands for mushroom supply are increasing because of the rising awareness on the nutritional value and medicinal benefit of mushrooms. In this study, mushroom yield, mineral contents, and nutritional contents were influenced by substrate combination. High yield was obtained with 100% RS substrate, and a combination of RS and PPF achieved a good amount of nutrient content in terms of protein. Mushroom growers can use RS or PPF for mushroom cultivation based on their business goal, and dependency to use 90-100% SD can be reduced by local agricultural product of PPF or RS.

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