

Efficiency of Charcoal as Supporting Growth Material in *Pleurotus Ostreatus* Mushroom Cultivation on Various Agricultural Wastes Mixed with Rubber Tree Sawdust (SR)

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Presently, the production of oyster mushroom in Malaysia is using rubber sawdust (SR) as the main medium to cultivate the oyster mushroom. Due to high demand and shortage of SR, other lignocellulosic wastes from oil palm wastes are used as an alternative substrate with the purpose of cultivating the oyster mushroom. This study was carried out to investigate the effect of additional charcoal as supporting growing material in the cultivation of the fungus *Pleurotus ostreatus* using various agricultural wastes mixed with SR. The SR, palm pressed fiber (PF), sugarcane bagasse (SB), corncob (CC) and charcoal were analyzed for their elemental compositions. The charcoal addition was varied to 0 wt%, 2 wt% and 4 wt% for 100 % SR, 50 % SR + 50 % PF, 50 % SR + 50 % SB and 50 % SR + 50 % CC substrates. It is hypothesized that the charcoal addition can boost up the yield and the mushroom quality as a growth supporting material. These substrates also have been supplemented with a fixed ratio of wheat bran and limestone to enhance the mushroom production. The number of days of mycelium growth, the number of days of pin head formation and yield for every flush were recorded. All the substrate containing charcoal, either 2 wt% or 4 wt%, showed a faster growth of mycelium rather than the substrate without the charcoal addition except for the 50 % SR + 50 % SB substrate. The 100 % SR with 2 wt% of charcoal substrate recorded the fastest mycelium growth which around 19 days. The 100 % SR, without the addition of charcoal, which acts as a control substrate, recorded the fastest pin head formation as well as fruiting body formation, around 27 d and 29 d, as compared to other substrate formulations. In terms of total average yield and biological efficiency (BE), the 50 % SR + 50 % SB without charcoal addition substrate shows the maximum average of total yield (207.96 g/bag) and the highest BE of 78.48 %. Meanwhile, the 100 % SR with 2 wt% of charcoal addition substrate produced an average total yield of 203.33 g/bag and BE of 64.55 % and these values are close to the 50 % SR + 50 % SB without charcoal addition substrate. In conclusion, the 50 % SR + 50 % SB without charcoal addition was found to be the best substrate for the cultivation of the oyster mushroom followed by 100 % SR with 2 wt% charcoal addition substrate based on the total average yield and BE. However, the other combination of the agricultural wastes and SR can be applied as an alternative substrate to cultivate the oyster mushroom since the availability of the SR is limited in Malaysia.

1. Introduction

Pleurotus ostreatus which is known as oyster mushroom continues to be grown in Malaysia because of their culinary, nutrition and health benefits. This oyster mushroom is widely known for its delicious taste and its high nutritive value. *Pleurotus spp.* is also a white rotten fungus that could be grown on numerous lignocellulosic substrates because of its lignocellulolytic enzymes degrading lignocellulosic matter into useful carbohydrates for fungi. The ability of these fungi in degrading lignin can vary to a great extent according to the species as well as the growing medium (Janusz et al., 2017). Any agricultural waste containing cellulose, hemicellulose and lignin can be a potential substrate. *P. ostreatus* species can grow on a wide range of agro waste materials like empty fruit bunch and also palm pressed fibre (Ali et al., 2013), oil palm fronds (Ali et al., 2018), sugarcane bagasse (Ahmad Zakil et al., 2020), rice straw (Utami and Susilawati, 2017) and corn cob (Rambe et al., 2019).

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Mycelia growth and fruit body formation of fungi are strongly influenced by the type of agricultural waste used as a base medium and the quality of the spawn. Ahmad Zakil et al. (2019) reported that an alternative substrate containing either 25 % or 50 % of EFB, PF as well as SB mixed with SR were successfully done for *Pleurotus ostreatus* mushroom cultivation and all the substrates produced the total average yields between 194 - 318.88 g/kg substrate BE between 48.5 – 79.72 %. According to Ali et al. (2013), the 100% EFB and 100% PF substrate cannot support mycelium growth and failed to produce any fruit body in the past. This is due to the presence of oil in EFB and PF fibre, and the inability of fungus to extract the cellulose entrapped in the fiber. Based on these findings, only 50 % of PF, SB and CC have been chosen to be used in this study.

Presently, Malaysia produces 44% of oil palms and it is the second largest exporter and also producer of oil palms (Shevade et al., 2019). The oil palm oil industry yields a huge number of solid lignocellulosic biomass like oil palm trunks (OPT), oil palm fronds (OPF), empty fruit bunch (EFB), palm pressed fibres (PF) and palm shells (OPS). The sugarcane bagasse (SB) is also abundantly available in rural as well as urban regions in Malaysia. SB is the fibrous by-product that remains after the extraction of high sucrose juice from sugarcane rods, produced in large quantities by many industries. 1 Mt of sugarcane can produce 280 kg of bagasse (Ameram et al., 2019). Corn cob (CC) is also another residue in Malaysia which is available abundantly after the removal of corn kernels and are easily available yearly. In 2012, corn production in Malaysia was 52,481 t, and in the following year, it escalated by approximately 5 % to 55,000 t (Shariff et al., 2016). All these types of agricultural waste, especially oil palm biomass, SB and CC in general, are high in hemicellulose, cellulose as well as lignin that could be converted to greater value-added products rather than throwing them away, which could lead to global warming and the creation of green houses.

Currently, in Malaysia, *P. ostreatus* is commercially grown using SR as a base medium. SR has a high demand but its availability is limited and this has become a critical issue for the growers of mushroom specifically *P. ostreatus*. PF, SB and CC are high in lignin, hemicellulose and cellulose which contributes to the carbohydrate or carbon source to ensure that *P. ostreatus* develops well. PF, SB and CC acquire high potentials to be alternate substrates for *P. ostreatus* mushroom cultivation in order to reduce the dependence on SR and simultaneously put the abundance of solid waste into good use and solve environmental issues in Malaysia. In, Malaysia, there is a high demand for oyster mushroom, which could be up to 50 t/day as the mushrooms nutritional values and antioxidant properties, but the rate of production at present is only about 24 t/day (Mohd Zaffrie et al., 2014). For the purpose of increasing the quality of mushroom and its yield, it has been hypothesized that charcoal may act as a growth supporting material in order to increase the growth as well as mushroom quality for this research. Nam et al. (2018) reported that 100 % SR with around 2 wt% of palm kernel shell (PKS) biochar was found to have better mycelium growth and enhance the oyster mushroom yield as compared to the 100 % SR without charcoal addition (control substrate). Based on this finding, the charcoal addition was varied from 0 – 4 wt%. To date, limited study on charcoal addition in mushroom cultivation is reported. This research's objective is to investigate the charcoal addition by varying the wt% of charcoal between 0 wt% to 4 wt% as a supporting growth material to the cultivation of *Pleurotus ostreatus* mushrooms using various agricultural wastes mixed with SR. The performance of growth in terms of mycelium growth, pin head formation, fructiferous body, yield and biological effectiveness of *Pleurotus ostreatus* on various fungal substrate mixtures were studied.

2. Materials and methodology

2.1 Source of raw materials

The PF has been taken from Kilang Sawit Panching, Kuantan, Pahang. The SB was collected at Local Growers in Kuantan, Pahang. and the CC was collected by weekly at the Gambang Night Market, Gambang, Pahang. The samples were then ground by using an industrial grinder at the Laboratory of Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang and sieved to obtain 1-2 mm samples' size. Calcium carbonate (limestone), *Pleurotus ostreatus* spawn and wheat bran were obtained from Pekan Mushroom, Pekan, Pahang. The charcoal was bought from the Giant Supermarket, Kuantan, Pahang.

2.2 Carbon (C), Hydrogen (H), Nitrogen (N) and Sulphur (S) Analysis

The CHNS analysis was run via an Elemental Analyzer (Elementar, Japan) according to ASTM D5373-02 to avail the elemental content of SR, PF, SB and CC. This analysis was done at the Central Laboratory, Universiti Malaysia Pahang (UMP).

2.3 Substrate preparation and sterilization

The mass ratio of SR to PF, SB and CC, respectively was set at 50:50 based on dry weight basis. A total of three bag logs were made ready for every substrate formulation. The material then was mixed with 5 wt% wheat bran and 1.5 wt% limestone and also 0 wt%, 2 wt% and 4 wt% charcoal. The tap water was added in order to increase the moisture content. Each substrate was packed in a clear 10 x 15 cm plastic bag. The substrate bags

were subsequently compressed and closed with PVC necks and securely covered with paper to block the entry of insects. All bag logs were sterilized at 100 °C for approximately 8 h in the sterilization chamber to kill insects and competing organisms in the substrates. After the bag logs were sterilized, the bag logs were cooled down to room temperature. The mushroom cultivation process is practiced according to Ali et al. (2013).

2.4 Spawning, incubation and harvesting

After the substrate is cooled to room temperature, about 10 g of oyster mushroom spawn was injected into the bag logs. The bag logs were then placed vertically in spawn running room at room temperature under dark room. The measurement and observation of the growth of mycelium (linear length) in each bag was performed continuously until the mycelium matured. After the substrate surface was completely covered with mycelium, the substrates were moved to a fructifying room. The bags were reorganized horizontally, and the upper portions of the bags were folded out to produce fruit for the initial crop. The water was dispersed as a fine mist on the mushroom bags in order to maintain relative humidity of 80-90 % twice daily through an automatic sprinkler. Mushrooms have been allowed to grow under ambient conditions. The experiment ended with 3 fructification flushes. The parameters that were measured for *P. ostreatus* efficiency included number of days needed for the mycelial growth completion (days to colonize the substrate), time to fruiting (initial of pin heads), time to harvest (first fruiting body harvest), fresh mushroom yield and biological efficiency (BE).

3. Results and discussion

3.1 Elemental analysis of SR, PF, SB and charcoal

Growing fungi needs nitrogen, carbon and inorganic compounds as the sources of nutrition, and the major nutrients are sources of carbon like lignin, cellulose and hemicellulose (Ali et al., 2018). Oyster mushrooms need less nitrogen and higher carbon. For instance, most organic materials that contain cellulose, hemicellulose and lignin must be utilized as a mushroom substrate. Elemental analysis and carbon-to-nitrogen (C/N) ratio for SR, PF, SB and CC are presented in Table 2. The % of C (44.90 wt%) was the highest in SB fiber and the lowest % of C (38.57 wt%) was identified in PF fiber. SR fibre showed the highest % of N (0.22 wt%) while CC fibre showed the lowest % of N (1.95 wt%). From the result, it can be seen that the C/N ratio for SR and SB was as high as 181.80 and 160.6, respectively, as compared to other fibers. The C/N ratio for CC (22.59) was low relatively when compared with other substrates employed, as illustrated in Table 1.

Table 1: Elemental analysis of PF, SR, SB and CC

| Element | SR (wt%) | PF (wt%) | SB (wt%) | CC (wt%) | Charcoal (wt%) |
|---------|----------|----------|----------|----------|----------------|
| C | 40.00 | 38.57 | 44.90 | 44.04 | 44.04 |
| N | 0.22 | 1.69 | 0.28 | 1.95 | 1.95 |
| H | 7.42 | 4.78 | 5.81 | 8.58 | 8.58 |
| S | 2.49 | 0.50 | 3.43 | 3.97 | 3.97 |
| C/N | 181.8 | 22.82 | 160.36 | 22.59 | 22.58 |

The C/N ratio was dependent on the C and N source (Tesfay et al., 2020). Cueva et al. (2017) recommended a C/N ratio of 32 to 150 as the most suitable for producing *P. ostreatus*. The C/N ratio had more important effects on the formation, growth of the mycelium and the development of the fructifying body (Rambey et al., 2019). In this study, 100 % SR that contains 2 wt% of charcoal with higher C/N ratio can support mycelium growth and fruiting bodies better than other mushroom substrate used in this study. However, these findings were contradicting to the results reported by (Atila, 2017) who found that higher C/N ratio favoured the mycelium growth, and lower C/N ratio favoured the fruiting body growth. Table 3 also shows the element analysis for charcoal sample. Charcoal containing high carbon (44.04 wt%) indicated that it is a highly carbonated material. Lam et al. (2019) reported that biochar with high carbon content might be employed in growing mushroom considering that high carbon content results in good stability and resistance to react with lime and water. The double carbon-carbon bond between charcoal is strong and would only be decomposed at very high temperatures or with high thermal energy.

3.2 Mycelium colonization, pin head formation and first harvest

Mycelium growth is the first stage in the fruit body development. Mycelium is used to absorb water, nutrients and organic matter from the substrate to promote the growth and development of *P. ostreatus*. Figure 1 illustrates the growth of mycelium cultivation of fungal baglog with a different % of charcoal (0 wt%, 2 wt% and 4 wt%) for 100 % SR, 50 % SR + 50 % PF, 50 % SR + 50 % SB and 50 % SR + 50 % CC. The results show that all the mushroom baglog containing charcoal either 2 wt% or 4 wt% showed a faster growth of mycelium except

for the 50 % SR + 50 % SB. 100 % SR that containing 2 wt% of charcoal required lesser than 19 d to complete the spawning cycle and this could be due to easy digestion and rapid decomposition of SR. The 100 % SR either with and without charcoal addition is easily and rapidly digested as compared to other lignocellulosic waste and this might be due to the uniform particle size among the SR, resulting in larger particle size in the substrate. The higher aeration and porosity of the SR also can promote easier digestion of the substrate. Most of the baglog added with charcoal supports yielded a greater growth owing to the porous charcoal that is easier to absorb water as well as the nutrients from wheat bran when mixing the charcoal with mushroom medium, subsequently enhancing water supply and nutrients to encourage the mycelium growth.

The pin heads are the tiny fruit bodies of the mushroom with a size larger than (0.01mm). They emerged after the completion of mycelial growth of the *P. ostreatus*. The lowest growth period (27 d) for pin head formation was recorded on 100 % SR that contained 0 wt% of charcoal (control substrate) and the longer duration (57 d) for primordial development was recorded on 50 % SR+ 50 % SB that contain 2 wt% of charcoal. Overall, in this study, most of the mushroom baglog containing either 2 wt% or 4 wt% required long duration for primordial development except for 100 % SR and 50 % SR + 50 % CC. This might be due to PF and SB having enough C/N ratio and do not need the charcoal addition as a supporting growing material to grow oyster mushrooms. The difference in the amount of days required for complete pin head formation in *P. ostreatus* mushroom on different substrates can be resulting from variation in the substrate nutrient availability, temperature and relative humidity (RH) of the growth room during bag transfer (Tesfay et al., 2020).

The development of the fruiting body was the last stage in the cultivation of the fungus. Days to fruiting body formation took place earlier in 100 % SR (control substrate) with 29 d while 50 % SR + 50 % SB that contained 2 wt% of charcoal took a longer time, which was 59 d for fruiting body formation. Most bags of mushrooms took about 2 d from the primary formation to the maturation of the fruiting body of mushrooms. After 2 d, the mushrooms were ready for harvest. According to Ahmad Zakil et al. (2019) the fruiting body formation of all the agro-industrial waste employed took between 2 and 4 d.

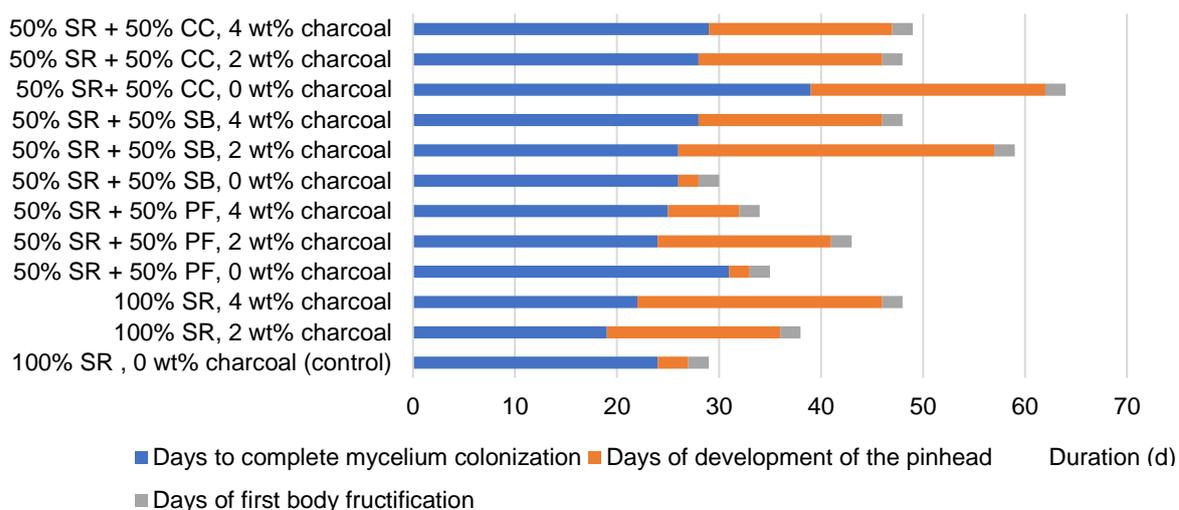


Figure 1: Number of days taken to complete mycelium growth, number of days taken for pin head formation, and number of days taken for the first fructification harvest

3.3 Total average yield and biological efficiency (BE) of *P. ostreatus*

Fructification yields were based on three replicates and averaged as shown in Table 3. The outcomes illustrated that the mycelium growth greatly affects the yield of *P. ostreatus*. The highest yields of mushrooms were mostly recorded from the first harvest, except those from 100 % SR with 4 wt% of charcoal, 50 % SR+ 50 % PF with 4 wt% of charcoal, 50 % SR + 50 % SB with 2 wt% and 4 wt% charcoal, 50 % SR + 50 % CC with 0 wt%, 2 wt% and 4 wt% charcoal which observed the highest yields either in the second or third harvest. This could be resulting from environmental factors like relative humidity and temperature which were difficult to maintain and could have affected the yield of the second and third flush. There was no fruiting body yield for the third flush from the mushroom grown on 50 % SR + 50 % SB with 4 wt% charcoal and 50 % SR + 50 % CC either with 0 wt% of charcoal or 2 wt% of charcoal, and this might be due the diminishing nutrient in the mushroom substrate. The highest total average yield was obtained from 50 % SR+ 50 % SB with 0 wt% of charcoal (207.96 g/bag),

which possessed the relatively fastest mycelium within 26 d. Surprisingly, the mushroom substrate that contained 2 wt% charcoal for 100 % SR also recorded high total average yield up to 203.33 g/bag with 19 d for mycelium to colonize the baglog as compared to the control substrate (100 % SR with 0 wt% of charcoal) that recorded the total average yield around 159.67 g/bag with 24 d for mycelium to colonize the baglog.

Overall, in this study, the best formulation with highest product yield was varied. Most of the mushroom baglog showed the highest total average yield at 0 wt% of charcoal except for the 100 % SR and 50 % SR + 50 % PF that recorded the highest total yield with 2 wt% and 4 wt% charcoal loadings. This could be due to the temperature and humidity surrounding mushroom house being not at the desired range especially when the weather was very hot throughout the full day. Water sprayed automatically using the sprinkler somehow did not maintain the temperature and humidity of the mushroom house and may affect the total average yield either with or without charcoal addition. The total average yield from other mushroom substrate (50 % SR + 50 % PF, 50 % SR + 50 % SB and 50 % SR + 50 % CC) that contained the charcoal either 2 wt% and 4 wt% was shown to be not successful in exceeding the total average yield of the mushroom substrate without adding charcoal. There are certain benefits to using additional charcoal in growing mushrooms because recent researches have indicated that biochar might reduce cadmium and toxins in crops, enhance soil fertility and allegedly contribute result in high crop yield and quality (Nigam et al., 2021).

It can be seen that the biological efficiency has been affected by different substrates tested from Table 2. Additionally, the highest biological efficiency of 78.48 % was achieved by 50 % SR+ 50 % SB substrate followed by 100 % SR with 2 wt% of charcoal (64.55 %). However, the minimum biological efficiency of 13.24 % was obtained from 50 % SR + 50 % SB with 4 wt% of charcoal substrates. The resulting values for biological efficiency have shown that biological efficiency is directly proportional to the average total yield. Most of these representations are in agreement with the findings reported by (Jamil et al., 2019) where the biological efficiency (BE) values ranged between 17 to 79 % except 50 % SR + 50 % SB with 4 wt% of charcoal substrates. The variation in the biological effectiveness of oyster mushrooms might be due to the composition of different substrates (Cyriacus et al., 2020).

Table 2: Yield for each flush and biological efficiency (%)

| Substrate | Average yields (g/bag) | | | Total average yield (g/bag) | Biological Efficiency (%) |
|------------------------------------|------------------------|--------------|-------------|-----------------------------|---------------------------|
| | First flush | Second flush | Third flush | | |
| 100 % SR, 0 wt% charcoal (control) | 67.13 | 51.07 | 41.47 | 159.67 | 53.22 |
| 100 % SR, 2 wt% charcoal | 94.01 | 59.75 | 49.57 | 203.33 | 64.55 |
| 100 % SR, 4 wt% charcoal | 29.38 | 67.94 | 34.4 | 131.72 | 39.91 |
| 50 % SR + 50 % PF, 0 wt% charcoal | 54.13 | 13.3 | 26.8 | 94.23 | 32.49 |
| 50 % SR + 50 % PF, 2 wt% charcoal | 53.29 | 42.55 | 32.7 | 128.54 | 41.47 |
| 50 % SR + 50 % PF, 4 wt% charcoal | 51.57 | 53.64 | 43.54 | 148.75 | 45.77 |
| 50 % SR + 50 % SB, 0 wt% charcoal | 100.29 | 61.1 | 46.57 | 207.96 | 78.48 |
| 50 % SR + 50 % SB, 2 wt% charcoal | 41.48 | 45.23 | 17.6 | 104.31 | 37.25 |
| 50 % SR + 50 % SB, 4 wt% charcoal | 32.57 | 6.48 | 0 | 39.05 | 13.24 |
| 50 % SR + 50 % CC, 0 wt% charcoal | 58.7 | 65.54 | 0 | 124.24 | 41.414 |
| 50 % SR + 50 % CC, 2 wt% charcoal | 32.15 | 33.7 | 0 | 65.85 | 20.904 |
| 50 % SR + 50 % CC, 4 wt% charcoal | 46.95 | 47.79 | 15 | 109.74 | 33.254 |

4. Conclusions

In general, the current research confirmed that oyster mushrooms (*Pleurotus ostreatus*) can grow on PF, SB and CC mixed with SR by varying the wt% of charcoal (0 wt%, 2 wt% and 4 wt%). 50 % SR + 50 % SB without charcoal addition have been identified as the best formulation substrate for oyster mushrooms with 19 d, 207.96 g/bag, 78.48 % of BE followed by 100 % SR with 2 wt% of charcoal that is capable to complete mycelium growth within 19 d and produce 203.33 g/bag of fresh mushroom with BE is about 64.55 %. The mushroom substrate from 100 % SR with 2 wt% charcoal addition and 50 % SR + 50 % PF with 4 wt% were found to have better of mycelium growth and enhanced oyster mushroom yield as compared to other mushroom substrates that were added with the addition of charcoal. The application of charcoal to the 50 % SR + 50 % SB and 50 % SR + 50 % CC did not give huge effect since the average yield at 50 % SR+ 50 % SB and 50 % SR and 50 % CC were higher than the addition of 2 wt% or 4 wt% of charcoal in the substrate. Nevertheless, the other substrate combinations also had promising potential as an alternate medium for mushroom farming in Malaysia. The utilization of PF, SB and CC as substrates for oyster mushroom cultivation can be a solution to the huge agricultural by-products available. It also can provide a promising way economically of converting low-quality

biomass into protein-rich food for humans. Charcoal can be characterized by using Brunauer, Emmett and Teller (BET) in the future in order to obtain the specific surface of the charcoal sample.

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