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# Cultivation of Pleurotus Ostreatus on Oil Palm Fronds Mixed with Rubber Tree Sawdust

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Pleurotus ostreatus (oyster mushroom) is cultivated in Malaysia using rubber tree sawdust (RS). Due to a shortage and the increasing cost of rubber tree sawdust, an alternative substrate is needed. This study was undertaken to evaluate the feasibility of oil palm frond (OPF) as an alternative substrate for the cultivation of Pleurotus ostreatus. The OPF and RS were analysed for their elemental compositions. The mycelium growth rate, the time for first pinhead formation and first fruiting bodies, yield, and biological efficiency (BE%) were studied for 100 % OPF, 90 % OPF - 10 % RS, 70 % OPF - 30 % RS, 50 %OPF – 50 % RS, 30 % OPF – 70 % RS, 10 % OPF - 90 % RS and 0 % OPF – 100 % RS substrates supplemented with rice bran and calcium carbonate. The proximate nutritional value of Pleurotus ostreatus first flush was also analysed for 100 % RS and 100% OPF. For mycelium growth rate, time for first pinhead formation and time to first Pleurotus ostreatus crop, the 0% OPF - 100 % RS substrate recorded the shortest time followed by the 10% OPF -90 % RS substrate. The 0 % OPF – 100 % RS substrate gave the highest yield of 135 g (22.6 % BE) whereas 125 g (20.9 % BE) was obtained from the 30 % OPF – 70 % RS substrate. The first flush of Pleurotus ostreatus harvested from the 100 % OPF substrate was richer in protein but lower in fat and carbohydrate compared to the mushrooms from the 100 % RS substrate making it an excellent food for low caloric diet, but OPF alone is not suitable as an alternative substrate for the cultivation of Pleurotus ostreatus as it took a longer growth performance with a lower yield of fruiting bodies compared to RS substrate.

## 1. Introduction

Pleurotus ostreatus also known, as oyster mushroom is the most cultivated mushroom contributing to 90.89 % of the total mushroom cultivated in Malaysia (Haimid et al., 2013). It is highly nutritious as it contains protein, fat, carbohydrates, minerals (ash), vitamins, essential amino acids and medicinal properties (Deepalakshi and Mirunalini, 2014). Table 1 shows the macronutrients of Pleurotus ostreatus. Pleurotus ostreatus is a fungus that can be cultivated on various lignocellulosic substrates due its lignocellulolytic enzymes which degrade the lignocellulosic matters into useful carbohydrates for the fungi. Any agricultural waste that contains cellulose, hemicellulose and lignin is a possible substrate. Malaysia is the world's second largest producer of palm oil amounting to 41 % of the global palm oil production. 343 palm oil mills produced 19.67 Mt of crude palm oil in 2014. In that year alone, 4.7 Mha of land in Malaysia are under oil palm cultivation amounting to 71 % of total agricultural land (Palm Oil Research, 2016). The palm oil industry generates a large volume of solid biomass. In 2012, 83 Mt of solid biomass are generated and it is expected to increase to 100 Mt in 2020 (Borneo Post, 2015). Oil palm frond (OPF) is generated from harvesting, pruning and replanting activities. In 2014, 47.06 Mt and 3.66 Mt (based on dry weight) were generated from the pruning and replanting activities (Aziz, 2015) where most of it is left on the ground for natural decomposition (Cheng et al., 2011). This huge underutilised biomass can be upgraded to higher value-added products instead of being disposed of, generating greenhouse gases and consequently to global warming.
Presently, in Malaysia, the commercial cultivation of Pleurotus ostreatus utilises rubber tree sawdust (RS) as the base medium. There is a shortage of rubber tree sawdust due to the limited availability of rubber trees and also competition from other industries. This has become a serious problem to the mushroom growers. A new

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alternative substrate to replace rubber tree sawdust is needed to overcome this shortage. Pleurotus ostreatus being a fungus has the ability to convert lignocellulosic materials using its lignocellulolytic enzymes into useful carbohydrates that it can use as an energy source. Since the typical lignocellulosic composition (% dry matter basis) of OPF consisting of 39.5 % cellulose, 29.8 % hemicellulose, 23.3 % lignin, 1.7 % extractives and 5.7 % ash (Saka et al., 2008) is very close to that of RS where there are 39 % cellulose, 29 % hemicellulose, 28 % lignin and 4 % ash (Petchpradab et al., 2009), OPF has the potential to be an alternative substrate in the cultivation of Pleurotus ostreatus and at the same time, solve the waste abundance and environmental issues. The objective of this study is to investigate the feasibility of using OPF as an alternative substrate for the cultivation of Pleurotus ostreatus. The growth performance in terms mycelium growth, formation of pinhead, fruiting body, yield, biological efficiency and nutritional value of Pleurotus ostreatus on various mixtures of OPF and RS were studied.

*Table 1: Macronutrients contents of Pleurotus ostreatus mushroom (Khan, 2010)*

| | Protein (%) | Carbohydrates (%) | Fat (%) | Crude fibre (%) | Minerals/Ash (%) | Moisture (%) |
|---|---|---|---|---|---|---|
| Content (g/100 g dried mushroom) | 17 - 42 | 37 - 48 | 0.5 - 5 | 24 - 31 | 4 - 10 | 85 - 87 |

## 2. Materials and methods

Fresh OPF was obtained from oil palm trees in Universiti Teknologi Malaysia and the RS, wheat bran and calcium carbonate were bought from Saifulam Mushroom Farm in Benut, Johor. The OPF were cut into 5 cm length, dried and ground. Seven different mediums of 100 % OPF, 90 % OPF - 10 % RS, 70 % OPF - 30 % RS, 50 % OPF - 50 % RS, 30 % OPF - 70 % RS, 0 % OPF - 90 % RS and 0 % OPF - 100 % RS were prepared with three replicates for each composition. The main medium was supplemented with rice bran and calcium carbonate in the ratio of 100 : 5 : 2. Tap water was added where the required amount of water was determined using the first test where after the substrate is pressed as a lump in the fist, upon releasing the fist, the lump does not crumble. The substrates were mixed thoroughly, and 1 kg of each substrate was then placed in an 8.5 cm x 35 cm polyethylene bag. The bags of substrates were compressed and closed with PVC-necks plugged with cotton wools and covered. All the substrates were steamed at 100 °C for about 6 h in a drum steamer. The bags were left to cool to ambient temperature before inoculating with 10 g of spawn. The bags of substrates were arranged vertically in a mushroom house. At the end of the colonisation period at 45 d after inoculation, the covers were removed, and the bags rearranged horizontally. Water was sprayed periodically on the floor of the house to maintain high humidity. The mycelium growth rate, the days for pinhead and fruiting bodies formations, the total weight of fruiting bodies from three flushes were recorded. The biological efficiency was calculated based on the total yield of Pleurotus ostreatus harvested per 1 kg of dry substrate.Elemental analysis of OPF and RS was done using a CHNS analyser. Carbon to nitrogen ratio was calculated from the result. The dried fruiting bodies from the first flush for the 100 % OPF and 100 % RS substrates were analysed for moisture, crude protein, crude fibre, and ash using the official Methods of the Association of Official Analytical Chemist (AOAC, 2005). Percentage carbohydrate is determined by subtracting the total percentage of moisture, crude protein, crude fat, crude fibre and ash from 100 in accordance with AOAC 2005.

## 3. Result and discussion

Pleurotus ostreatus requires carbon, nitrogen and inorganic compounds as its nutritional sources and the main nutrients are carbon sources such as cellulose, hemicellulose, and lignin. The elemental analysis and the calculated C/N ratio of OPF and RS are as shown in Table 2.

*Table 2: Elemental analysis of OPF and RS*

| | OPF | RS |
|---|---|---|
| C | 42.7469 | 45.4238 |
| N | 0.2272 | 0.2564 |
| H | 5.6247 | 5.7244 |
| S | 0.1524 | 0.1542 |
| C/N ratio | 188.15 | 177.16 |

OPF has a higher C/N ratio compared to RS even though RS contains more carbon and nitrogen than OPF. Narain et al. (2009) related that the development of mycelium growth and pinhead formation is dependent on C/N ratio. Royse (2002) and Mintesnot et al. (2014) reported low C/N ratio gives better performance on fruiting bodies and supports better yield.

Figure 1 shows the length of mycelium growth in 45 d. All the substrates except for the 50% OPF - 50% RS, 90 % OPF - 10 % RS, and 100 % OPF completed mycelium growth in 45 d. The mycelium growth rate increased gradually with the amount of RS where the 0 % OPF - 100 % RS substrate displayed the fastest mycelium growth followed closely by the 10 % OPF - 90 % RS and 30 % OPF - 70 % RS substrates. The chemical composition of OPF and RS in terms of cellulose and hemicellulose is the same with the exception of lignin where lignin is slightly higher in RS. This finding is in contrast with Atila (2017) that stated that high lignin content would result in reduced mycelium growth as it reduces the bioavailability of other cell wall constituents and cellulose. The lower C/N ratio of RS compared to OPF promotes better mycelium growth that is in agreement with the findings of Atila (2017) and Narain et al. (2009) as ligninolytic activity is increased in nitrogen-rich medium. This will induce rapid growth of the mycelium as the carbon sources are easily degraded. As the amount of OPF increases in the substrates, the nitrogen content decreases resulting in higher C/N ratio, resulting in the slower mycelium growth as insufficient nitrogen inhibits mycelium growth.

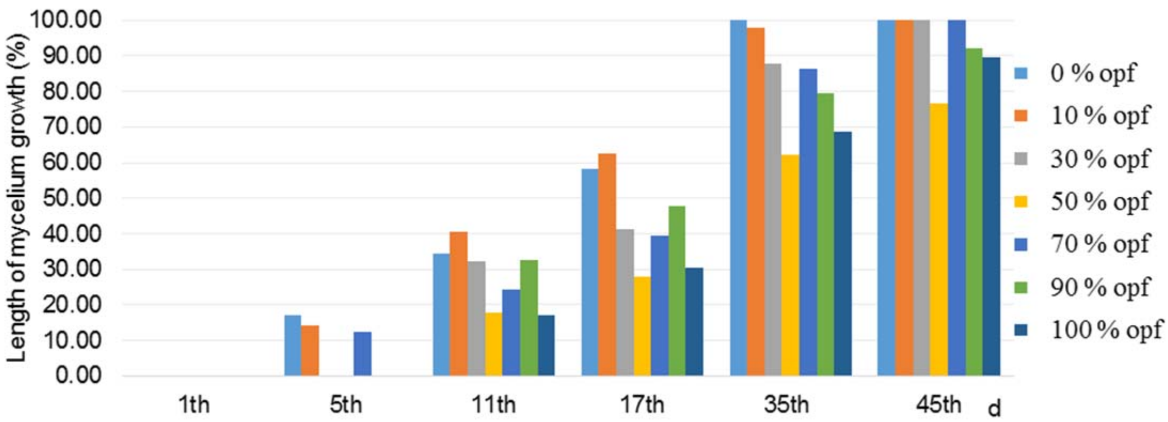


*Figure 1: The length of mycelium growth in 45 d*

Table 3 and 4 show the time for pinhead formation, time for first fruiting bodies crop, yield and total weight of fruiting bodies for three flushes together with the calculated percentage of biological efficiency (% BE). Different pinhead and fruiting times were recorded depending on the composition of the substrates. Increasing the amount of OPF in the substrates slowed the pinhead and fruiting bodies formation. The fastest time for pinhead formation and first fruiting bodies crop were obtained for the 100 % RS substrate while the 50 % OPF - 50 % RS and the 100 % OPF substrates took the longest time. Ashraf et al. (2013) reported nitrogen rich source substrate speed up the mycelium growth and pinhead formation. Narain et al. (2009) noted that mycelium growth and pinhead formation are dependent on C/N ratio. The low C/N ratio of RS gives a better growth performance as compared to the high C/N ratio of OPF. According to Yang et al. (2013) though, excess nitrogen will delay the fruiting bodies formation as it slows the degradation of lignin.

*Table 3: The growth performance in pinhead and first fruiting bodies formation of Pleurotus ostreatus*

| Composition | Pinhead formation (d) | Fruiting bodies (d) |
|---|---|---|
| 0 % OPF - 100 % RS | 50.00 | 51.00 |
| 10 % OPF - 90 % RS | 56.50 | 58.00 |
| 30 % OPF - 70 % RS | 56.50 | 58.00 |
| 50 % OPF - 50 % RS | 62.67 | 65.33 |
| 70 % OPF - 30 % RS | 62.50 | 64.50 |
| 90 % OPF - 10 % RS | 62.00 | 64.00 |
| 100 % OPF- 0 % RS | 62.67 | 65.00 |

*Table 4: Weight for every flushes and biological efficiency*

| Substrate | Average yields (g) | | | Total yield (g) | Biological Efficiency (%) |
|---|---|---|---|---|---|
| | 1st flush | 2nd flush | 3rd flush | | |
| 0 % OPF - 100 % RS | 60.60 | 35.5 | 39.47 | 135.57 | 22.60 |
| 10 % OPF - 90 % RS | 41.94 | 12.65 | 21.02 | 75.61 | 12.60 |
| 30 % OPF - 70 % RS | 42.52 | 55.40 | 27.44 | 125.36 | 20.89 |
| 50 % OPF - 50 % RS | 57.03 | 10.06 | 10.22 | 77.31 | 12.89 |
| 70 % OPF - 30 % RS | 32.94 | 42.55 | 15.67 | 91.16 | 15.19 |
| 90 % OPF - 90 % RS | 21.84 | 14.44 | 32.22 | 68.50 | 11.42 |
| 100 % OPF - 0 % RS | 32.30 | 17.35 | 29.67 | 79.32 | 13.22 |

The yields of fruiting bodies were recorded from three replicates and calculated as an average. The highest mushroom yields were mainly from the first flush except those from the 90 % OPF - 10 % RS, 70 % OPF - 30 % RS and 30 % OPF -70 % RS substrates which recorded the highest yields from the second flush. The highest yield and % BE was from the 100 % RS substrate followed by the 30 % OPF - 70 % RS substrate while the other substrates recorded almost similar % BE. The % BE for the 100 % OPF substrate was much lower than that of the 100 % RS substrate. This finding is similar to Ibrahim et al. (2015) where the biological efficiencies were 39.21 % and 30.57 % for sawdust and OPF. Yang et al. (2013) reported the highest yield of 121.5 (unsterilised) and 125.6 (sterilised) at the lowest C/N ratio of 34.87 for a substrate mixture of 80% cotton seed hull with 20% wheat bran. A negative relationship was observed between the C/N ratio and the biological efficiencies for both the sterialised and unsterilised mixture of cotton seed hull, rice straw and wheat bran, and the mixture of cotton seed hull, wheat straw and wheat bran. Hoa et al. (2015) also reported that low C/N ratio supported better yield of fruiting bodies resulting in higher biological efficiencies for both Pleurotus osteatus and Pleurotus cystidiosus from substrate mixtures of 100%, 80%, 50% and 0% sawdust with corncob and sawdust with sugarcane bagasse  Again, the trend was increasing amount of OPF decreases the yield and % BE due to the decreasing nitrogen content and resulting in higher C/N ratio.

The nutritional analysis of the first fruiting bodies of 100 % RS and 100 % OPF substrates is as shown in Table 5. The crude fibre and ash content were not statistically different in both RS and OPF substrates, but comparing the % crude fibre and % ash obtained for OPF are lower and higher, than the values given by Khan (2010). The first flush fruiting bodies from the 100 % OPF substrate has higher protein content compared to that from the 100 % RS substrate. 100 % OPF substrate has a higher C/N ratio than 100 % RS substrate that contributes to the higher protein content. This finding is in agreement with Li et al. (2015). Hoa et al. (2015) and Kurtzman (2005) found low C/N resulted in high protein content of mushroom, as nitrogen is essential for the production of protein in the mushroom. Wang et al. (2001) revealed that the protein content in the mushroom is influenced by the content and nature of protein in the starting substrate. Ragunathan and Swaminathan (2003) found the protein content of mushroom depended on the C/N ratio and the chemical composition of the substrates.

*Table 5: Nutritional contents of oyster mushroom*

| Substrate | Crude Fibre (%) | Protein (%) | Fat (%) | Moisture (%) | Total carbohydrate (%) |
|---|---|---|---|---|---|
| 100 % OPF | 17.95 | 17.61 | 1.82 | 12.03 | 42.71 |
| 100 % OPF | 16.64 | 11.74 | 8.99 | 1.76 | 54.30 |

The % total carbohydrate was slightly lower in the mushroom from the 100 % OPF substrate compared to that from the 100 % RS that is in agreement with Li et al. (2015) finding where low C/N ratio resulted in high carbohydrate content.

The fat content of the mushroom from the 100 % OPF substrate is 1.82 % that is significantly lesser than 8.99 % in the mushroom from the 100 % RS substrate. According to Bellettini et al. (2016) increased protein content in the mushroom resulted in low fat content.

The moisture content of mushrooms from the 100 % OPF and the 100 % RS substrates is 12.03 % and 1.76 %. Both moisture contents differ from Khan (2010) finding due to the high temperature of 30 °C and the low relative humidity of 67 % recorded during the fruiting phase as the growing environment also influences the moisture content of the mushroom. The higher moisture content in the mushroom from the 100 % OPF substrate is probably due to the greater water-holding capacity of the OPF compared to the RS substrate.

## 4. Conclusions

It is found that the growth and yield performance of Pleurotus ostreatus are dependent on C/N ratio of the substrate. Increasing the amount of OPF in the substrate mixture of OPF - RS slowed down the mycelium rate, pinhead and fruiting bodies formations as well as the yield and % BE due to low nitrogen in OPF resulting in high C/N ratio. 100 % RS is still a better substrate compared to 100 % OPF for the cultivation of Pleurotus ostreatus in term of growth performance, yield and % BE with 50 d, 135 g, 22.6 % compared to 63 d, 79 g and 13.2 %.

100 % OPF alone is not a suitable alternative to RS in the cultivation of Pleurotus ostreatus mushroom as it took the longest time in formation of pinhead and fruiting bodies and gave low yield. OPF mixed with a very high percentage of RS can be an alternative substrate. The mushrooms harvested from the 100 % OPF substrate was better nutritionally than that from the 100 % RS substrate having 50 % more protein, 20 % more ash (minerals), 8 % more crude fibre, 80 % less fat and 21 % less carbohydrate.

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