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## THE USAGE OF EMPTY FRUIT BUNCH (EFB) AND PALM PRESSED FIBRE (PPF) AS SUBSTRATES FOR THE CULTIVATION OF *PLEUROTUS OSTREATUS*

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**Abstract.** At present Malaysia is the largest exporter of palm oil in the international market. In the process of extraction of palm oil from oil palm fruit, biomass materials such as palm empty fruit bunch (EFB) and palm pressed fibre (PPF) are generated as waste products. This research was undertaken to evaluate the use of empty fruit bunch (EFB) and shredded palm pressed fibres as the substrates for the cultivation of *Pleurotus ostreatus* (oyster mushroom) which currently use sawdust of rubber trees. Five different substrates were prepared either alone or in combinations with rubber tree sawdust. These substrates were supplemented with fixed ratio of rice bran and limestone to increase the yield of *Pleurotus ostreatus*. The highest yield observed is from substrate comprising 50% PPF and 50% rubber tree sawdust with a mean yield of 0.19375 kg. Both shredded palm pressed fibre and empty fruit bunch show potential as substrate for the cultivation of *Pleurotus ostreatus*.

**Keywords:** *Pleurotus ostreatus*; oyster mushroom; substrates; palm pressed fibre; palm empty fruit bunch

**Abstrak.** Pada masa kini, Malaysia merupakan pengeksport terbesar minyak kelapa sawit di pasaran antarabangsa. Dalam proses untuk mengekstrakkan minyak sawit dari buah kelapa sawit, bahan biojisim seperti tandan buah kosong (EFB) dan sabut tertekan sawit (PPF) dijana sebagai produk buangan. Kajian ini dilakukan untuk menilai kegunaan tandan buah kosong (EFB) dan sabut tertekan sawit yang tersiat sebagai substrat untuk penanaman *Pleurotus ostreatus* (cendawan tiram) yang semasa ini menggunakan habuk kayu getah. Lima substrat berbeza disediakan sama ada bersendirian atau dalam gabungan dengan habuk kayu getah. Substrat tersebut ditambah dengan nisbah tetap dedak padi dan batu kapur untuk meningkat pengeluaran *Pleurotus ostreatus*. Hasil yang tertinggi dilihat dari substrat yang mengandungi 50% PPF dan 50% habuk pokok getah dengan min hasil 0.19375 kg. Kedua-dua sabut tertekan sawit yang tersirat dan tandan buah kosong menunjukkan potensi sebagai substrat untuk penanaman *Pleurotus ostreatus*.

**Kata kunci:** *Pleurotus ostreatus*; cendawan tiram; substrat; sabut tertekan sawit; tandan buah kosong

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## 1.0 INTRODUCTION

At present Malaysia is the largest exporter of palm oil in the international market. A total of 79.3 million tonnes of fresh fruit bunch is processed in 2006 to produce 15.9 million tonnes of crude palm oil (CPO) and 2.0 million tonnes of palm kernel oil (PKO). In the process of extracting these oils, 4.3 million tonnes of palm kernel shell, 10.7 million tonnes of palm pressed fibres (PPF), 17.4 million tonnes of empty fruit bunch (EPF) and 53.1 million tonnes of palm oil mill effluent (POME) are generated [1]. In practice the palm kernel shell and palm pressed fibres are used as fuels to generate steam and electricity for the palm oil mills. The empty fruit bunch are either incinerated or applied to the field. These practices create environmental pollution problems as incineration and boiler emit gases with particulates such as tar and soot droplets of 20-100 microns and a dust load of about 3000 to 4000 mg/nm [2] and indiscriminate dumping of EFB causes additional methane emission into the atmosphere [3]. To minimize pollution, a new usage for these wastes ought to be looked into. These biomasses from palm oil mills contain cellulose, hemicellulose and lignin.

*Pleurotus ostreatus* or oyster mushroom is an organism with the ability to bio convert various lignocelluloses materials as substrates [4]. This capability of the oyster mushroom is due to the presence of its lignocellulolytic enzymes, also named fibrolytic enzymes, including: xylanases, cellulases, and laccases [5] which help it to convert cellulose and lignin into useful carbohydrates such as glucose that can be used as an energy source for the fungi [6]. A wide range of plant waste, such as sawdust, paddy straw, bagasse, cornstalks, waste cotton, stalks and leaves of bananas, can all be used for *Pleurotus* mushroom production without a requirement for costly processing methods and enrichment materials [7]. Any agricultural waste that contains cellulose and lignin is a possible substrate for growing this fungus. However, Stajic *et al.* [8] describes that lignocellulolytic enzymes production by *Pleurotus ostreatus* depends strongly on the strain, substrate composition and conditions of cultivation. Enzyme actions on the substrate depend upon the physical properties of the materials including the crystalline or amorphous nature, accessible area, surface area, porosity and mainly particle size [9 - 10].

Presently, in Malaysia, the commercial cultivation of *Pleurotus ostreatus* utilizes sawdust from rubber palm as the base medium. However, the low availability of rubber palm has become a serious problem to the mushrooms grower. Thus a new alternative substrate ought to be looked into to overcome the shortage of sawdust from rubber trees.

Mushroom requires carbon, nitrogen and inorganic compounds, as its nutritional sources and the main nutrient are carbon sources such as cellulose, hemicelluloses and lignin as shown in Table 1.

Table 2 shows the composition of nutrients in EFB, PPF and palm kernel shell. PPF and EFB both contain 47.2% carbon and 1.4% nitrogen, and 48.4% carbon and

**Table 1** Chemical composition on dry basis of palm oil waste [11]

<b>Nutrients</b>		<b>Materials</b>	
Organic	C-source	cellulose	Humus materials such as wood, straw, leaf, etc.
	N-source	hemicellulose	Humus materials such as wood, straw, leaf, etc.
Inorganic		proteinamino nitrogen	K.P, Si, Fe, Mg, etc.

**Table 2** Chemical composition on dry basis of palm oil waste [12]

<b>Element</b>	<b>Empty Fruit Bunch (%)</b>	<b>Fibre (%)</b>	<b>Shell (%)</b>
H	6.3	6.0	6.3
C	48.8	47.2	52.4
S	0.2	0.3	0.2
N	0.2	1.4	0.6
O	36.7	36.7	37.3
Ash	7.3	8.4	3.2

0.2% nitrogen, respectively as shown in Table 2. This means that the palm pressed fibres and empty fruit bunch have the potential to be used as substrate for the *Pleurotus cultivation*.

*Pleurotus ostreatus* is a fungus which is valuable with highly nutrients and is able to grow at a wide range of temperature [13 - 15]. It contains most of the essential amino acids, valuable vitamins, minerals and low energy carbohydrates. In addition, the cultivation process of the *Pleurotus* is free from chemical substances and this makes the *Pleurotus* to be one of the healthy food available.

## 2.0 EXPERIMENTAL PROCEDURES

Fresh empty fruit bunch and pressed palm fibres are obtained from Kilang Sawit Kulai, Felda Taib Andak, Kulai Johor. The fresh empty fruit bunch are cut into smaller pieces, dried and then shredded using a shredder whereas fresh palm pressed fibre are only shredded. The sawdust of rubber trees is obtained from a rubber tree-processing mill. Five different substrates are prepared as shown in Table 3 with a total of fifteen replicates prepared for each substrate. The preparation of substrate

**Table 3** Composition of substrate

<b>Substrate</b>	<b>Composition of substrate</b>
A	100% EFB
B	50% EFB, 50% rubber tree sawdust
C	100% PPF
D	50% PPF, 50% rubber tree sawdust
E	100% rubber tree sawdust

involved mixing of the main material with the addition of 5% rice bran. The substrates were mixed thoroughly until no lumps of rice bran were found. Then, 2% calcium carbonate was added to the mixture. The calcium carbonate neutralizes the acidity of the substrate. The mixture was again stirred until no calcium carbonate was visible. Water (80% of the total weight of mixture) was then added to increase the moisture content. The substrates were mixed again until all the water was absorbed. 1kg of each substrate was then placed in a polyethylene bag. The bags of substrate were then compressed and closed with PVC necks which were covered with cotton plugs and wrapped with papers to prevent the entry of insects.

All the substrates were placed on steel shelves in an upright position and sterilized at 100 °C for about 8 hours in the sterilization chamber. After sterilization, the bags were left to cool and then inoculated with 10 g of spawn. The substrates were subsequently placed vertically in a spawn running room maintained at 25 °C and relative humidity at 85%. This is done by periodically spraying water on the floor of the room. The substrates were left in a hut covered with nets specifically designed so as to allow only 70% sunrays for mycelium to grow throughout the bags.

At the end of the colonization period, the bags were rearranged horizontally. After 70 days, the upper parts of the bags were unfolded to induce fruiting for the first cropping. A tiny pinhead will be seen on the surface of the substrates and these will grow into full size mushrooms within a day or two. Water was sprayed in the form of fine mist to maintain the moisture and lower the temperature. The fruit bodies were ready for picking just when the periphery of caps started turning upwards. This will be evident as small crinkles appeared on the side of the pileus (cap). The bags that had already been cropped were closed for another 10 days until the next harvest. In total, 4 harvests/flushes were collected.

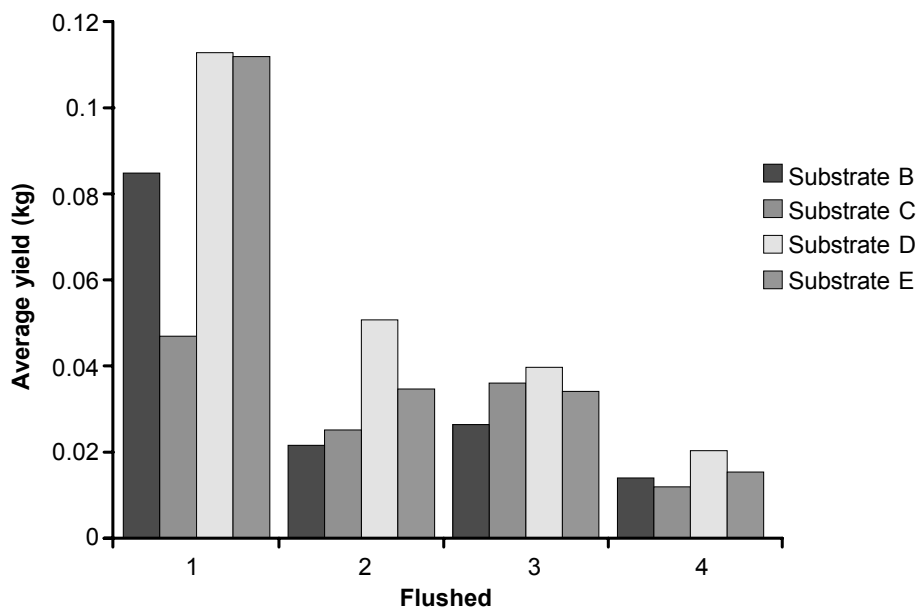
### 3.0 RESULT AND DISCUSSIONS

In this research, *Pleurotus ostreatus* were cultivated on substrates with different composition of fine palm pressed fiber, palm empty fruit bunch and sawdust from rubber trees. These substrates were supplemented with fixed ratio of rice bran and calcium carbonate.

It is observed that Substrate B took the longest time for the mycelium to fully colonize the bag, which is 60 days followed by Substrate C, 56 days, and Substrate D, 52 days. Substrate E took the shortest time for spawn running which is 49 days.

The first flush was harvested 5 days after the bags were opened, while the second flush was harvested in about 15 days after the first harvest. The third and fourth flushes were obtained in similar manner.

Substrate A did not yield any result as all replicate failed to complete spawn running (mycelia fully colonized substrate) and thus could not produce *Pleurotus ostreatus* fruit bodies. For Substrate C and D, six and three replicates, respectively,



**Figure 1** Mean yield of pleurotus fruit bodies produced from each flush

were identified to be contaminated. The production of *Pleurotus ostreatus* from Substrate B and E, on the other hand, shows no sign of contamination.

The yields are based on the average yield of all the replicates, which successfully produces *Pleurotus ostreatus* fruit bodies. As shown in figure 1, for substrates B, D and E, more than 50% of the total yields were produced during the first flush while the fruit body obtained in the second, third and fourth flushes were of lower quality as well as in a smaller amount. As the number of flushes increase, the yields obtained from each type of substrates decreases due to the diminishing nutrient in the substrate.

Chang *et al.* [16] defined the term “biological efficiency” to express the yield of fresh fruit bodies per 100 g dry substrate. The biological efficiencies of the fruit bodies of *Pleurotus ostreatus* harvested from the various substrates based on the first flush are shown in Table 4.

According to the results listed in Table 4, the different substrates caused a difference in biological efficiency. Substrate D and E produced the highest biological efficiency

**Table 4** Effect of different substrates on the biological efficiency (first flush)

Substrate	Biological efficiency (%)
A	Non available
B	8.6%
C	4.6%
D	11.3%
E	11.3%

**Table 5** Average and total weight of mushroom yield for each substrate

Substrate	Average Weight (kg)	Total Weight (kg)
A	Non available	Non available
B	0.0920	1.38
C	0.06556	0.59
D	0.19375	2.325
E	0.1550	2.325

of 11.3% compared to that of Substrate B and C. This might be due to the presence of higher content of nutrient in substrate D when rubber tree sawdust was added to palm pressed fibre. The control substrate, substrate E, is generally used in the commercial cultivation *Pleurotus ostreatus* of in Malaysia.

Based on Table 5, Substrate D produces the highest yield of *Pleurotus ostreatus*, which is 0.19375 kg per replicates followed by the controlled substrate, Substrate E (0.155 kg) and Substrate B (0.092 kg). The lowest mean yield of 0.06556 kg is produced by Substrate C. However, in terms of total yield, both Substrate D and E produced 2.325 kg of *Pleurotus ostreatus* even though three of the replicates of Substrate D were contaminated.

Generally, *Pleurotus ostreatus* requires carbon, nitrogen and inorganic compound, as its nutritional sources and the main nutrient are carbon sources such as cellulose, hemicelluloses and lignin. For optimal growth and fruiting, they require significant amounts of nitrogen, which may be inadequate in the crop residues. The function of rice bran is to provide nitrogen especially during the formation of fruiting bodies. Both palm empty fruit bunch and fine palm pressed fibre are identified to contain nitrogen as shown in Table 2. According to Truong [17], the content of nitrogen for the rubber tree is about 1.68% as compared to that of palm pressed fibre (1.4%) and that of palm empty fruit bunch (0.2%). The difference in the nitrogen content in sawdust of rubber tree, palm pressed fibre and palm empty fruit bunch could be the factor for the difference in the results obtained.

As mentioned previously, nitrogen is the main nutrient for the growth of mycelium. The result shows that, all the replicates for Substrate E has no difficulty for the mycelium to fully colonize the bags. This is because the content, which is sawdust from rubber trees, has the highest nitrogen content. The substrate also requires the shortest time about 49 days to complete the spawn running compared to the other substrates.

Since Substrate A which is 100% empty fruit bunch contains the least amount of nitrogen (0.2%), it is possible that this amount is insufficient for the growth of the mycelium as compared to Substrate C, 100% pressed palm fibre, which managed to produce *Pleurotus ostreatus* fruit bodies.

Another factor that might contribute to the difference in the mycelium growth could be the particle size of the substrates. Empty fruit bunch is observed to be

much finer as compared to that of palm pressed fibre and rubber tree sawdust with rubber tree sawdust being the coarsest. It is also observed that the substrate became much more compact when water was added, thus, reducing the voidage available.

Substrate C which is 100% PPF gives the lowest yield as compared to the other substrates (with the exception of 100% EFB). This is most probably due to the low carbon content (47.2%) resulting in less nutrient for the fruiting bodies to form. The highest yield of *Pleurotus ostreatus* is from Substrate D (50% PPF and 50% rubber tree sawdust) due to the fact that the carbon content is much higher when palm pressed fibre is combined with rubber tree sawdust as the rubber tree sawdust provides the richest source of lignin and cellulose.

Critical examination of other environmental growth requirements such as temperature, relative humidity, pH and light requirement need to be done on these substrates so as to have a better understanding on the mechanism of *Pleurotus ostreatus* fruiting in the future. Further research needs to look into the effect of difference in the particle size of the substrate, the C/N ratio and also in the chemical composition of the substrate in terms of trace elements. It is also recommended that the composition of the mixture of palm pressed fibre and rubber tree sawdust to be more varied in order to determine the optimum mixture for the cultivation of the *Pleurotus ostreatus*.

#### 4.0 CONCLUSION

From this research, both palm oil mill wastes, shredded empty fruit bunch and palm pressed fibre, applied in this study show potential as viable substrates for mushroom cultivation either alone or in combinations of both as substrate for the cultivation of *Pleurotus* as both substrates produce *Pleurotus ostreatus* fruit bodies. However, Substrate D with the composition of 50% of PPF and 50% of rubber tree sawdust produces the highest yield of *Pleurotus ostreatus* fruit bodies per replicate compared to the other substrates.

With optimum use of the biomass generated from the palm oil mills, it will not only solve the environmental pollution problem but it can also offer a promising way to convert low quality biomasses into a valuable high protein food.

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