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Growth and Yield Performance of *Pleurotus ostreatus* on Various Agro-Industrial Wastes in Malaysia

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Abstract. The utilization of low value agro-industrial waste as valuable end product has become a key research priority in the recent years. Currently, the commercial cultivation of *Pleurotus ostreatus* using sawdust from rubber tree as the base substrate in Malaysia has been reported. However, the price and demand of the rubber tree sawdust (RS) have been increasing, affecting the overall production cost. Thus, a new inexpensive alternative substrate yet as effective as rubber tree sawdust is needed to replace the RS as the base substrate for the cultivation of *P. ostreatus*. In this study, the investigation on substrates from various agro-industrial waste in Malaysia was carried out. Nine different substrates were prepared in combinations in the ratio of 0.25 w/w and 0.5 w/w with empty fruit bunch (EFB), palm pressed fibre (PPF), sugarcane bagasse (SGB) and RS as an alternative substrate for the cultivation of *P. ostreatus*. These substrates were supplemented with fixed ratio of wheat bran and calcium carbonate to increase the yield of *P. ostreatus*. The number of days mycelium growth, yield (g/kg substrates), BE (%) and nutrient compositions were studied for different the nine combinations of EFB, PPF and SGB to RS. The substrate of 25%PPF + 25%SGB + 50%RS was found as the best formulation in term of growth performance, yield and biological efficiency (BE) (%) with 35 days for first harvest at 318.88 g/kg and 79.72%, respectively. Thus, the combination of EFB, PPF and SGB demonstrated a great potential as an alternative substrate for the cultivation of *P. ostreatus* when incorporated with RS.

INTRODUCTION

Pleurotus ostreatus, or also known as oyster mushroom, (*Basidiomycetes*: *Agaricaceae)* is the second widely cultivated mushrooms worldwide after *Agaricus bisporus* [1]. Haimid *et al.* [2] reported the *P. ostreatus* is the most cultivated mushrooms contributing to 90.89 % of the total mushrooms cultivated in Malaysia. It has high nutritional values as an important source of protein, carbohydrates, vitamins, calcium, and iron [3]. *P. ostreatus* is a fungus that can be cultivated on various lignocellulosic substrates due to its lignocellulolytic enzymes which degrades the lignocellulosic matters into useful carbohydrates for the fungi [4]. Therefore, any types of organic matters that consist of lignocelluloses such as hemicellulose, cellulose and lignin can be used for oyster mushroom substrates, and this includes almost all agro-industrial wastes in Malaysia.

At present, Malaysia accounts for an overwhelming contributions to world's oil palm production and export which are 39% and 44%, respectively [5]. Though, palm oil industry has boosted the national economy in Malaysia, it also generated abundant of oil palm biomass, including empty fruit bunches (EFBs) and palm pressed fibers (PPFs). For every ton of palm oil produced from fresh fruit bunch (FFB), approximately 1 ton of oil palm empty fruit bunch (EFB), 0.7 ton of palm fibers, 0.3 ton of palm kernels and 0.3 ton of palm shells were generated [6]. In particular, EFB is the residual fruit bunch generated after fruits are removed from the FFB [7], while the PPF is a form of recovered fibrous residue from palm fruit during palm oil extraction which accounts for about 11% of the fresh fruit bunch [8]. The fruit fiber has been shown to possess high potential to be used as mushroom growing substrate without any further treatment [9]. The preparation of the substrates can be directly inoculated with little pre-treatment, whereas in some

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cases, they required microbiologically and physically pre-treatment [9]. Wood [10] explained that microbiological pre-treatment of substrate generally comprised of some form of controlled bulk composting process, while physical pre-treatment may include sterilization by autoclaving. Besides solid waste from palm oil mill industries, the SGB are available in abundance both in rural and urban areas, particularly in Perlis and Kedah states in Malaysia. All these kinds of waste generally have high content of hemicellulose, cellulose and lignin which can be upgraded to higher value-added products instead of being disposed resulting in the alarming greenhouse effect and global warming.

Presently in Malaysia, the commercial cultivation of *P. ostreatus* utilises RS as the base medium. However, the high demand of RS leads to increase in its production cost which is a serious problem to the mushroom growers in particular *P. ostreatus* that requires carbon, nitrogen and other inorganic compounds for its nutritional sources. This species of mushrooms has the ability to convert lignocellulosic materials using its lignocellulolytic enzymes into useful carbohydrates that can be used as an energy source. PPF, EFB and SGB have high contents of hemicellulose, cellulose and lignin for carbohydrates or carbon source in order to ensure the *P. ostreatus* grows well. Therefore, the EFB, PPF and SGB have the great potentialsto be an alternative substrate in the cultivation of *P. ostreatus* in the future and at the same time, able to reduce the solid waste abundance and environmental issues in Malaysia.

In this present study, three locally available organic wastes; oil palm fruit fiber, oil palm empty fruit bunch and sugarcane bagasse were utilized for the cultivation of *P. ostreatus*. The influence of these agro-industrial waste on the mycelium growth, yield, biological efficiency and nutrient compositions of the fruit bodies were evaluated.

METHODOLOGY

Preparation of The Substrates

Fresh EFB and PPF shredder were obtained from Kilang Sawit Lepar Hilir, Gambang, Pahang, while the SGB was obtained from local farmers in Kuantan, Pahang. Meanwhile, the RS, wheat bran, calcium carbonate and *P. ostreatus* spawn were purchased from Pekan Agro Farm, Pahang. While the SGB was shredded using a grinder in the Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, the rest of the agro-industrial waste were sun dried. Nine different substrate compositions were prepared as shown in Table 1 with a total of three replicates prepared for each substrate composition. Substrate of 100% RS was used as a control treatment.

TABLE 1. Composition of substrate.

Substrate	**Composition of substrate**
RS	100% RS
EFB + RS	25% EFB + 75% RS, 50% EFB + 50% RS
PPF + RS	25% PPF + 75% RS, 50% PPF + 50% RS
SGB + RS	25% SGB + 75% RS, 50% SGB + 50% RS
EFB + PPF + RS	25% EFB + 25% PPF + 50% RS
PPF + SGB + RS	25% PPF + 25% SGB + 50% RS

All the substrates were mixed in the fix ratio of wheat bran (5%) and calcium carbonate (1.5%) supplements. The substrates were mixed until no lumps of wheat bran were found and no calcium carbonate were visible. The tap water that contained a small amount of life light (80% of the total weight of mixture) was then added into the mixing substrate to increase the moisture content until all of the water was absorbed. The life light was made from woody material which was used to promote faster growth of the plants. About 600-700 g of each substrate was placed in polyethylene bag at fix height of 15 cm and a volume of 1,178 cm^3. The bags of substrate were then compressed and closed with PVC-necks, which were wrapped with papers to prevent the entry of insects.

Sterilization of Substrates

All of the substrates were placed on the steel shelves in an upright position and sterilized at 100 °C for 8 hours in sterilization chamber. Then, all the substrates bags were left to cool at room temperature.

Inoculation

After sterilization, each of the prepared polypropylene bag was inoculated at the centre of the substrate with 7-10 g of spawn under aseptic condition. After the spawn was added to the substrates, the bags were shaken for proper distribution of spawn.

Incubation and Harvest

The substrates were placed vertically in the dark room and maintained at room temperature and relative humidity in the range of 80-85%. At the end of the colonization period, the bags were rearranged horizontally. After the mycelium fully colonized and the substrates were completely white, the upper parts of the bags were unfolded to induce fruiting for the first cropping. The water was sprayed in the form of fine mist towards the bags to maintain the moisture and lower the temperature. The pin head formation can be seen on the surface of the substrates after a two or three days. The pinhead of the mushrooms grew into the full size within three to five days interval after pinhead formation. The bags that already cropped were closed for another 7-10 days until the next harvest. In total, 4 harvests were collected in this study. The mushrooms were harvested by hand. The days for the growth of mycelium as well as the pin head and fruiting bodies formations, total weight of fruiting bodies from four flushes were recorded. The biological efficiency (BE) was calculated based on the total yield of *P. ostreatus* harvested per dry substrate used and expressed in percentage.

Raw Materials Analysis and Substrates Analysis

Elemental analyses of RS, EFB, PPF and SGB were done using CHNS analyzer at Central Laboratory, Universiti Malaysia Pahang. The pH and the moisture content of the substrates were also determined using pH meter and the moisture analyzer, respectively.

Fruiting Body Analysis

The nutritional compositions (protein, total fat, total carbohydrate, moisture, energy and crude fiber) of fruiting body for first flush for every composition were analyzed using Proximate Analysis at UNIPEQ, Universiti Kebangsaan Malaysia.

RESULTS AND DISCUSSIONS

P. ostreatus requires carbon, nitrogen and inorganic compounds as its nutritional sources with the main nutrients being carbon sources such as cellulose, hemicellulose, and lignin. The elemental analysis and the carbon to nitrogen ratio (C/N) of RS, EFB, PPF and SGB are presented in Table 2.

TABLE 2. Elemental analysis of RS, EFB,PPF and SGB.

Element	RS	EFB	PPF	SGB
C	38.7	42.62	38.57	44.5
N	4.97	4.66	4.78	4.21
H	0.39	1.29	1.69	0.39
S	0.33	0.69	0.5	0.84
C/N ratio	7.79	9.15	8.07	10.57

Table 2 showed that the SGB had the highest C/N ratio compared to PPF and EFB. Meanwhile, the lowest C/N ratio was observed for RS (7.79). The *P. ostreatus* needs the optimum C/N ratio to grow well on substrates. The SGB contains more carbon than EFB, RS and PPF. Naraian *et al.* [11] relate that the development of mycelium growth and pinhead formation is dependent on C/N ratio. Philippoussis and Diamantoppoulou [12] reported that oyster mushrooms required more carbon and less nitrogen, however most of the substrates must be supplemented with nitrogen and carbon source to reach the optimal C/N ratio for the mushrooms to grow. Therefore, the types and the formulation of the substrates for *P. osteratus* cultivation should contain a balance content of carbon and nitrogen in order to achieve optimum C/N ratio. Table 3 tabulates the pH and moisture of block for various substrates compositions.

TABLE 3. Chemical properties of substrates compositions

Substrate	pH	Moisture content (%)
25% EFB + 75% RS	8.50	76.47
25% SGB + 75% RS	8.75	68.74
50% SGB + 50% RS	7.50	78.35
25% EFB + 25% PPF + 50% RS	9.30	74.89
25% PPF + 25% SGB + 50% RS	8.20	70.92

These were the two known contributing factors on the growth of *P. ostreatus* [13]. The highest pH substrate (pH 9.30) was obtained from substrate of 25% EFB + 25% PPF + 50% RS, followed by 25% SGB + 75% RS (pH 8.75, 25% EFB + 75% RS (pH 8.50), 25% PPF + 25% SGB + 50% RS (pH 8.20) and the lowest pH was obtained by the composition of 50% SGB + 50% RS which was around pH 7.50. These result concurs with [14] where the pH of substrates should be in the range of pH 3 – 10 for the mushrooms to grow in the tropics and thus, the ability of the mycelia to tolerate the temperature of 28 °C. Besides, oyster mushrooms can grow at moderate temperatures, ranging from 18-30 °C [15]. On the other hand, water is also one of the main factors that influence the success of mushrooms to grow well. The highest moisture content was obtained from the substrate compositions of 50% SGB + 50% RS which was around 78.35 % and the lowest is from the 25% SGB + 75% RS substrates which was 68.74% as shown in Table 3. According to Urben [16], the high moisture content in the substrate would result in difficult breathing for the mycelium, inhibiting perspiration, thus rendering the development of fruiting body impossible and resulting the development of non-desired organism such as bacteria and nematodes. However, the low moisture content may also result in death of the fruiting body [13]. It was reported that the suitable moisture content should be in the range between 50% and 75% for substrates [17].

Figure 1 represents the average days for completion of spawn running, first pinhead formation and first fruiting body formation for different substrates formulation. Beside substrate of 100% RS, 50% EFB + 50% RS, 50% PPF + 50% RS and 25% EFB + 25% PPF + 50% RS, other substrates managed a complete mycelium growth in less than 30 days. Theoretically, the mycelium growth rate increased gradually with the amount of RS. However, in this study, the substrates that contained SGB amount of either 25% or 50% showed the fastest mycelium growth, attributable to the blending of RS and PPF to SGB which helped at optimizing the C/N ratio and the carbon source in the substrates. Moreover, the SGB had higher sugar content that can influence the rapid growth of mycelium as compared to others that were not incorporating with SGB. The substrate composition of 50% PPF + 50%RS displayed the lowest mycelium growth, followed closely by the 50% EFB + 50% RS , 100% RS and 25% EFB + 25% PPF + 50% RS substrates which were in contrast with reported literature [18] which stated that the combination of 50% RS and 50% SGB had slightly slower the growth of mycelium than in 100% RS substrate. This was due to the difference in total C and N of substrate formulas, hence the difference in C/N ratio. The C/N ratio had more effects on the mycelium growth, the formation and development of fruiting body [18]. Moreover, as the amount of RS decreased in the substrate formulations, slower mycelium growth was observed due to the larger particles size of the EFB and PPF in the substrates. The particle size of EFB and PPF should be sufficient enough in order to mix well with RS. However, if the particles sizes are too small, the wet substrate can becoming over compact and hence, reducing the porosity and aeration available [19].

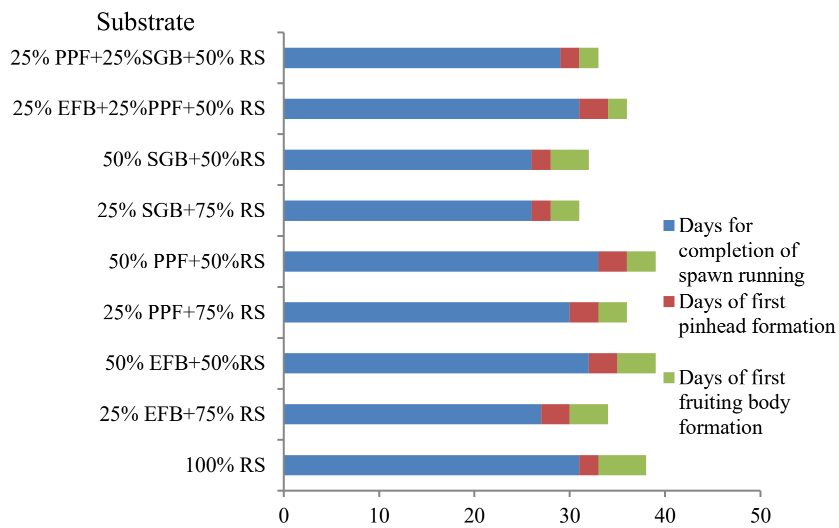

FIGURE 1. Average days for completion of spawn running, first pinhead formation and first fruiting body formation.

Figure 1 shows the time for first pinhead formation and first fruiting bodies formation as well as crop. The different pinhead and fruiting times were recorded, depending on the composition of the substrates. The substrates that contained SGB showed the fastest pinhead and fruiting bodies formation i.e. only within 28-32 days. On the other hand, decreasing the amount of RS in the substrates reduced the pinhead and fruiting bodies formation rate, for example the substrates of 50% EFB+50% RS and 50% PPF+50% RS. The nitrogen rich source substrate speeded up the mycelium growth and pinhead formation [20]. The fruiting body formation from almost all these substrates took between 2-4 days after pinhead formation (Figure 1). The fast growth of the mushrooms also was influenced by addition of supplement (wheat bran mixed) [21].

TABLE 4. Yield for every flushes and biological efficiency of different substrates.

Substrate	Average yield (g/kg substrate)				Total yield (g/kg substrate)	Biological efficiency (%)
	1st flush	2nd flush	3rd flush	4rd flush		
100% RS	55.67	62.53	48.14	36.43	202.77	67.59
25% EFB+75% RS	107.58	55.8	46.62	40.82	250.82	62.71
50% EFB+50%RS	88.17	39.12	45.72	41.67	214.68	53.67
25% PPF+75% RS	75.23	58.16	53.38	52.11	238.88	59.72
50% PPF+50%RS	80.67	66.38	30.00	16.95	194.00	48.50
25% SGB +75% RS	92.86	64.90	48.86	43.57	250.19	62.55
50% SGB+50%RS	100.29	61.10	46.57	42.86	250.82	62.71
25% EFB+25%PPF+50% RS	111.05	30.83	57.05	42.67	241.60	60.40
25% PPF+25%SGB+50% RS	123.60	72.83	64.62	57.83	318.88	79.72

The yields of fruiting bodies were recorded from three replicates and calculated as an average as shown in Table 4. Theoretically, the yield was gradually decreased over the flush. The highest mushrooms yields were mainly from the first flush, except those from the 100% RS substrates which recorded the highest yields from the second flush. The highest total yield and biological efficiency (BE) (%) was from the 25% PPF + 25% SGB + 50% RS substrate, while the lowest total yield and BE (%) was obtained from 50% PPF + 50% RS. However, in this study, the 50% EFB + 50% RS and 25% EFB+25% PPF+50% RS had resulted in the third flush being higher than second flush which might be due to the environmental factor such as temperature and relative humidity which was difficult to maintain, and thus might had affected the second flush yield. All of the substrates that contained SGB resulted the higher total yield and BE (%) compared with other substrates due to the higher sugar content in SGB itself. In general, the BE (%) in this present study was far lower in comparison with other studies. Bhattacharjya *et al*. [22] observed that BE (%) of *P. ostreatus* grown on different RS substrates ranged from 187 % to 213.2 %. However, the results of this study were similar to those reported in literature [23] which found that BE (%) of *P. pulmonarius* that was grown on the stalks of three grass plants in Taiwan was between 39.55 to 58.33% .

The nutritional analysis of the first fruiting bodies of 100 % RS and 50% RS that combine either EFB, PPF and SGB substrates is shown in Table 5. There was non-detectable fat content of the mushrooms from all of the substrates 50%, except the substrates from 50% PPF + 50% RS that only had a small amount of the fat content (0.09). The first flush fruiting bodies from the 50% PPF +50% RS had higher protein content compared to other substrates. Ali *et al.* [4] reported that the substrate that had higher C/N ratio contributed to the higher protein content. Ragunathan and Swaminathan [24] found that the protein content of mushrooms was influenced by the C/N ratio and the chemical compositions of the substrates. The results in this present study were found to be in contrast with the finding from Bellettini *et al.* [13] which stated increasing protein content in the mushrooms resulted in low fat content.

TABLE 5. Nutritional contents (%) and Energy (Kcal/100g) of *P. ostreatus* for first flush.

Substrate	Energy (kcal/100g)	Nutritional contents of *P. ostreatus* (%)					
		Prote in (%)	Total Fat (%)	Total Carbohydrate (%)	Crude Fiber (%)	Moisture (%)	Ash (%)
100% RS	42.5	2.90	0	7.67	6.19	88.78	0.66
50% EFB+50%RS	50.5	2.77	0	9.81	6.07	86.42	1.00
50% PPF+50%RS	48.5	3.82	0.09	8.19	6.06	87.09	0.82
50% SGB+50%RS	30	2.06	0	5.43	6.15	91.78	0.73
25% EFB+25%PPF+50% RS	35	3.14	0	5.63	4.90	90.27	0.97
25% PPF+25%SGB+50% RS	35.5	2.32	0	6.58	6.59	90.33	0.79

The mushrooms that grows from 50% EFB + 50% RS substrate had the highest total carbohydrates (9.81%), followed by 50% PPF + 50% RS, 100% RS, 25% PPF + 25% SGB + 50%, 25% EFB + 25% PPF + 50% RS. Meanwhile, the lowest content of total carbohydrates was in mushrooms that grew from the substrates of 50% SGB + 50%RS. Wen *et al.* [25] found that the low C/N ratio resulted in high carbohydrate content. The mushrooms from 25% EFB + 25% PPF + 50% RS substrates had the lowest crude fiber content and the others substrates were not statistically different in term of crude fibre content. It can be seen in Table 5 that the moisture contents of mushrooms from all substrates almost similar to each other's which were in the range of 92 - 87%. The higher moisture content in the mushrooms from the 50% SGB + 50% RS substrate was probably due to the greater water-holding capacity of the combination of SGB and RS itself. The highest ash composition obtained from the 50% EFB + 50% RS substrate and the lowest 100% RS which were 1.00% and 0.66%, respectively.

The substrates were not only affected by the protein, carbohydrate, and fat content in mushrooms, but also the total energy of *P.ostreatus* (as shown in Table 5). The total energy contribution of the samples ranged between 30-50.5 kcal/100 g dry weight for *P.ostreatuss*. Thus, it can be deduced that *P.ostreatus* was low in calorie food because they provided almost zero content of fat. This healthy food is one of appropriate food to be consumed for those who are watching on diet.

CONCLUSION

It is found that the growth and yield performance of *P.otreatus* were dependent on C/N ratio of the substrate. The substrates that contain SGB in the ratio of 0.25 w/w and 0.5 w/w gave better the mycelium rate, fastest pinhead and fruiting bodies formations as well as the yields and BE(%) due to high sugar content in sugarcane bagasse which resulted in the optimum C/N ratio for mushrooms to grow well. The substrate of 25% PPF + 25% SGB + 50% RS had showed the best formulation in terms of growth performance, yield and BE(%) which were at 35 days for first harvest, 318.88 g/kg substrate and 79.72% respectively, as compared to other formulations of the substrates. However, the other combinations of the substrates also had tremendous potentials as an alternative substrates for mushroom cultivation in Malaysia. The particle size, C/N ratio, pH, moisture content can significantly affect the growth of *P. ostreatus* and mineral content on the substrates. The advantages of using the biomass generated from the palm oil mills which is available throughout the year at little and no cost, and sugarcane bagasse that abundance in the rural and urban areas in Malaysia will not only solve the environmental pollution problem, but it can also offer an economically promising way to convert low quality biomasses into a valuable high protein food for human. In addition, it can also help in generating income for the mushroom growers.

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