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Growth and yield of *Pleurotus ostreatus* using sugarcane bagasse as an alternative substrate in Malaysia

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Abstract. This paper looks at the feasibility of using sugarcane bagasse (SGB) for the cultivation of *Pleurotus ostreatus* (oyster mushrooms) as an alternative for rubber tree sawdust (RS). Three different compositions of oil palm trunk and rubber tree sawdust were studied with each composition having three replicates. A substrate containing only rubber tree sawdust acted as the control variable. The substrates were supplemented with 5% rice bran and 1.5% calcium carbonate. There are four steps involved in the cultivation of *P. ostreatus* which were bagging, sterilization, spawning and harvesting the substrates. The mycelia growth, spawn run, pin head formation, yield and biological efficiency were observed. The 100% composition of RS gave the best yield in terms of the mean height of stipe, which was 7.75cm. The mean fresh weight of sawdust was the highest with 50.4g with the number of fruiting bodies around 6. The least yield was recorded for 50% composition of substrates with a mean height of stipe 9.12cm. Among the substrates used, the RS showed the highest biological efficiency with 56.3% followed by 45.1% and 44.8% RS having mixed compositions of 25% and 50% SGB substrates, respectively. Hence, it is proven that in terms of quantity and quality, the unmixed substrates produced better edible mushrooms. However, the results obtained by SGB also showed a great potential as an alternative substrate for the cultivation of *P. ostreatus* when mixed with RS.

1. Introduction

Oyster mushrooms are saprophytic fungi naturally found on rotten wood material and can be utilized as substrates [1]. According to Chang [2], the mushroom industry is generally classified into three main categories: medicinal, edible and wild mushrooms. *P. ostreatus,* which is also known as "oyster mushroom" is the second most cultivated edible mushroom worldwide after *Agaricus bisporus* [3]. It is highly nutritious as it contains protein, fat, carbohydrates, minerals (ash), vitamins, essential amino acids and medicinal properties [4]. Mushrooms have been identified as one of the high-value commodities under Malaysia's National Agro-Food Policy (2011-2020). Currently, seven varieties of mushrooms are grown commercially in Malaysia and the most popular varieties are grey oyster mushroom, black jelly, ganoderma, and shitake [5].

Mushroom cultivation is one of the immense potential agricultural activities in Malaysia. High valued crop in terms of both food and medicine aspects with low cost production technology can bring high return within short time interval. The agro climatic conditions in Malaysia is suitable for the cultivation of mushrooms throughout the year. Thus, Malaysia has the potential to be a large mushroom producer in the market as its environment is suitable for mushroom cultivation.

P. ostreatus is a fungus that can be cultivated on various lignocellulosic substrates [6]. Any agricultural waste that contains cellulose and lignin is a possible substrate for growing this fungus. About 90 different kinds of agricultural wastes worldwide are studied for their potential as oyster mushroom substrates [7]. Since the typical lignocellulosic composition (% dry matter basis) of SGB consisting of 46 % cellulose, 27 % hemicellulose, 26 % lignin, and 4 % ash [8] is very close to that of the RS where it has 39 % cellulose, 29 % hemicellulose, 28 % lignin and 4 % ash [9], SGB has the potential to be an alternative substrate in the cultivation of *P ostreatus* and at the same time, solve the waste abundance and environmental issues.

Presently, in Malaysia, the commercial cultivation of *P. ostreatus* utilizes RS as the base medium. There is a shortage of RS due to the limited availability of rubber trees and the increasing price of RS. This has become a serious problem to the mushroom growers. A new alternative substrate to replace rubber tree sawdust is needed to overcome this shortage. The objective of this study is to investigate the effectiveness of using SGB as an alternative substrate for the cultivation of *P. ostreatus*. The growth performance in terms mycelium growth, formation of pinhead, fruiting body, yield and biological efficiency of the *P. ostreatus* on various mixtures of SGB and RS were studied.

2. Material and Method

2.1. Sample preparation
The sample of sugarcane bagasse was obtained from local farmers in Kuantan, Pahang. Meanwhile, the sample of RS, wheat bran and calcium carbonate were obtained from Pekan Agro Farm, Pahang. The raw materials were soaked with tap water to remove dirt and impurities. Then, sugarcane bagasse was dried, cut and shredded into smaller pieces by using a grinder at FKKSA laboratory. The samples of mixed composition (100:5:1.5) were mixed well until no lumps were found. The compositions were mixed and 700g of each substrate was placed in clear plastic bag. The bag was compressed and closed with PVC necks. These materials prepared consist of different composition of sawdust (RS) and sugarcane bagasse (SGB) as shown in Table 1:

Table 1. Substrate composition.

Substrates
100% RS
75% RS + 25% SGB
50% RS + 50% SGB

2.2. Preparation of substrates
The substrates were mixed with wheat bran (5%) and calcium carbonate (1.5%) supplement. All the substrates were placed in polypropylene bags and sterilized at 100°C for 8 hours in order to kill all fungi, microbes, bacteria, viruses and bacteria spores in the substrates before the spawning process began. After sterilization, each experimental polypropylene bag was inoculated at the center of the substrate with 10g of spawn of oyster mushroom. They were kept in a dark room at room temperature.

2.3. Sugarcane baggase characterization
The sugarcane baggase was characterized by using thermogravimetric analysis (TGA). The TGA of sugarcane bagasse was carried out at the Centre of Excellence for Advanced Research in Fluid Flow (CARIFF), Universiti Malaysia Pahang. The main aim of thermogravimetric was to study the thermal stability of cellulose, hemicellulose and lignin present in lignocellulosic. This equipment is purged with nitrogen gas and air to simulate conventional combustion with a flow rate of 50mL/min and a heating rate of 10^oC/min. The analysis was performed at a temperature within 30^oC to 900^oC under nitrogen flow while the air flow temperature was set at 900^oC for a duration of 30 minutes. The decomposition of lignocellulosic structure started at 200°C and completed above 600°C.

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2.4. Data collection

The yield of oyster mushroom on different composition was determined by recording the number, weight and size of the fruit bodies after sprouting. The measurements from the various replicates were added and their mean value was calculated. The parameters of growth such as number of fruit bodies, height of stipes, diameter of the pileus and biological efficiency were measured and calculated. The number of fruit bodies was measured by directly counting the number of fruit bodies on each substrate. The height of stipes was measured in centimetres (cm) using a ruler by measuring from the base of the stipe to the pileus. The diameter of the pileus was also measured in centimetres (cm) with a ruler by measuring from one edge of the pileus across the stripe to the other edge. The biological efficiency (BE) that expresses the yield of fresh fruit bodies per g of dry substrate was calculated according to the following equation as given by [10]:

$$\text{BE (\%)} = \frac{\text{Fresh weight of mushroom x 100\%}}{\text{Dry weight of mushroom}} \quad (1)$$

3. Results and discussion

3.1 Thermal dedgradation analysis

Thermogravimetric analysis is an accurate method for investigating the decomposition pattern and thermal stability of cellulose, hemicellulose and lignin present in lignocellulosic over a wide range of temperature. Figure 1 shows the weight loss (%) of raw sugarcane bagasse versus temperature. Each of the lignocellulosic structure has its own degrading temperature which varies, starting with hemicellulose (220-315˚C) followed by cellulose (315-400˚C) and lastly lignin in wide temperature range between (160-900˚C) [11]. The estimation of the hemicellulose, cellulose and lignin content in the fiber were determined by using the weight loss percent. Raw sugarcane bagasse started its degradation at 250°C with 12.29% weight loss and completed at a temperature above 600 °C. The estimation of weight loss percentage of hemicellulose and cellulose content for the sugarcane bagasse was around 69.13%. At the same time, the lignin content showed the initiation of degradation at 375°C with 17.32% weight loss.

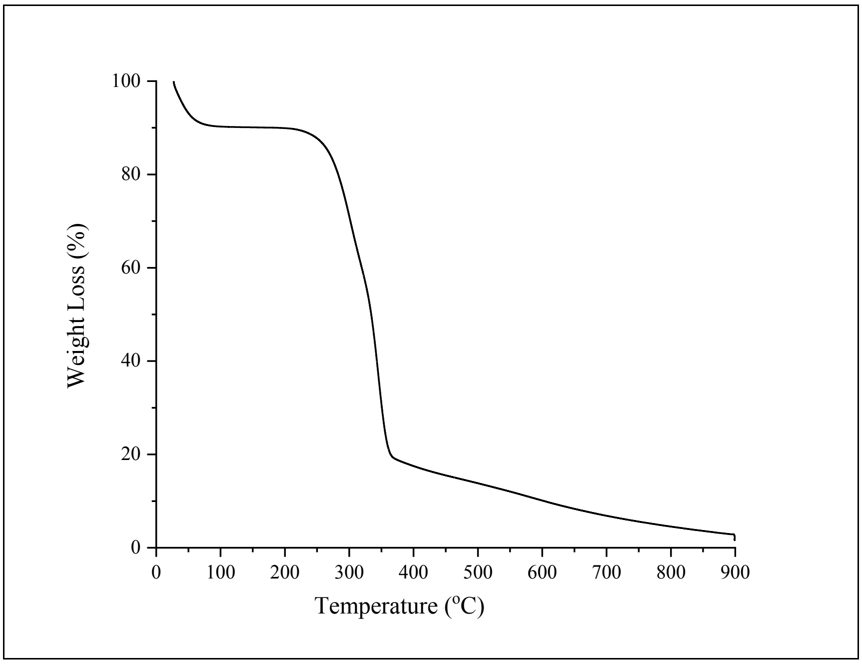

Figure 1. Thermogravimetric curve of raw SGB

3.2 Spawn running, pinhead formation and fruiting body formation

The findings of this research indicated that the sugarcane bagasse responds well when it is used with sawdust as it took 25 days and 29 days for a combination of 75% RS + 25% SGB and 50% RS + 50% SGB, respectively while sole sawdust took a longer time for mycelia to fully colonize, as shown in Table 2. Verma and Marschner [12] stated that the particle size of the substrates might influence the mycelia development.

Table 2. Days for completion of spawn running, pinhead formation and fruiting body formation on different substrates

Substrates	Days of completion of spawn running	Days for pinhead formation	Days of fruiting body formation	Average number of fruiting bodies
100% RS	30	34	38	6.17
75% RS + 25% SGB	25	29	31	5.08
50% RS + 50% SGB	29	30	34	5.50

Pinhead formation is the second stage of mycelia growth in the cultivation of mushroom. The days of pinhead formation were lesser; 29 days and 30 days for a composition of 75% RS + 25% SGB and 50% RS + 50% SGB when compared to 34 days for the sole sawdust as shown in Table 2. This study is supported by Vetayasuporn [13], who reported that the time taken for pinhead formation of oyster mushroom was between 6–7 days. The observation also coincides with [14] when he cultivated oyster mushroom on different substrates consisting a combination of wheat straw and sawdust.

In the study of [14], the fruiting body formation took 3–6 weeks after pinhead formation. It contrasts with the results obtained whereby the fruiting body formation was observed in 25% of SGB with 75% RS followed by 50% SGB and 50% RS, which took 2–3 days after the pinhead formation, as shown in Table 2. The fast growth of mushroom was influenced by the addition of supplement by wheat bran mixed [15].

3.3 Average length, diameter and thickness of fruiting bodies of mushrooms on different compositions

Table 3 shows the length and diameter of stipe as well as the thickness of the caps and stipes of the mushrooms harvested, while Figure 2 shows the fruit bodies of *P. ostreatus* grown on different substrates. There was a significant difference in stipe length and cap diameter of *P. ostreatus* grown on different substrate compositions. The stipe length of 9.12 cm from the composition of 50% RS + 50% SGB was the highest and the least was 7.75 cm from the sole sawdust. The oyster mushroom physical quality depends on the length of stipe [16]. Mondal [17] noted that the higher the stipe length, the poorer the quality of the mushrooms.

Table 3. Average length, diameter and thickness of fruiting bodies of mushrooms on different compositions

Substrates	Stipe length (cm)	Stipe thickness (cm)	Cap diameter (cm)	Cap thickness (cm)
100% RS	7.75	0.93	6.77	0.57
75% RS + 25% SGB	9.00	1.17	7.52	0.53
50% RS + 50% SGB	9.12	0.81	7.80	0.70

On the other hand, the thickness of stipe showed a different pattern of results where the composition of 75% RS + 25% SGB composition gave the highest stipe thickness, which is 1.17 cm while for the cap diameter and thickness, the 50% RS + 50% SGB composition gave the highest value with 7.80 cm and 0.7 cm, respectively. Averagely, the sole sawdust substrates produced mushrooms with better physical parameters than the mixed composition substrates. The physical parameter of unmixed composition was poor compared to the mixed composition of substrates and this might be due to the very high carbon to nitrogen ratio observed in these substrates.

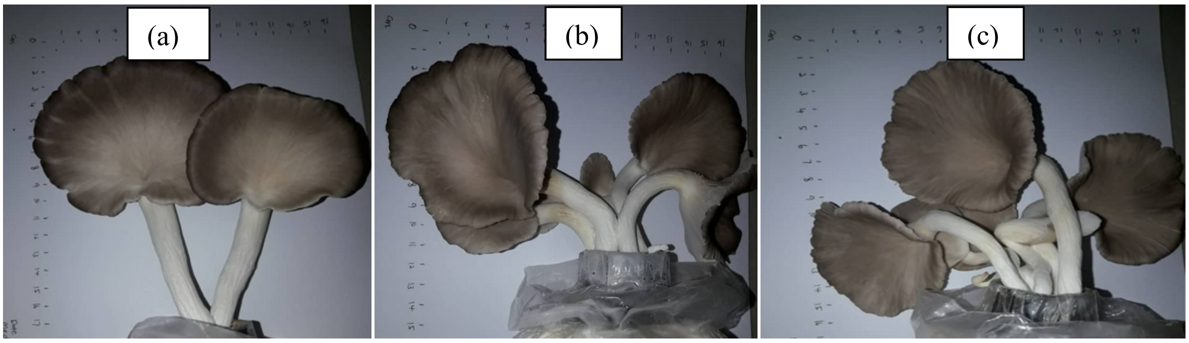

Figure 2. Fruiting bodies of *P. ostreatus* grown different substrates. (a); 100% RS, (b); 50% RS + 50% SGB, (c); 75% RS + 25% SGB

3.4 Weight, average yield and biological efficiency of different substrates

Table 4 indicates that the sole sawdust substrate gave the highest yield in grams of mushrooms per bag of substrates (g/bag) which was 50.4g/bag and this was significantly higher than the 41.3g/bag and 41.4g/bag of mixed substrates of 25% and 50% SGB composition, respectively. This is in contrast with the reports of Shashirekha and Rajarathnam [18] who observed that mixed substrates composition will have a higher yield compare to sole substrate. This higher yield observed in the sole substrate may be due to good physical and chemical qualities of these substrate.

Table 4. Weight, average yield and biological efficiency of different substrates

Substrates	Weight of each substrate (g)	Average yield (g)	Biological efficiency (%)
100% RS	700	50.4	56.3
75% RS + 25% SGB	700	41.3	45.1
50% RS + 50% SGB	700	41.4	44.8

Biological efficiency is the yield of mushrooms per g of substrates on dry weight basis. Table 4 indicates that the sole sawdust substrates were more efficient than the mixed substrates. It is evident that the 100% sawdust substrates showed the highest biological efficiency of 56.3%, followed by 75 % RS + 25% SGB with 45.1% and 50 % RS + 50% SGB with 44.8%. It has been reported by [14] that oyster mushroom gave the maximum biological efficiency on sawdust.

4. Conclusion

The results of the present study showed that the unmixed sawdust performs better substrate than the mixed substrate in terms of the growth performance, yield and % BE with 38d, 50.4 and 56.3% respectively. Increasing the amount of SGB in the substrate mixture of SGB-RS yields the fastest mycelium rate, pin head and fruiting bodies formations. However, the yield and percentage of BE decreased when the amount of SGB increased in the mixed substrate, which might be due to low nitrogen content in SGB resulting in high C/N ratio. Over all, the results of this study showed that the SGB mixed with a very high percentage of RS, can be an alternative substrate in Malaysia. However, the particle size, C/N ratio, pH, moisture content of the substrate needed to be critically studied in the future.

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