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PRODUCTION OF *PLEUROTUS SAJOR-CAJU* ON SAWDUST OF RUBBER TREE AND EMPTY PALM FRUIT BUNCH

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ABSTRACT

Cultivations of the grey oyster mushroom, *Pleorotus sajor-caju*, on sawdust, empty palm fruit bunch (EPFB), and combination of sawdust and EPFB (1:1; w/ w) without any nutrient supplementation were studied. The effects of different types of media on mushroom yield, time taken for mushroom growth, nutrient content in media and mushroom, and water activity were compared. The sawdust media yielded about 25% more mushrooms than EPFB under similar cultivation condition. However, mushroom failed to bloom fruit from the combination media of sawdust and EPFB. Mushroom grew in the sawdust media 28 days faster than EPFB media. The water loss of the media after mushroom growth varied from 44.6 % to 40.2 % and 51.2 % to 32 % for sawdust and EPFB media, respectively.

Key Words : Oyster mushroom, Pleorotus sajor-caju, Saw dust, Cultivation, Empty palm fruit bunch

1.0 INTRODUCTION

Mushrooms have been used for their beneficial properties for over 2000 years, including studies that suggest an important therapeutic role in cancer and other diseases. They are good source of protein, vitamins and minerals. Some types even contain significant amount of vitamin C, as well as appreciable amount of minerals such as potassium, phosphorus, calcium, magnesium, copper and iron, but low level of calcium [1]. Mushrooms contain about 85-95% water, 3% protein, 4% carbohydrates, 0.1% fats, 1% minerals and vitamins. The mushroom protein is intermediate between that of animals and vegetables [2]. They have been part of human diet for thousands of years and recently their consumption has been increasing, involving a great number of species apart from the popular mushrooms.

More than 2000 edible species of mushroom are known, but only 25 are commercially cultivated [1,3]. Mushroom cultivation is a profitable agribusiness and oyster mushroom is an edible mushroom having excellent flavour and taste. The technology of artificial cultivation of mushroom is somewhat recent innovation; incorporation of non conventional crops in existing agricultural system can help in improving the social as well as economic status of small farm. The choice of species to rise depends both on growth media available and on market considerations [2, 4].

Oyster mushrooms or *Pleurotus sajor-caju* can grow on many substrates. In Malaysia, rubber tree dust is the main source of media for growing oyster mushroom.

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However, the increasing price of rubber tree sawdust in market has affected the production cost. Thus, new alternatives of media those are inexpensive but as effective as rubber tree dust have to be explored. This can help farmers and entrepreneurs to expand their business in this highly competitive world. So, this preliminary research has been done to investigate the cultivation on different media preparation in order to see its effects in the production yield by studying the formulation of media, and the transformation of nutrient and metal element in the media for mushroom healthy growth.

2.0 EXPERIMENTAL

2.1 Media Preparation

Formulation of media and mushroom cultivation.

Three types of media were prepared as listed in Table 1. In each formulation, 2 kg of wheat bran was added into 20 kg basic material. Then 200 g lime was added into the mixture of media. The mixture was mixed well after predetermined amount of water had been added to keep the final moisture content at 40 % - 50 %. About 50 ml of effective microorganism activated solution (EMAS) was added into the mixture. After that, each media was filled into polythene bags and their mouths were plugged by inserting water-absorbing-cotton or waste paper with the aid of plastic rings. The bags were steriled at 80-90 °C for 7-8 hrs and allowed to cool. After the sterilization process completed, the spawning process was performed on the media. The seed of oyster mushrooms were injected into the bags after cooling process. Then the mouths of bags were closed with caps and the bags were arranged on racks for mycelia run. The bags were then inoculated for spawn running under full darkness at controlled temperature of 25 °C. The temperature was controlled by electric heaters at 25°C for spawn running and 20 - 22 °C for fruiting body formation. The humidity of bags was accomplished by spraying of water on them twice a day. The design of the mushroom house is ensured to have air holes because oxygen is essential for mushroom during fructification. The bags were watered three times in a day during cropping. After the body completely formed, the mushroom was harvested. Then, the weight of mushroom was measured and recorded. The yield of mushroom was determined by using weighing scale after harvest.

Table 1 Composition of media used for mushroom cultivation

Basic materials	Weight percentage (%)
Rubber tree sawdust (SD)	100
Empty palm fruit bunch(EPFB)	100
SD + EPFB	50 + 50

2.2 Nutrient Content Analysis

The analysis in media are divided in two: physical (pH, moisture content, C/N ratio) and chemical analysis (nutrient analysis namely nitrogen (N), phosphorus (P),and potassium (K). The media is weighed by using weighing scale before and after the mushroom growth to study the dry matter loss in the media [5]. A spectrophotometer (Model Hach

DR 2000) was used to determine total nitrogen, phosphorus and potassium using photometric detector to read the results.

2.3 Determination of Moisture Content

Moisture Analysis
About 100 g of sample was weighed in a broad shallow pan and spreaded evenly. The initial mass was recorded as W_1. The sample was heated at 110 ± 5 °C for 2 to 3 hours. After that, the sample was cooled down and re-weighed. The mass of the sample after drying is measured and recorded as W_2.

$$\text{Moisture content, \%} = ((W_1-W_2) / W_1) \times 100\% \quad (1)$$

where, W_1 is the initial weight in grams of the sample taken, W_2 is the weight in grams of the sample after drying to constant weight.

Rate of Water Absorption
About 20 g of sample was weighed in a broad shallow pan and spreaded evenly. 100 ml of water was measured in 100 ml beaker. The sample was then put into the beaker that contain water for 10 minutes. After 10 minutes, sample was weighed and remaining water in the beaker was measured. The procedure was repeated for time intervals 15, 20, 25 and 30 minutes.

2.3 Ash Content Analysis

The ash content generally represents the mineral content of the sample. The muffle furnace was heated to 425 °C. A crucible was ignited for an hour in the furnace and then cooled in the desiccators. Then the crucible was weighed accurately and the same procedure was repeated. After that, the crucible was transferred to the muffle furnace where the temperature being increased until 600 °C and samples were further heated until becoming white grey ash. The sample was then cooled in the desiccators and re-weighed [5].

$$\% \text{ Ash} = \frac{[(\text{weight of ash + crucible, g}) - (\text{weight of crucible, g})]}{\text{Weight of original sample, g}} \times 100 \quad (2)$$

2.4 Analysis of Fiber Content

The weight of ashless filter paper (Whatman No. 541) was measured. About 5g sample was put in conical flask after the water was removed and mixed with about 200 ml 0.25N H_2SO_4 as solvent. The sample was crushed with spatula. Then the conical flask was connected to air reflux condenser and sample was boiled for about 30 minutes. The flask was slowly being rotated for five minutes and the sample was kept attached to the acid. The mixture then was washed and filtered till non acidic condition is achieved. The left-over then was brought to the flask again and 200 ml of 0.313N NaOH was added. Then the conical flask was connected to air reflux condenser and the boiling process was done for 30 minutes. Ashless filter paper with the digest mixture was removed again and

filtered with new ashless filter paper. The left over was cleaned with hot distilled water until non alkaline condition is achieved. Then the left over was transferred into the crucible and dried at temperature 105 °C until its weight was constant. The weight of crucible with lid and its content was measured after the drying process. The crucible with lid and its content then was transferred into the muffle furnace. The burning process was started at temperature 550 °C until there was no black particles were left.

2.5 Analysis of Trace Metal

Trace metal is analyzed using Inductive Coupled Plasma Mass Spectrometry (ICP-MS). 0.1 g sample was boiled with continuously stirred for 15 minutes at 100 °C in 25ml deionized water for extracting trace metal components. By using Whatman Filter no. 542; the solids were separated to obtain the solution for the sample in 100 ml volumetric flask. Deionized water was added until the 100 ml solution of sample reached. The solution then was filtered using 15 ml syringe with 0.2 µm filter before injected into the instrument.

3.0 RESULTS AND DISCUSSION

3.1 Effects of Media Characteristics

Three types of media were compared to determine the differences between sawdust and empty palm fruit bunch in *pleorotus sajor-caju* cultivation. The periods of various phases of the cultivation of grey oyster mushroom are illustrated in table 1. The most rapid spawn running took place in 25 days with the EPFB followed by 30 days with sawdust and 35 days with combined media (SD +EPFB). Previous research reported that *P. ostreatus* and other mushroom species cultivated on cotton waste took 2-3 weeks for fruit body formation after mycelium complete run [6].

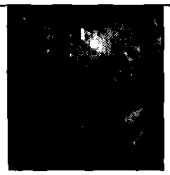
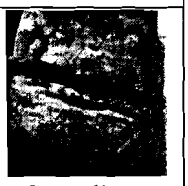
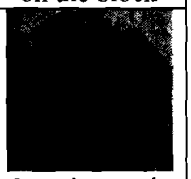
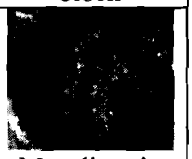
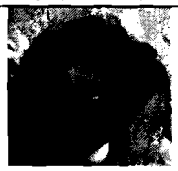
Table 1 Records of the different phases of grey oyster mushroom cultivation and mushrooms yield

Media	Composition of weight (%)	Time for mycelium complete run, (days)	Pinhead formation, (days)	Time for 1st harvest after inoculate seed spawn, (days)	Mushroom yield, (gram)
SD	100	30	57	60	265
EPFB	100	25	85	88	200
SD + EPFB	50: 50	35	-	-	-

Results indicated that using EPFB as media is suitable for cultivation but produced lower mushroom yield compared to sawdust. This result is consistent with the previous report [6, 10]. However, the mixture of (1:1) EPFB and sawdust as media for mushroom growth

surprisingly cannot obtain any yield of mushroom. It is clearly observed that time of fruiting body formation and harvesting for cultivation on sawdust are much shorter than that for EPFB. The physical observation on the sawdust, EPFB and combination of sawdust and EPFB media, and mushroom produced is illustrated in table 2.

Table 2 Physical Observation on the Media and Mushroom

Observation on the media and mushroom			
Media/ Phase	SD	EPFB	SD + EPFB
1	 Mycelium complete run on the block	 Mycelium complete run on the block	 Mycelium complete run on the block
2	 View on the top of block	 Burning peak on the top of block.	 Burning peak on the top of block
3	 Mycelium in the middle of the block.	 Mycelium in the middle of block.	 Mycelium in the middle of the block
4	 Mushroom on the first harvest	 Mushroom on the first harvest	Mushroom fail to bloom on the first time harvesting
5	 Mushroom on the second harvest	 Mushroom on the second harvest	Mushroom fail to bloom on the second time harvesting

Mycelium has performed complete run on the block of the sawdust but much slower in the EPFB, and combination of sawdust and EPFB media (phase 1). In phase 2, it could be observed that a burning-like sign appeared on top of the EPFB media and more severe on the combination media where no mycelium has successfully grown. This indicates unevenness of water absorption and excessively dry zone on top of both media. However, no such mark on the seeding point was observed on the sawdust media where mycelium performed the highest yield complete run.

In phase 3, mycelium did not perform complete run and showed some accumulation in the middle of block for EPFB, and more in the combination media, but completed run was demonstrated in the middle of the block for sawdust media. For that reason, there is no mushroom bloom from the combination media of sawdust and EPFB. At the first time harvesting (phase 4), the quality of mushroom from each media were similar. Nonetheless, the second time harvest (phase 5) showed a large difference in the quality of mushroom. Mushroom growth on the sawdust media was remarkably better than that on the EPFB media which was less bloom, larger individual size but with softer texture. Besides that, mushrooms that grow on the EPFB media bloomed on the side of the media block and the quality of the mushroom is much lower than expected.

3.3 Nutrient Analysis

The nutrient analysis results for N, P, and K from initial content of media, content of media after harvest and the mushroom content from each media are given in figure 1 and figure 2 for sawdust and EPFB media respectively, and table 3. Compositional mixture of sawdust and EPFB cannot be fully analyzed since no mushroom has been produced. The initial content of nutrients show a quite similar form for each type of media. It was calculated that the N, P, and K assimilated into mushrooms accounted for 64%, 49%, and 23% respectively, for the sawdust media and 62.5%, 70.5%, and 21.8 %, respectively, for EPFB media. In addition, any of the N, P and K further loss might be due to the mushroom mineralization process [7].

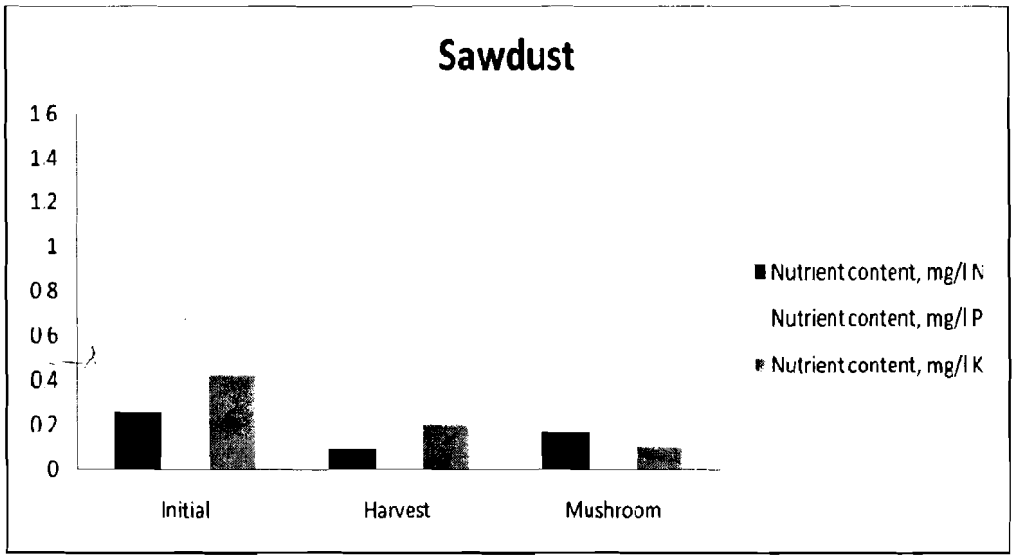


Figure 1 Nutrient Content in Sawdust Media during Initial and Harvest Stage, and in Mushroom on the Harvest.

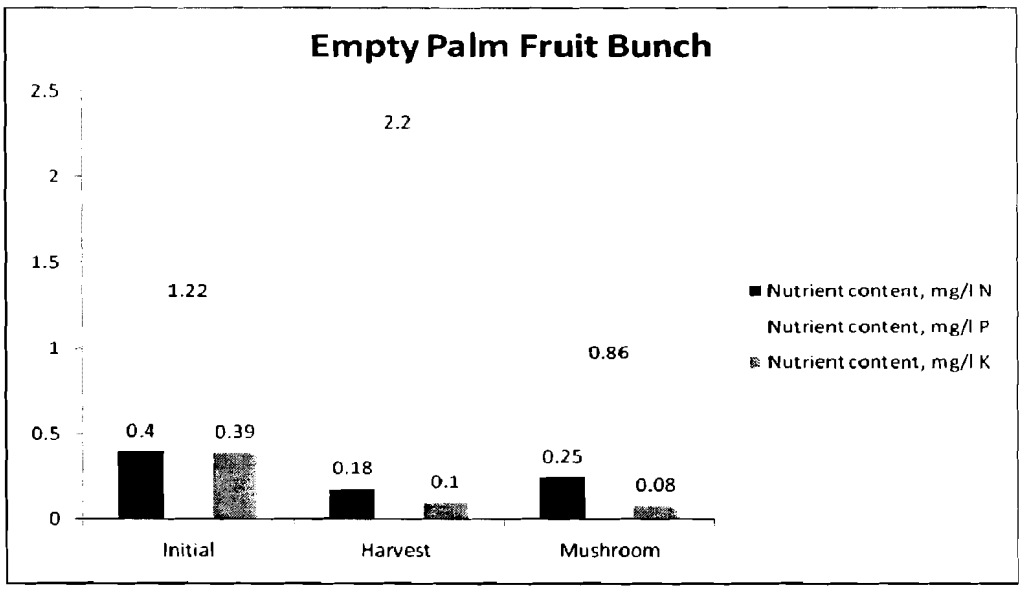


Figure 2 Nutrient Content in Empty Palm Fruit Bunch Media during Initial and Harvest Stage, and in Mushroom on the Harvest.

Table 3 Results of the Nutrient Contents in Media and Mushroom

Media		Nutrient content (mg/l)		
		N	P	K
SD	Initial	0.25	1.52	0.42
	Harvest	0.09	0.97	0.20
	Mushroom	0.16	0.75	0.10
EPFB	Initial	0.40	2.20	0.39
	Harvest	0.18	1.22	0.10
	Mushroom	0.25	0.86	0.08
SD + EPFB	Initial	0.45	1.18	0.23
	Harvest	-	-	-
	Mushroom	-	-	-

Table 4 shows data for moisture content, total fiber and total ash of the media before and after mushroom cultivation and on harvested mushroom. The water loss is in agreement with the increase in mushroom growth and yield. The higher mushroom yield corresponded to the higher water absorption. The water content was partly assimilated into mushroom fruit bodies and partly lost into the atmosphere as carbon dioxide due to the mushroom respiration [8].

Table 4 Results of the Moisture Content, Total Fibre, and Total Ash

Media		%			Weight (g)
		Moisture content	Total fiber	Total ash	
SD	Initial	44.6	21.87	2.42	980
	After 2nd Harvest	40.2	21.73	4.94	700
	Mushroom	86.8	17.29	1.73	265
EPFB	Initial	51.2	23.37	3.91	1000
	After 2nd Harvest	32.0	16.04	7.56	650
	Mushroom	69.7	15.90	5.37	200
SD + EPFB	Initial	43.1	15.04	3.07	1000
	After 2nd Harvest	-	-	-	-
	Mushroom	-	-	-	-

From table 4, it shows that moisture content of EPFB media was higher than sawdust media at initial stage. After second harvest of the mushroom, the moisture content in the EPFB was lower than moisture content of sawdust but less moisture being successfully absorbed by the mushrooms.

Initially EPFB has slightly higher fibre content than sawdust. After harvesting, total fibre content were decreased for both. The total fibre content in EPFB was decreased more than sawdust. Total ash content for both media was increased after harvesting. The moisture content of mushroom from sawdust media is greater than the value in mushroom from EPFB media. Perhaps this is due to the higher initial fiber content of EPFB where the mushroom and fiber matrices in the media competing each other in absorbing the existed water. This could be investigated from the properties of the rate of water absorption by the media [9]. Figure 3 shows the relationship between the weight of EPFB sample and volume of absorbed water as a function of time.

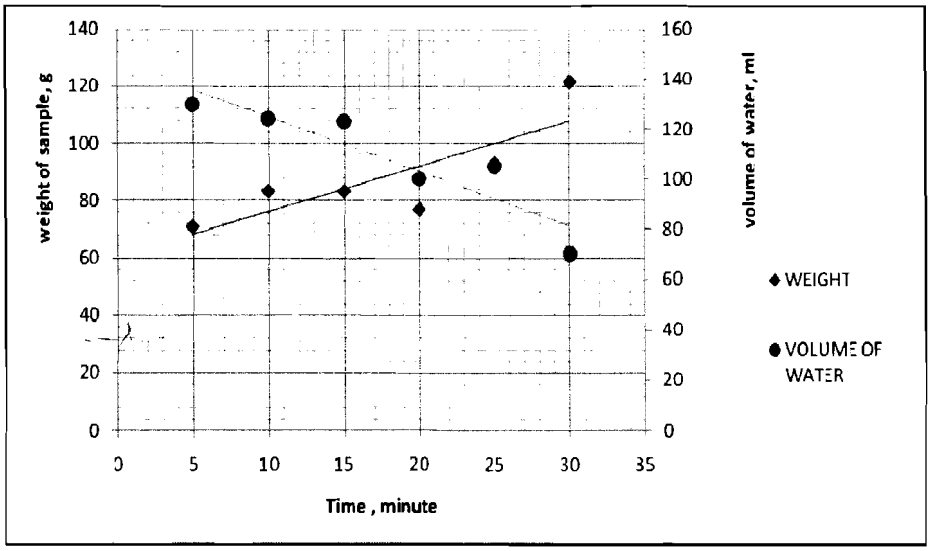


Figure 3 Volume of Water and Weight of EPFB versus Time

From the result, weight of sample was increased by time and the volume of existing water was decreased because EPFB fibrous matrices are highly absorptive towards water, which supports the previous assumption that EPFB was competing with mushroom to absorb water. Sufficient water or moist condition in the cultivation period is a crucial factor to allow the success of complete run of mycelium on the whole media in order to move to the phase of fruiting or mushroom blooming healthily [10].

3.5 Analysis of Trace Metal

From the analysis of trace metal element in media for both samples, it was found that the minimum and maximum values of cadmium concentration in the SD and EPFB media were 0.0038 - 0.0044, and 0.0031 – 0.0039 ppm respectively. The concentration of cadmium is much lower than the limit allowed as reported from literature that is in the range 0.14 – 0.95 ppm [11].

For Plumbum (Pb), the concentration limit as reported from literature is in the range 16.5-33.5 ppm [12]. The maximum and minimum Pb concentrations in the harvested mushroom were 0.0469 and 0.0222 ppm respectively. This result shows that the media and mushroom produced have a much lower concentration of major heavy metals that could be neglected and considered as safe.

4.0 CONCLUSIONS

The sawdust yielded better mushroom production than the EPFB and mixture of sawdust and EPFB. The mushroom grew faster on the sawdust media with their growth cycles being 28 days shorter and yielded about 25% more mushrooms than on the EPFB and combined media SD and EPFB. It can be concluded that EPFB highly fibrous characteristic is a disadvantage as much amount of water is needed because mushroom that grew on the EPFB is competing with the EPFB in absorbing moisture. Moisture content of the mushroom grew on SD is 20 % higher than that on the EPFB under the controlled conditions. The nutrient elements such as N, P, and K in the SD media is higher than in EPFB media. The trace metal analysis shows that the media used and mushrooms produced have very low concentration of heavy metal such as cadmium and plumbum relative to the approved limit.

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