Journal of Engineering Science and Technology
Vol. 12, No. 4 (2017) 1104 - 1110

EFFECT OF USING VARIOUS SUBSTRATES ON CULTIVATION OF *PLEUROTUS SAJOR-CAJU*

S. N. FASEHAH[1,*], A. SHAH[2]

[1]Faculty of Bioscience and Medical Engineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Malaysia
[2]Faculty of Technical and Vocational Education, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia
*Corresponding Author: sitinurulfasehah@gmail.com

Abstract

The unmanageable agricultural waste comprises of structural polymers, cellulose, hemicellulose and lignin can be led to pollutions, thus it can be used as a mushroom substrate. Lignocellulosic materials are most favorable feedstock as renewable and natural resource. Forestry and agricultural practices generated a large amount of ligncelluosic waste and promoted to serious problematic environmental pollution. It can be easily broken down by lignocellulotic enzymes. In this study, an attempt was made to evaluate the effect of various substrates on cultivation of *Pleurotus sajor-caju.* The substrates used in this study were tissue paper, rice husk ash and rubber sawdust. All of the substrates were added with rice bran and calcium carbonate ($CaCO_3$). Then, the mixtures were transferred into plastic sized 8 cm × 4.5 cm and were pasteurized in the steamer for 1 hour at 60 °C - 100 °C. After that they were cooled overnight at 25 °C - 30 °C. The spawn were inoculated into the bag and incubated in incubation room. The media bags were incubated until mycelium fully colonized and watering was done twice a day. The parameters studies were included spawn running, number of fruit body, total of stipe length, weight of fruit body and biological efficiency. Results showed that the fastest spawn running and highest number of fruits body, total of stipe length, weight of fruit body and biological efficiency are found using tissue paper substrates. In contrast the rubber sawdust showed the lowest values of spawn running, total of stipe length, weight of fruit body and biological efficiency. It can be concluded that the tissue paper is one of promising substrate which can be used in growing of *Pleurotus sajor-caju* due to lower cost and easy to purchase as compared to other substrates.

Keywords: *Pleurotus sajor-caju*; Spawn Running; Stipe Length; Weight of fruit

Abbreviations

ANOVA	Analysis of Variance
BERNAS	Padiberas Nasional Berhad
BE	Biological Efficiency
EPFB	Empty Palm Fruit Bunch
SD	Standard Deviation

1. Introduction

Agricultural wastes are increased due to the development of agricultural sector. However, agricultural wastes were discarded without using it. If agricultural wastes are not managed properly, it will cause the pollution of air, water and affect human health. Nevertheless, the uses of wastes are still lacking [1]. Consequently, the idea is generated that the agricultural wastes can be used again for the purpose of making fertilizer, mushroom substrates, animal food, source of biogas energy and furniture. The cheapest way to convert these wastes to nutritious food is by growing mushroom [2].

Agro-wastes comprise of structural polymers, cellulose, hemicellulose and lignin. It can be easily broken down by lignocellulotic enzymes [3]. Recently, there are many types mushroom available in industries and the famous one is *Pleurotus sajor- caju* (one of the *Pleurotus* species). Mushrooms are consumed widely as a source of food and medicine [4]. Oyster mushrooms or *Pleurotus sajor- caju* have very short lateral stalk and oyster-shaped pileus [5]. The oyster mushroom cap is shell-like sized about 5 to 20 cm in diameter and their colour can be yellow, pink, white, cream or dark grey. The most efficient lignin-degrading organisms are *Pleurotus* species. They are one of the most diverse groups of cultivated mushrooms. They show the typical life cycle of Basidiomycetes begins with germination of basidiospore in substrate [3]. Oyster mushroom needs substrates abundant in polysaccharides and lignin for their growth because they have ability to utilize cellulose and hemicelluloses. Several studies have been conducted to evaluate the effect of substrate on cultivation of *Pleurotus* species. Muhammad et al. [6] cultivated *P. sajor-caju* on rubber sawdust, empty palm fruit bunch (EPFB) and combination of sawdust and EPFB in the ratio 1:1 by weight without any supplement. As a result, mushroom grew in sawdust faster than EPFB. Frimpong et al. [7] evaluated rice husk as a possible additive to composted sawdust of *Triplochiton scleroxylon* to determine the biological efficiency (BE) and nutrient content of *Pleurotus ostreatus*. Therefore, Frimpong et al. [7] recommended that rice husk can be used as an additive in the rice growing areas for production of mushrooms. Dehariya and Vyas [8] studied the effect of different types of spawns on oyster mushroom production using three types of substrates conventional (Soybean straw, Wheat straw and Paddy straw) and non-conventional (Domestic wastes, Fruit waste and Used Tea leaves). Spawn run time (mycelia development), pinhead formation, fruit body formation and yield are the parameters in their study. They found that wheat grain spawn produced better results compared to spawn grown on the maize and sorghum for spawn running, pinhead formation, fruit body formation and increased yield. Domestic wastes showed the best substrate in their studied. Alanabeh et al. [9] studied on the use of date palm wastes mixed with other agricultural wastes such as wheat straw, saw dust, and boobialla *Myoporum serratum* leaves. They discovered that wheat straw mixed with date palm and agro-waste at ratio of 25: 75 presented the best results in most of the parameters

measured such as pin appearance, fruiting bodies, biological efficiency, and total yield. Kulshreshtha et al. [10] investigated the cultivation of *Pleurotus citrinopileatus* on the sludge of handmade paper and cardboard industrial waste. They found that the use of combination of sludge and wheat straw able to increase the biological efficiency of mushroom.

Most of the previous studies focus the use of substrate such as rubber sawdust, (EPFB), sawdust of *Triplochiton scleroxylon* on cultivation of *Pleurotus* species. Nevertheless, there is limited report available in the literature uses tissue paper as mushroom substrate. Therefore, it is needed to study the effect of tissue paper on cultivation of *Pleurotus sajor-caju*. The aim of this study is to evaluate the effect of various substrates on cultivation of *Pleurotus sajor-caju*.

2. Materials and Methods

2.1. Substrates preparation

Spawn of mushroom, strain *Pleurotus sajor-caju* and rubber sawdust were obtained from Nutri Agrotech Enterprise, Kelantan. Rice husk ash was purchased from Padiberas Nasional Berhad (BERNAS) in Kelantan. Tissue paper bought from the same supermarket and the same brand. All substrates were dried under the sun until its moisture down to 0-5%. Then, they were cut 1-2 cm long bits [11]. Rice bran as a supplement and calcium carbonate were purchased from Nutri Agrotech Enterprise.

Three different substrates were prepared such as tissue paper, rice husk ash and rubber sawdust. Control media was prepared (rice bran and $CaCO_3$) in the total weight of 111 g. All of the substrates were added with rice bran and calcium carbonate ($CaCO_3$). Substrates, rice bran and $CaCO_3$ were mixed in ratio 100:10:1 by weight [12]. The pH tested and should be between 5 and 6.5 [5].About 100 grams of substrate, 10 g of rice bran and 1 g of calcium carbonate were used. Then, they were mix together meanwhile the water was added until the moisture raise up to 75%-80% into the mixture [13]. Then, the mixtures were transferred into plastic sized 8 cm × 4.5 cm. The substrates were compressed until the height reaches 10 cm. The plastic cap and neck were put on the plastic filled with mixed substrates or media bag. The sponge was put into the cap hole. This experiment was conducted in three replications.

2.2. Pasteurization and incubation

All the media bags were pasteurized in the steamer for 1 hour at 60 °C - 100 °C. After steamed them, they were cooled overnight at 25 °C - 30 °C [5]. The spawn were inoculated into the bag about 16% of substrate's dry weight that is 16 g [14]. After that, all media bags were incubated in incubation room. The incubation process was done at room temperature that is (27±2) °C in a room with sufficient light about 1 lux - 5 lux [11]. The media bags were incubated until mycelium fully colonized. Watering was done twice a day. The days of spawn running to fully colonize the media bag of all substrates were observed. When the mushrooms or fruit bodies fully developed, they were harvested. After that, number of fruit bodies and total stipe length were calculated. Then, weight of fruit body was measured using weighing scale and Biological Efficiency (BE) was calculated as fresh weight of mushroom (g) / dry weight of substrate x 100.

2.3. Data Analysis

All the data obtained were subjected to one way analysis of variance (ANOVA) by using software PASW Statistics 18**.**

3. Results and Discussion

Mushroom (*P.sajor-caju*) was successfully grown using various types of substrates. The use of supplement can enhance the mushroom production due to low of protein content and need various minerals such as phosphorus, potassium, and nitrogen in lignocellulosic materials [15]. Table 1 shows the effect of various substrates on *P.sajor-caju* growth characters. The growth characters include spawn running, number of fruits body, total of stipe length, weight of fruits body and biological efficiency. It was clearly showed that the use of different types of substrates significantly effects on mycelial growth. It was observed that the tissue substrate provides the shortest day to complete the spawn running (12 days). The spawn running of tissue is about two folds shortest as compared to control (26 days). Dehariya and Vyas [8] reported that the quickest spawn running can be found in soybean straw substrate which took about 17 days to complete spawn running compared. Thus, their results were longest than our finding that took only 12 days for spawn running. This result was found better than that of previous attempted using 100 % of rice husk which took 20 days to complete mycelial growth. Mycelial were colonized the media bags during incubation process. It is believed that the variation of spawn running is due to biochemical changes takes place during development of mycelial which convert or degrade large component of lignocellulosic materials into low molecular weight compound by extracellular enzyme [16]. The effect of various substrates on number of fruits body collected at the first batch is summarized in Table 1. It was noted that the number of fruits body increased using tissue substrate (3.67) as compared to rice husk ash (1.33) which was the lowest number of fruits body. The number of fruits body not depends on spawn running as can be seen on rice husk ash and rubber sawdust substrate. The spawn running of rice husk ash was faster than rubber sawdust but the number of fruits body for rice husk ash lower as compared to rubber sawdust. The fruiting bodies (mushrooms) of the *Pleurotus* genus are valued for their taste and high nutritional value. They have big amounts of high quality protein, essential amino acids, various vitamins and minerals and can contribute to the intake of vitamins B1 and B2 [17]. In addition, fruiting bodies of mushrooms that grow in different waste are varied in protein content and other nutrients [18]. Oyetayo and Ariyo [19] found high carbohydrates and fiber in fruit bodies of *Pleurotus* sp. The decomposition of total carbohydrate, cellulose, hemicellulose, and fiber during inoculation stage promoted to high protein content in fruit bodies of mushrooms [20].

The total of stipe length, weight of fruits body and biological efficiency are shown in Table 1. It was observed that the highest of total stipe length, weight of fruit body and biological efficiency can be found on tissue substrate while the lowest was rubber sawdust substrate. These different characteristics of mushrooms were explained by [19] that the chemical, functional and organoleptic characteristics of mushrooms affected by substrate used. Spawn running influenced the stipe length, weight of fruit body and biological efficiency. The shorter days to complete spawn running can lead to increase of stipe length, weight of fruit body and biological efficiency. The increasing of growth characters of mushroom is might due to the rapid growth and colonization of mycelia [21]. It is believed that more

production of enzyme such as hemicellulases, cellulose and ligninase during the colonization of mycelial causes the rapid spawn running.

This statement was supported by [16] who had reported that the increasing of enzyme production can increase biodegradation of lignocellulosic waste thus increase development of mushroom mycelial. The lower of growth character of mushroom is due the excess nitrogen, which can slow down the growth of mushroom in excessive amount. Table 1 also shows the standard deviation (SD) of the effect of different substrate on *P.Sajor-caju* characters. It can be seen that the value of SD for all data was smaller indicating that the result of these experiment consistence.

Table 1. Effect Substrate on *P.sajor-caju* growth characters.

Substrate/ Treatment	Spawn running (days)	Number of Fruit Body	Total of Stipe Length (cm)	Weight of Fruit Body (g)	Biological Efficiency (%)
Tissue	12.00±1.00	3.67±1.00	25.30±3.58	71.3±3.48	62.79±2.54
Rice husk Ash	16.00±1.00	1.33±1.15	8.83±2.60	51.17±4.86	46.10±4.38
Rubber Sawdust	18.67±3.00	1.67±1.15	5.33±0.76	42.83±3.33	38.60±3.00
[control]	26.00±1.00	1.00±0.00	3.40±2.13	28.43±2.00	25.19±2.03

Values in the table show the mean. T indicated treatment.

Table 2 exhibits ANOVA for various substrates. The ANOVA table was used in these studies to analyse the significant of data or results. Results showed that all the response variables have significant level which the significant value is ($p \leq 0.05$).

Table 2. One way ANOVA for effect of various substrates on *P.sajor-caju* growth characters.

Growth characteristic		Sum of Squares	df	Mean Square	F	Sig.
Spawn Running (days)	Between Groups	313.000	3	104.333	78.250	.000
	Within Groups	10.667	8	1.333		
	Total	323.667	11			
Number of Fruit Body	Between Groups	12.917	3	4.306	5.741	.022
	Within Groups	6.000	8	.750		
	Total	18.917	11			
Total Stipe Length	Between Groups	896.203	3	298.734	48.424	.000
	Within Groups	49.353	8	6.169		
	Total	945.557	11			
Total Weight of Fruit Body	Between Groups	2885.147	3	961.716	75.711	.000
	Within Groups	101.620	8	12.703		
	Total	2986.767	11			
Biological Efficiency	Between Groups	2212.456	3	737.485	76.157	.000
	Within Groups	77.470	8	9.684		

($p \leq 0.05$) indicates that the data was significantly influence growth characteristic of *Pleurotus sajor-caju.*

4. Conclusions

Mushroom (*P.sajor-caju*) was grown successfully using various types of substrates. The effect of various substrates on *P.sajor-caju* cultivation has been analyzed. Results showed that tissues significantly increases the number of fruit body, total of strip length, weight of fruits body, biological efficiency and speed up the mycelia growth as compared to others substrates. On the other hand, rubber sawdust exhibited the lowest value for total of strip length, weight of fruits body, biological efficiency and longer time taken for spawn running. Further research is needed to look into the experimental on nutrient content and chemical composition in all of these substrates.

Acknowledgment

Authors would like to express the highest gratitude to Nutri Agrotech Enterprise for the supplying of their oyster mushroom spawn and Faculty of Agro Based Industry, UMK for funding and providing their facilities for this research respectively**.**

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