EVALUATION OF AGRICULTURAL AND FOOD WASTES AS ALTERNATIVE SUBSTRATE COMPONENTS FOR CULTIVATION OF WHITE OYSTER MUSHROOM *Pleurotus floridanus* SINGER

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KUALA LUMPUR

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EVALUATION OF AGRICULTURAL AND FOOD WASTES AS ALTERNATIVE SUBSTRATE COMPONENTS FOR CULTIVATION OF WHITE OYSTER MUSHROOM *Pleurotus floridanus* SINGER

ABSTRACT

The demand for oyster mushrooms in Malaysia is increasing due to the health benefits. However, there are some challenges and obstacles faced by the mushroom industry including lack of supply and increasing price of raw materials which greatly burden the mushroom growers. Thus, mushroom growers are seeking alternative lignocellulosic substrates to reduce the cost of production. The objectives of this study were to optimise the substrate formulation, to evaluate the growth performance and production yield as well as to analyse the nutrient profile of *Pleurotus floridanus* (strains PF1 and PF2) basidiocarps grown in the standard substrate supplemented with coffee waste (CW), food waste compost (FWC) and empty fruit bunch compost (EFBC) of varying concentrations (10%, 20%, 30% and 40% w/w). In order to select the suitable concentration of these three supplements, linear growth study were conducted. Strain PF1 displayed variable growth rate and mycelium density on each of the waste formulations. The selected CW, FWC and EFBC used for cultivation of PF1 strain were10% (w/w) at 0.37 ± 0.02 cm/day, 10% (w/w) (0.52 ± 0.02 cm/day) and 20% (w/w) (0.61 ± 0.02 cm/day) respectively. Meanwhile for PF2 strain, the most suitable concentrations of standard substrate supplemented with CW, FWC and EFBC were 10% (0.38 ± 0.02 cm/day), 20% (0.52 ± 0.02 cm/day) and 30% (0.65 ± 0.02 cm/day), respectively. In yield of fruiting bodies, the highest yield per bag obtained was PF1 strain grown in standard substrate supplemented with 10% (w/w) FWC (83.37 ± 24.25g), while the lowest production recorded in PF1 strain grown in substrate supplemented with standard substrate (79.26 ± 14.16).PF2 strain produced the highest of77.62 ± 34.23g yield per bag in standard substrate and the lowest

yield of 52.93 ± 25.53g in standard substrate supplemented with 30% (w/w) EFBC. In terms of number of harvests per cycle, PF1 fruiting bodies were able to harvest until 4th harvests. Fruiting bodies of PF1 strain were selected for nutrient profiling compared to fruiting bodies of PF2 strain, as it had the fastest spawn run time, produced more harvest cycles, obtained higher yield and BE as well as zero contamination rate in bags. Total sugar content and beta glucan content (75.12 ± 0.03 mg/g and 33.19%, respectively) were found the highest in the fruiting bodies grown on standard substrate while total phenolic content at 21.99 ± 0.01 mg/g and total protein content (200 ± 0.01 mg/g) were found the highest in standard substrate supplemented with CW and FWC, respectively. The findings from this study showed that these wastes can be considered as supplements in standard substrates for *P. floridanus* cultivation.

Keywords: coffee waste, food waste compost, empty fruit bunch compost, nutrient analysis, *Pleurotus floridanus*

PENILAIAN SISA PERTANIAN DAN MAKANAN KOMPOS SEBAGAI KOMPONEN SUBSTRAT ALTERNATIF DALAM PENANAMAN *Pleurotus floridanus* SINGER

ABSTRAK

Permintaan penghasilan cendawan tiram di Malaysia semakin meningkat kerana kebaikannya terhadap kesihatan. Walaubagaimanapun, terdapat kekangan yang dihadapi oleh industry cendawan termasuklah kekurangan sumber dan peningkatan harga pasaran bahan mentah yang amat membebankan pengusaha cendawan. Oleh itu, pengusaha cendawan mencari substrat alternative untuk mengurangkan harga penghasilan. Objektif kajian ini ialah untuk mengoptimumkan formulasi substrat, mengetahui kadar pertumbuhan dan penghasilan jana buah serta menganalisa kandungan nutrient dalam jana buah. Kajian dijalankan untuk menanam cendawan *Pleurotus floridanus* (PF1dan PF2) dengan menggunakan substrat ditambah dengan sisa kopi (CW), kompos sisa makanan (FWC) dan kompos tandan kosong (EFBC). Empat berlainan kepekatan CW, FWC dan EFBC digunakan iaitu 10%, 20%, 30% dan 40% dalam kajian ini. Untuk memilih kepekatan sisa yang sesuai, kajian pertumbuhan linear dijalankan. PF1 menunjukkan kepelbagaian dari segi kadar pertumbuhan miselia dan ketebalan miselia dalam setiap satu formulasi sisa. Kepekatan sisa CW, FWC dan EFBC yang sesuai digunakan untuk PF1 ialah 10% (w/w) at 0.37 ± 0.02cm/ hari, 10% (w/w) (0.52 ± 0.02 cm/ hari) dan 20% (w/w) (0.61 ± 0.02 cm/ hari). Selain itu, untuk PF2, kepekatan sisa CW, FWC dan EFBC yang amat sesuai ialah10% (0.38 ± 0.02 cm/ hari), 20% (0.52 ± 0.02 cm/ hari) and 30% (0.65 ± 0.02 cm/ hari). Selain itu, dari segi penghasilan jana buah, jumlah penghasilan terbanyak PF1 ialah dalam substrat ditambah dengan 10% FWC (83.37 ± 24.25g) dan jumlah penghasilan yang sedikit dimiliki oleh control iaitu 79.26 ± 14.16g. PF2 mengeluarkan jumlah penghasilan yang paling maksimum ialah control

(77.62 ± 34.23g) dan paling minimum dalam substrat ditambah dengan 30% EFBC (52.93 ±25.53g). Jana buah PF1 berjaya melalui proses penuaian selama 4 kali. Oleh disebabkan mempunyai masa kolonisasi substrat yang singkat, pengeluaran bilangan jana buah tertinggi dan tiada beg kontaminasi direkodkan, jana buah PF1 dipilih berbanding dengan jana buah PF2 untuk menjalankan kajian menganalisa kandungan nutrien. Jumlah kandungan gula dan beta glucan (75.12 ± 0.03 mg/g dan 33.19%) tertinggi dijumpai dalam jana buah yang tumbuh dalam control manakala jumlah kandungan phenolic (21.99 ± 0.01 mg/g) dan kandungan protein ((200 ± 0.01 mg/g) masing-masing direkodkan tertinggi dalam jana buah yang tumbuh dalam substrat ditambah dengan CW dan FWC. Daripada kajian ini, boleh dirumuskan bahawa penggunaan sisa-sisa ini dalam substrat boleh digunakan untuk penananam *P. floridanus.*

Kata kunci : sisa kopi, kompos sisa makanan, kompos tandan kosong, analisa nutrien, *Pleurotus floridanus*

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LIST OF SYMBOLS AND ABBREVIATIONS

BE	:	Biological Efficiency
Cd	:	Cadmium
$CaCO_3$	:	Calcium Carbonate
C/N	:	Carbon to Nitrogen ratio
CW	:	Coffee Waste
Cu	:	Copper
EFBC	:	Empty Fruit Bunch Compost
FFB	:	Fresh Fruit Bunch
FWC	:	Food Waste Compost
Pb	:	Lead
Mg	:	Magnesium
Ni	:	Nickel
NA	:	Not Available
OM	:	Organic Matter
POME	:	Palm Oil Mill Effluent
P	:	Phosphorus
K	:	Potassium
PDA	:	Potato Dextrose Agar
Na	:	Sodium
T-C	:	Total Carbon
T-N	:	Total Nitrogen
w/w	:	Weight per weight
Zn	:	Zinc

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CHAPTER 1: INTRODUCTION

1.1 Introduction

Pleurotus genus is the second most popular edible mushroom in world mushroom market (Bellettini et al., 2019). This genus is usually called as 'Dhengri' in India due to the shape which looks like an oyster (Ahmed et al., 2009). *Pleurotus* is a white rot fungus and is usually cultivated on non-composted lignocellulosic substrates. Besides that, this they are well-known in decomposing organic matter by degrading hemicellulose, cellulose and lignin in wood. Mushroom production using agro wastes as substrates and converts these wastes to value added products. Hence, oyster mushroom cultivation is an effective alternative compared to recycling, which can lower environmental effects due to the organic waste disposal problem (Souza et al., 2016).

Oyster mushroom production in Malaysia is receiving attention in conjunction to the demand it receives from local due to the health benefits. However, there are some challenges and obstacles faced by the mushroom industry include insufficient supply and ever increasing price of raw materials like rice bran and sawdust which burden the mushroom growers (Mohd Zaffrie et al., 2014; Rosmiza et al., 2016). Thus, mushroom growers are searching for alternative ways to reduce the cost of their production by supplementation of mushroom substrates with alternative biomass/wastes (Royse, 2010). Besides that, structure and types of wastes used as supplements in substrate are vital parameter for the mycelium growth since it provides mycelium a conducive medium to grow (Tripathy et al., 2009) and develop into fruiting bodies (Subbu Lakshmi & Sornaraj, 2014). Food waste and agricultural industry by-products such as empty fruit bunch (EFB) have become a serious issue globally and researchers have reported the potential of using food waste and EFB as alternative substrates for mushroom growing (Chae & Ahn, 2013; Kavitha et al., 2013).

Malaysia is generating large amounts of food waste at an alarming rate. In order to curb the problem, one of the ways is by recycling food waste and turn them into compost or fertilizer. Food waste compost (FWC) is rich in nutrients and low in toxicity, it can be fed to heterotrophic organisms like mushrooms as substrate (Stoknes et al., 2008). In other words, mushroom cultivation can be a good management for organic waste to overcome the disposal problem (Das & Mukherjee, 2007). Among the wastes, coffee and oil palm wastes are lignocellulosic in nature and may be explored as alternate substrates for mushroom growing.

Exporting and importing of coffee among countries have been actively carried out, made coffee as the second biggest trade in the world (Nabais et al., 2007). Therefore, increase in coffee consumption contributed to huge quantities of waste (Fernandes et al., 2017). Due to the presence of tannin and caffeine, coffee wastes are not properly used, and their disposal faced huge environmental disturbance. Among the ways that can reduce coffee waste (CW) is by utilising CW in mushroom cultivation as cheaper substrate (Murthy & Manonmani, 2008).

Malaysia is one of the biggest producer of oil palm in the world, regarded for about 36% of the total world palm oil production in 2011 (Sharma et al., 2012). Large scale oil palm productions contributes to a large amount of residues especially empty fruit bunches (EFB) (Ahmad Yahaya et al., 2017). The usual practice of EFB disposal is either by applying to the field or by being incineration. Since EFB is categorised under agricultural by-products and contain cellulose, hemicellulose and lignin, it can be used to cultivate mushroom (Amal Nafissa et al., 2008; Kavitha et al., 2013).

1.2 Significance of Research

Standard substrate or also known as control formulation (consist of 89% (w/w) SD, 10% (w/w) RB and 1% (w/w) $CaCO_3$) supplemented with CW, FWC and EFBC for *P. floridanus* cultivation can further enable mushroom farmers to reduce their cost production and increase their profit. At the same time, recycling the wastes as additives can be helpful in eradicating waste disposal problems.

1.3 Objectives of Study

The main objectives of this study were:

a) to optimise the substrate formulations of standard substrate supplemented with varying concentrations of CW, FWC and EFBC for growth of *P. floridanus*.

b) to evaluate the growth performance and production yield of *P. floridanus* in the substrate supplemented with CW, FWC and EFBC.

c) to analyse the nutrient profiles of *P. floridanus* fruiting bodies grown on substrate supplemented with CW, FWC and EFBC.

CHAPTER 2 : LITERATURE REVIEW

2.1 Edible Mushroom

Mushroom has been regarded as food and it is a largely consumed delicacy since centuries due to the taste and flavour (Das et al., 2012). It is even considered as meat for vegans. Mushroom are rich in macronutrients and micronutrients such as protein, fibre, minerals, vitamins (Guadarrama-Mendoza et al., 2014). Mushroom is well-known for having bioactive compounds like phenolic compounds, steroids, polysaccharide and terpenes (Bellettini et al., 2019). Besides that, high amount of beta glucan, secondary metabolites and chitin found in mushrooms reported to exhibit medicinal properties such as antioxidant, antidiabetic, anticancer, antiobesity and cholesterol reducer (Agricultural Food Development Authority, 2013; Valverde et al., 2015).

According to Kalac (2013), there are more than 200 mushroom species are edible but only 35 species have been grown commercially. Asia is the main mushroom producer contributed to the world mushroom market where China is leading in exporting mushroom with 40% contribution to the total world mushroom production (Rosmiza et al., 2016).

2.1.1 Mushroom Production in Malaysia

Malaysia is producing approximately 1000 tonnes yearly for local and exports purposes (Mohd Zaffrie et al., 2014). Since the demand of mushroom industry in Malaysia is getting higher due to the awareness towards the health benefits as well as a tasty dish, small-scale mushroom growers considered mushroom cultivation as profit-making industry. Climate condition in Malaysia is very conducive for the growth of 17 different types of mushroom with only eight are commercially cultivated which are *Auricularia polytricha, Ganoderma applanatum, Lentinula edodes, Volvariella volvacea, Pleurotus cytidiosus, P. pulmonarius, P. floridanus* and *P. flabellatus* (Mohd Zaffrie et al., 2014).

2.2 *Pleurotus* spp.

Mushroom species under the *Pleurotus* genus is the second most cultivated around the world (Barshteyn & Krupodorova, 2016). Miles &Cheng (1997) stated that all *Pleurotus* or oyster mushrooms in general are having morphological characteristics such as decurrent gills, usually seen with or without stipe and possessed white spore print. *Pleurotus* is extensively cultivated and studied due to its ability and adaptation to survive and grow at various temperatures and climate as well as on broad range of substrates (Adebayo & Martinez-Carrera, 2015).

2.2.1 Origin of *Pleurotus floridanus*

Origin of this mushroom species can be dated back to 1958 where majority of this mushroom strains from wild specimens were cultivated by S.S Block of Gainesville, Florida. Hence, the name of Florida was given to this species where it was first collected (Stamets & Chilton, 1983). A scientist named Eger did comparison of *P. floridanus* strains with *P. ostreatus.* He stated that both strains have similarity in many ways like colour, smell, taste, and physical appearance (Stamets & Chilton, 1983) as well as similar in terms of cross fertile spores, production of fertile basidiomes from mating and capable of forming clamp connections (Li & Eger, 1978 as cited by Kashangura, 2008).

Figure 2.1: *P. floridanus* PF1 fruiting bodies grown in mushroom house at Mushroom Research Center, University of Malaya. 5 October 2018

2.2.2 Cultivation of *P. floridanus* using Agricultural Wastes.

Mushroom cultivation using agricultural wastes as substrates is a value-added process where it converts the waste into useful products (Tripathy et al., 2009). *Pleurotus floridanus* or white oyster mushroom is an excellent lignin-decomposing organism, where it produces ligninolytic enzymes such as manganese peroxidases (MnP), laccase and lignin peroxidase (LiP) (Adebayo et al., 2012; Isroi et al., 2012).

To date, *P. floridanus* has been reported to be cultivated on different agricultural wastes (Table 2.1).

Table 2.1: Wastes that has been reported as substrates supplements for *P. floridanus* cultivation.

Wastes	References
Fruit peel (pineapple peel)	(Souza et al., 2016)
Agricultural wastes - palm fruit shaft (*Elaeisguineensis)*, plantain leaves (*Musa paradisiaca*) and kenaf stem (*Hibiscus cannabinus*)	(Adedokun & George-David, 2016)
Oil palm empty fruit bunch (OPEFB)	(Isroi et al., 2012)
Combinations of wheat straw, paddy straw and wheat bran	(Zaman et al., 2017)
Combinations of wheat straw and oyster shell powder.	(Naraian et al., 2014)

2.3 Substrate for Mushroom Production

The usage of substrates in mushroom production is also known as food which aid in mushroom growing (Kashangura, 2008). Oyster mushroom in the wild, grow on woods. But they can thrive on a wider range of materials such as seed hulls, paper and pulp by-products, paddy straw, cereal straw, coffee wastes and many more (Stamets, 2011). Oyster mushrooms gets their nutrients from the substrate they are growing on. Adequate nutrients are taken by the mushroom through mycelium. Therefore, substrates play important role in terms of functional and chemical characteristics of mushroom (Bellettini et al., 2019). Preparation of substrate is important cost input. The selection of substrates are mainly based on the quantity and cost of the substrate. In order to gain more profit, the cost of production should be reduced and the increase the rate of production (Atila, 2018). There are large quantities of agricultural wastes and by-products remain after their harvesting and main processing. These lignocellulosic wastes are easily and readily available with low cost, which can be utilized in mushroom production (Ishak et al., 2018). Over the years, many studies on the usage of lignocellulosic wastes for mushroom cultivation have been conducted. Among them are utilization of barley straw, wastepaper, wheat straw, and sinar straw in *P.* ostreatus cultivation (Tesfaw et al., 2015). Iqbal et al. (2016), used wheat straw, sugarcane bagasse, rice straw, maize straw and sorghum straw in cultivation of *P. florida* mushroom where the shortest spawn run and the maximum biological efficiency were found in wheat straw. Besides that, Girmay et al. (2016), conducted a study on *P. ostreatus* cultivation using four agricultural wastes namely cotton seed, paper waste wheat straw and sawdust. The result obtained was the oyster mushroom that grown on cotton seed observed to have the highest yield and biological efficiency. Moreover, several studies have been reported using agricultural by-products in oyster mushroom cultivation, especially *P. floridanus* (Table 2.1).

Another factor that enhances the utilization of other available lignocellulosic waste in mushroom cultivation is scarcity of sawdust. Sawdust is used as a sole basal substrate for mushroom cultivation in most countries like Indonesia (Rizki & Tamai, 2011) and Malaysia. Currently, sawdust is widely utilized and is the most favourable substrate to be used in mushroom cultivation commercially (Pathmashini et al., 2008). Therefore, the large amount of sawdust usage leads to loss of wooded areas (Rizki & Tamai, 2011) and thus increase the demand of sawdust supply (Harith et al., 2014). High demands in sawdust affected the increment of cost in sawdust supply, which could burden the mushroom growers especially the small scale growers. Besides that, the researchers further stated that impure contents in sawdust like chemicals addition during processing of sawdust might affect the mushroom production in terms of yield loss and high contamination (Harith et al., 2014). Hence, the sawdust supplies shortages have diverted the researches' attention to the potential uses of lignocellulosic wastes as supplements in mushroom cultivation (Rizki & Tamai, 2011).

2.4 Agricultural by-Products and Food Waste Compost

Agro based industries produced huge amount of waste which contained lignocellulosic materials. Lignocellulosic materials is a major environmental concern as the disposal method includes incineration or apply to the fields leads to pollution problems as well as harmful to human beings. In recent years, lignocellulosic materials have been given undivided attention due to the renewable properties (Adebayo &Martin-Carrera, 2015). Utilization of these lignocellulosic wastes in mushroom cultivation is one of the way to convert these wastes into value added materials (Tesfaw et al., 2015; Hoa et al., 2015).

2.4.1 Empty Fruit Bunch

Malaysia is one of the biggest palm oil producers in the world. According to Sharma et al. (2012), Malaysia produced palm oil in estimation of 37% from the total world palm oil production in 2011. Besides that, Ng and Ng (2013) as cited by Abdulrazik et al. (2017) mentioned that five million hectares of palm oil plantation had generated 93 million tonnes of palm oil fruits.

Palm oil fruits or known as fresh fruit bunches (FFB) that has been harvested, are processed in palm oil mill process. FFBs underwent sterilization then went through a stripper to remove the fruits and produced empty fruit bunches (EFB) (Ng & Ng, 2013). Next, the fruits are screwed pressed before transferred to clarification tank and purification plant in order to extract oil from the fruit and produce crude palm oil. Then pressed cake that is generated from screw press process went through the nut/ fiber separator to separate the palm mesocarp fiber. The nuts are cracked by the nut cracker to remove the palm kernel shells. The crude palm kernel oil are produced by crushing the remaining palm kernels. Waste or by-products obtained at the end of the process are EFB, palm oil mill effluent (POME), mesocarp fiber and palm kernel shell (Ng & Ng, 2013) (Figure 2.2).

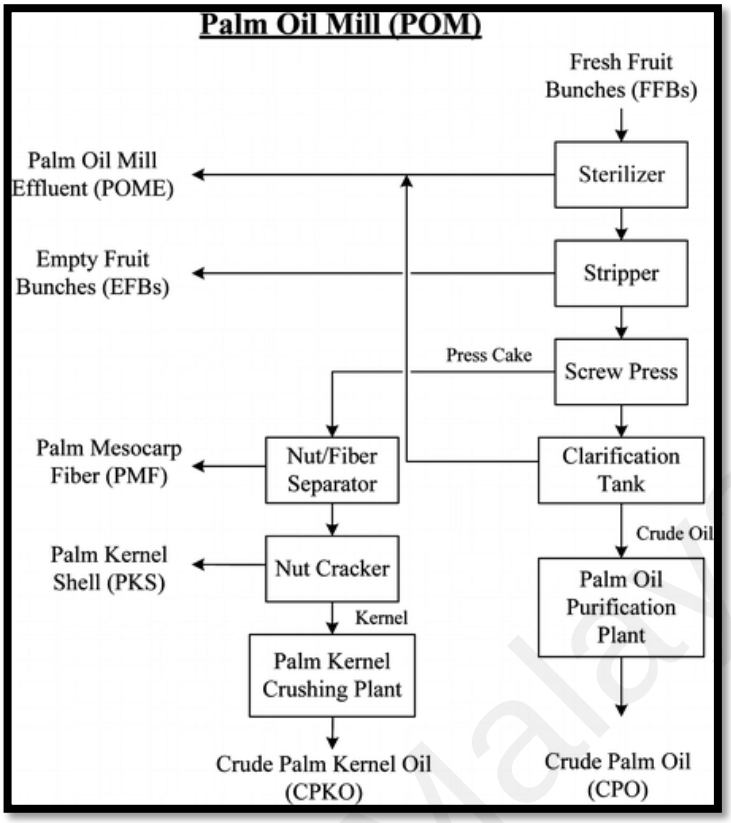


Figure 2.2: Flowchart of fresh fruit bunches in palm oil mill process (Cited from Ng & Ng, 2013)

Crude palm kernel oil and crude palm oil can be considered as raw materials for biodiesel and basic oleochemicals productions (Rupilius & Ahmad, 2007). Generally, palm fibers and kernel shell are utilised as fuel to produce electricity and steam to the mills while EBFs and POME are regarded as unwanted wastes. According to Ng & Ng (2013), for every 1000kg of FFBs processed, approximately 23% or 230kg of EFBs are generated. The EFBs wastes are either incinerated or applied to the field which can impose major threat to environment in terms of pollution (Kavitha et al., 2013). One of the methods to reduce the wastes is by converting them into useful products. EFBs consists of lignocellulosic components which could utilize for mushroom cultivation. Furthermore, EFB do not possess wood component called sap which could inhibit the growth of mushroom (Lin Marlina et al., 2015).

2.4.2 Food Waste Compost

Malaysia is generating large amount of food waste at alarming rate. According to The Star Online (2016), Malaysians generates approximately 15000 tonnes of food waste per day, where about 3000 tonnes of food waste are still edible. Since food waste are considered part of municipal solid waste and does not have specific management system for food waste in Malaysia (Azlina et al., 2012), party in-charge of waste management are facing difficulties in managing food waste wisely. In addition, food wastes generally consist of rich in moisture content and organic content. If the food wastes are not treated wisely, they can be rotten easily and produce unpleasant smell (Kim & Kim, 2010). If this situation left untreated, food waste can produce greenhouse gases which can lead to adverse effects towards climate change (Lim et al., 2016).

In order to curb the problem, one of the ways is by recycling and composting the food waste. Composting are the only way to break down solid organic waste to get an end product known as compost (Nur Fatin Mat Saat, 2014) which can be executed to land without having any side effects to the surrounding (Siti Noratifah et al., 2017).Korea is one of the countries that actively involved in food waste composting due to the limited area of landfill site and also prevention of groundwater and soil from leachate (Kim & Kim, 2010).

Eventhough food waste composting in Malaysia is still at infancy, Zero Waste Campaign implemented by University Malaya has been running successfully in minimizing food waste and the wastes are turned into useful compost or fertilizer (The Star Online, 2015). Food waste compost (FWC) is highly rich in nutrients (Table 2.2) and low in toxicity which can be fed to heterotrophic organisms like mushrooms (Stoknes et al., 2008). Mushroom cultivation can be a good management to overcome the disposal of organic waste (Das & Mukherjee, 2007).

Utilisation of FWC in mushroom cultivation was first reported by Block (1965) by utilising garbage mixtures of newspaper and vegetable wastes *Agaricus campestris.* Since then, a number of food wastes such as seafood processing wastes (Subbu Lakshmi, 2013; Subbu Lakshmi & Sornaraj, 2014), anaerobically digested food wastes (Stoknes et al., 2013) and aerobic food waste composting (Chae & Ahn, 2013; Jo et al., 2013) have been utilised for mushroom cultivation.

2.4.3 Coffee Waste

Coffee is one of the most popularly consumed beverages throughout the world. Coffee trading among countries have been actively carried out, made coffee as the second biggest trade in the world (Nabais et al., 2007). Affinity towards coffee has mushroomed with the presence of Starbucks and other coffee outlets. Hence, increase in coffee consumption contributed to huge quantities of waste (Fernandes et al., 2017). Wastes like coffee grounds, coffee cherry wastes, coffee parchment wastes and coffee silver skin are generated from harvesting stage to coffee consumption (Murthy &Manonmani, 2008). Cruz et al. (2012) mentioned that coffee ground residue is the byproduct of brewing process. Due to the presence of toxic compounds like tannin and caffeine, coffee wastes are not properly used and the disposal faced huge environmental disturbance. Organic compounds like lignin, hemicellulose and cellulose (Table 2.2) are present in larger quantities in coffeewaste (Campos-Vega et al., 2015). Therefore, utilisation of CW in mushroom production can lower the toxic characteristics as well as be one of a potential alternative substrate for mushroom cultivation (Murthy &Manonmani, 2008).

Table 2.2: Proximate chemical composition of various wastes from different studies

Parameter	Content			
	Sawdust [1]	CW [2]	FWC [1]	EFBC
pH	6.9 ± 0.1	6.1 3	7.4 ± 0.1	8.12 ± 0.8[6]
Water Content (%)	15.9 ± 0.2	NA	19.5 ± 0.1	51.8 ± 3.7[6]
OM (%)	80.3 ± 0.1	98 ± 1.5 [3]	68.3 ± 0.6	NA
T-C (%)	46.6	44 [3]	39.6	28.81 ± 3.3[6]
T-N (%)	0.08 ± 0.01	2.79 ± 0.10	3.10 ± 0.21	2.31 ± 0.08[6]
C/N ratio	615.9	16.91 ± 0.10	12.8	12.4[6]
P (g/kg)	0.27 ± 0.01	1.8 ± 0.00	2.68 ± 0.02	1.36 ± 0.5%[6]
K (g/kg)	0.34 ± 0.02	11.7 ± 0.01	0.90 ± 0.01	2.84 ± 0.6%[6]
Ca (g/kg)	3.26 ± 0.21	1.2 ± 0.00	25.22 ± 0.25	1.04 ± 0.3%[6]
Mg (g/kg)	0.33 ± 0.03	1.9 ± 0.00	1.58 ± 0.02	0.90 ± 0.1%[6]
Na (g/kg)	NA	1.1 ± 0.00 [3]	6.05 ± 0.01	11.0 ± 0.4 [4]
Pb (mg/kg)	NA	<1.60	NA	NA
Zn (mg/kg)	12.4 ± 6.9	8.40 ± 0.20	32.7 ± 3.5	157.32 ± 56.0[6]
Cu (mg/kg)	4.6 ± 2.2	18.6 ± 0.94	11.9 ± 1.5	74.30 ± 10.2[6]
Cd (mg/kg)	0.41 ± 0.01	<0.15	0.41 ± 0.01	NA
Ni (mg/kg)	NA	1.23 ± 0.59	NA	19.32 ± 2.4[6]
Cellulose (%)	40 - 55 [5]	12.40 ± 0.79	NA	33.86 ± 4.7[6]
Hemicellulose (%)	24 - 40 [5]	39.10 ± 1.94	NA	15.92 ± 2.5[6]
Lignin (%)	13 - 25 [5]	23.90 ± 1.70	NA	38.14 ± 3.1[6]

Sources 1: Jo et al., 2013a; 2: Ballesteros et al., 2014; 3: Hachicha et al., 2012;
; 4: Law et al., 2007; 5: Deraman, 1993; 6: Baharuddin et al., 2010
OM, organic matter; T-C, total carbon; T-N, total nitrogen; NA, not available

2.5 Chemical Contents of Mushrooms

Mushrooms in general, contain good nutritional values as they are rich in proteins, vitamins, minerals and low in fat and cholesterol (Raya et al., 2014). Many of them have been used in traditional medicines for ages. Most of them can be a good source of nutraceuticals (consume in the form of food) and nutriceuticals (consume in the form of capsules) (Ribeiro et al., 2007). Mushrooms are regarded as the least exploited resources where out of 15,000 mushroom species, only 2000 are found edible (Rai et al., 2005).

It is known worldwide besides possess good nutritional values, mushrooms are taken as a functional food. Carbohydrates which provide energy in daily basis, consists of 50% to 65% in mushrooms in terms of dry weight (Wani et al., 2010). Carbohydrate in mushroom consist both digestible like mannitol and glucose while non digestible carbohydrate such as ß-glucans and chitin are the largest component of carbohydrate as the both of them are major fungal component in mushroom (Wang et al., 2014). The protein content in mushroom is said to be higher compared to plant protein but lower to animal protein (Samsudin & Abdullah, 2018; Correa et al., 2016). According to Wani et al. (2010), the protein content in mushroom may vary depending on the type of substrates used and its composition, harvesting period, pileus as well its species. Fibre on the other hand is a type of non-digestible carbohydrate. According to Wang et al. (2014), the importance of fibre in daily diet is such that it improves the digestive system as well as lowers the cholesterol and blood glucose levels.

Authors have reported distinguished chemical compositions in *P. floridanus* (Table 2.3).

Table 2.3: Chemical composition of *P. floridanus* from different studies

Fat	Protein	Carbohydrate	Fiber	Ash	Reference
-	448µg/g	225µg/g	2.8%	6.1%	Das et al, 2005
1.6%	27%	58%	11.5%	9.3%	Bano et al, 1981
4.30 g/100g	20.56 g/100g	42.83 g/100g	23.29 g/100g	9.02 g/100g	Alam et al, (2007)
3.92 g/100g	20.6 g/100g	40.3 g/100g	26.8 g/100g	8.3 g/100g	Khan et al, (2008)
4.10 %	22.70 %	39.07 %	25.77 %	8.33 %	Ahmed et al, (2016)

CHAPTER 3 : RESEARCH METHODOLOGY

3.1 Mushroom Strains

In this study, two locally grown dikaryotic *P. floridanus* strains were used. PF1 strain was obtained from Mushroom Research Centre (MRC), University of Malaya while PF2 fruiting bodies was purchased from the local market. Identity of the mushroom were confirmed by mycologists of MRC.

3.1.1 Tissue Culture Technique

The fruiting body was split in half and the tissues were taken using forceps and was placed on the surface of potato dextrose agar (PDA). The culture was kept at room temperatures (28 ± 2ºC) for 14 days (Subbu Lakshmi & Sornaraj, 2014). White mycelia can be seen growing from the tissue after three days. The mycelium were fully grown and cover the agar's surface in 7-10 days. The grown mycelium was then ready to be used in spawn substrate preparation (Subbu Lakshmi, 2013). The tissue culture was maintained on PDA media by regular subculturing.

3.2 Spawn Production

The sorghum grains were cleaned and washed thoroughly to remove debris and unwanted matter. The cleaned grains were soaked in water overnight. The excess water from the grains was drained out until the moisture content is approximately 60-70% (Asghar et al., 2007). About 1% (w/w) calcium carbonate (CaCO3) were mixed to the grains. The grain mixture was filled in Erlenmeyer flasks and covered using cotton and aluminium foil. Then the flasks were autoclaved at 121 ºC for 15 minutes. The flasks were allowed to cool overnight before inoculating10 days old mycelial culture of *P. floridanus* (Pathmashini et al., 2008; Subbu Lakshmi & Sornaraj, 2014). About six plugs of 1x1 cm mycelial culture were inoculated into one flask and incubated at 25℃ for 10 -

14 days or until the mycelium covered the grains completely. The flasks were shaken periodically for maximise the mycelia distribution throughout the grains and to minimise the clumping of the grains (Tesfaw et al., 2015).

3.3 Substrate Source

The growth media used consisted of sawdust (SD), rice bran (RB) and $CaCO_3$supplemented with food waste compost (FWC), coffee waste (CW) and empty fruit bunch compost (EFBC). FWC was obtained from Zero Waste Site at University Malaya. CW was obtained from Starbucks and EFBC was obtained from Dr Fauziah Shahul Hamid, Solid Waste Management lab in UM (Refer to Table 1.1 in Appendix C).

3.4 Substrate Preparation

3.4.1 Linear Growth Study

Linear growth study was conducted to evaluate the effects of supplements' contents on the mycelia growth rate and thus select the suitable concentration for further bag cultivation. The contents of supplements were 10, 20, 30, 40 % (w/w) (Refer to Table 1.2 in Appendix C). Standard substrate consisting 89% (w/w) SD, 10% (w/w) RB and 1% (w/w) $CaCO_3$ are usually practiced conventionally in mushroom farms and serves as control. Standard substrate supplemented with FWC, CW and EFBC at different concentrations and the controls were prepared in glass test tubes. The moisture content was maintained around 75-85% (Das & Mukherjee, 2007). The substrate was compressed to 10 cm in length in race tubes and sealed using non-absorbent cotton. The race tubes were autoclaved at 121 ºC for 15 minutes. 1x1 cm of one mycelial plug of 10 days old culture grown on PDA were then inoculated. The race tubes were incubated at 25 ±2ºC under dark conditions. Visible mycelial growth was measured every 3 days until it was fully grown. All treatments were conducted in five replicates.

3.4.2 Bag Cultures

The suitable concentration of substrates with FWC, CW and EFBC for mycelium growth from the linear growth study were selected for mushroom bag cultivation. About 300g of homogenized substrates with moisture content of 75%-85% (Das & Mukherjee, 2007) was placed into polyethylene bags. The bags were autoclaved at 121 ºC for 15 minutes and allowed to cool down for 24 hours, the bags were then inoculated with fully colonized spawn. The inoculation process was done aseptically in laminar flow. The bags then capped and incubated under dark condition at 25 ± 2ºC until spawn run is completed (Jo et al., 2013a).

3.5 Harvesting

After completion of spawn run, the bags were transferred to mushroom house and kept open for fruiting. The relative humidity was maintained above 80% and temperature at 28 ± 2ºC (Jo et al., 2013a) by periodic misting using a sprinkler system. The pileus diameter, stipe length, days required for complete spawn run, primordial initiation, harvesting were observed and recorded (Pathmashini et al., 2008; Subbu Lakshmi & Sornaraj, 2014).

The biological efficiency (BE) was calculated using the formula (Subbu Lakshmi & Sornaraj, 2014):

$$Biological\ efficiency\ (\%) = \frac{Fresh\ weight\ of\ harvested\ mushrooms}{Dry\ matter\ content\ of\ the\ substrate} \times 100$$

3.6 Nutrient Analysis

Nutrient analysis was conducted by using the best strain, PF1. A total of 200g of freeze dried *P. floridanus* fruiting bodies were outsourced to external laboratory to analyse the nutrient (total protein content, total sugar content, total phenol content and beta glucan content) profile (Refer methodology in Appendix B).

3.7 Statistical Analysis

All experiments were conducted in five replicates for linear growth study and fifteen replicates for mushroom bag cultivation. Data obtained was analysed using one-way ANOVA method with Duncan's multiple range (DMR) for testing the significance of the treatments as compare to control.

CHAPTER 4 : RESULTS

4.1 Effects of Supplements on Linear Growth Rate

The selection of best supplementation to standard substrate were based on the mycelium growth rate and density. The density of mycelium growth was shown in Figure 4.1.

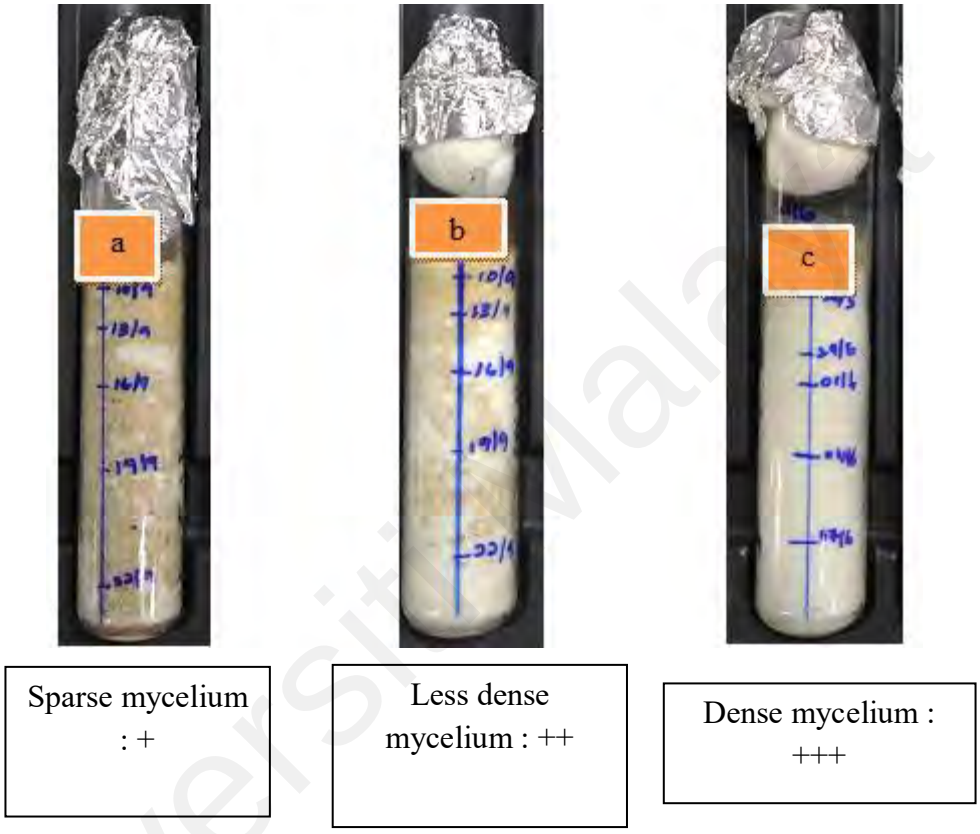


Figure 4.1: Degree of density of mycelium grown on substrates in race tubes: a) sparse mycelium ; b) less dense mycelium ; c) dense mycelium

4.1.1 PF1 Strain

Linear growth rate of PF1 strain varied among the different supplemented substrates used. Strain PF1 grown on standard substrate formulation recorded the highest growth rate at 0.40 ± 0.02 cm/day. The lowest growth rate for this strain at 0.24 ± 0.01cm/day, was observed in substrate supplemented with 40% (w/w) CW (Table 4.1). All the concentrations of CW tested had significant ($p<0.05$) effect on mycelia growth rate. However, PF1 strain grown on standard substrate supplemented with 10% CW with growth rate of 0.37 ± 0.02cm/day recorded dense mycelium and thus selected for mushroom bag cultivation.

The combination of substrate with 40% (w/w) FWC supplementation supported the highest mycelia growth rate at 0.57 ± 0.01 cm/day by strain PF1 and the lowest growth rate for the strain was recorded in standard substrate (0.42 ± 0.01cm/day) (Table 4.1). The standard substrate supplemented with 40% (w/w) FWC that supported the highest growth rate of PF1, however did not support dense mycelia growth. Substrate supplemented with 10% (w/w) FWC supported dense mycelium that grew above the surface of the substrate in the race tubes (Figure 4.2). Therefore, standard substrate combined with 10% (w/w) FWC was chosen as the most suitable concentration for mushroom bag cultivation.

Table 4.1: Linear growth rate (cm day^{-1}) of PF1 strain mycelium grown on substrate supplemented with various concentrations of CW, FWC and EFBC

Supplements	Concentration	Average growth rate (cm/day)	Mycelium density
CW	Control	0.40 ± 0.02^{e}	++
	10% CW	0.37 ± 0.02^{d}	+++
	20% CW	0.31 ± 0.01^{c}	++
	30% CW	0.27 ± 0.01^{b}	+
	40% CW	0.24 ± 0.01^{a}	+
FWC	Control	0.42 ± 0.01^{a}	++
	10% FWC	0.52 ± 0.02^{b}	+++
	20% FWC	0.55 ± 0.03^{c}	++
	30% FWC	$0.54 \pm 0.02^{b,c}$	++
	40% FWC	0.57 ± 0.01^{c}	+
EFBC	Control	0.54 ± 0.01^{a}	++
	10% EFBC	0.54 ± 0.01^{a}	++
	20% EFBC	0.61 ± 0.02^{b}	+++
	30% EFBC	0.59 ± 0.05^{b}	++
	40% EFBC	0.54 ± 0.02^{a}	+

Values are means of 5 replicates ± standard deviation. Values with different small alphabets are significantly different at $p<0.05$.

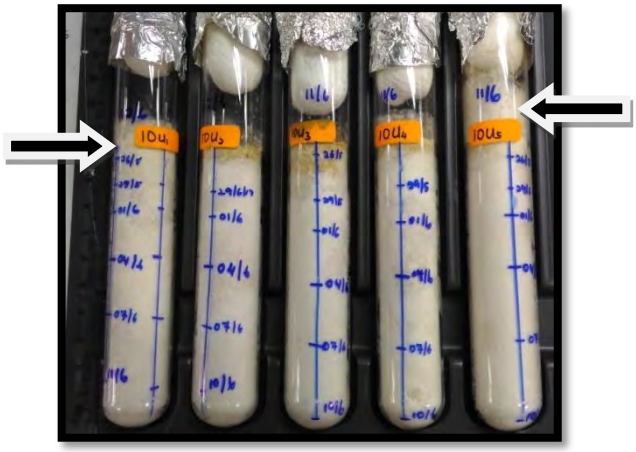


Figure 4.2: Mycelium grew above the medium on the wall of the test tube (as indicated by the arrow)

Strain grown in substrate supplemented with 20% (w/w) EFBC exhibited the highest growth rate which was at 0.61 ± 0.02 cm/day. The lowest mycelia growth rate of PF1 strain was observed in the standard substrate (Table 4.1). Substrate supplemented with 10% and 40% (w/w) EFBC showed no significant difference ($p<0.05$) on mycelia growth rate. White cottony mycelia mat of PF1 strain was dense in 20% (w/w) EFBC supplementation among all the concentrations tested. Therefore, standard substrate with20% (w/w) EFBC was chosen as the most preferred concentration to be used for fruiting body yield.

4.1.2 PF2 Strain

Mycelia linear growth rate of PF2 strain cultivated in various supplemented substrate (Table 4.2). It was observed that the linear growth rate varied considerably among the substrates used.

Table 4.2: Linear growth rate (cm day^{-1}) of PF2 strain mycelium grown on standard substrate supplemented with various concentrations of CW, FWC, and EFBC

Supplements	Concentration	Average growth rate (AGR)(cm/day)	Mycelium density
CW	Control	0.42 ± 0.00^{d}	++
	10% CW	0.38 ± 0.02^{c}	+++
	20% CW	0.33 ± 0.01^{b}	++
	30% CW	0.29 ± 0.03^{a}	+
	40% CW	0.27 ± 0.00^{a}	+
FWC	Control	0.44 ± 0.03^{a}	++
	10% FWC	0.50 ± 0.03^{b}	++
	20% FWC	0.52 ± 0.02^{b}	+++
	30% FWC	0.52 ± 0.03^{b}	++
	40% FWC	0.54 ± 0.02^{b}	+
EFB	Control	0.52 ± 0.01^{a}	++
	10% EFBC	0.54 ± 0.02^{a}	++
	20% EFBC	0.61 ± 0.04^{b}	++
	30% EFBC	0.65 ± 0.02^{c}	+++
	40% EFBC	0.55 ± 0.03^{a}	+

Values are means of 5 replicates ± standard deviation. Values with different small alphabets are significantly different at $p<0.05$.

PF2 strain showed the similar growth pattern as PF1 strain where control or standard substrate comprising of 89% SD, RB and $CaCO_3$supported the highest growth rate of mycelia (0.42 ± 0.00 cm/day). The lowest growth rate of 0.27 ± 0.00 cm/day was observed in standard substrate supplemented with 40% (w/w) CW (Table 4.2). The mycelia growth rate of PF1 strain grown in the standard substrate with 10% CW (0.38 ± 0.02cm/ day) were significantly ($p<0.05$) different from the standard substrate and all the concentrations used. The strain also exhibited the dense mycelium when grown in 10% CW supplementation. Thus, substrate supplemented with 10% (w/w) CW was selected as the most suitable concentration for mushroom bag cultivation.

The highest mycelia growth rate was exhibited by PF2strain grown in standard substrate supplemented with 40% (w/w) FWC at 0.54 ± 0.02 cm/day. The lowest mycelia growth rate was shown by PF2 strain grown in the standard substrate at 0.44 ± 0.03 cm/day. There was no significant difference in the mycelia growth rate among the four concentrations used. Hence, 20% (w/w) FWC supplementation was chosen for its dense mycelium and also due to pinhead formation on the surface of the substrate (Figure 4.3).

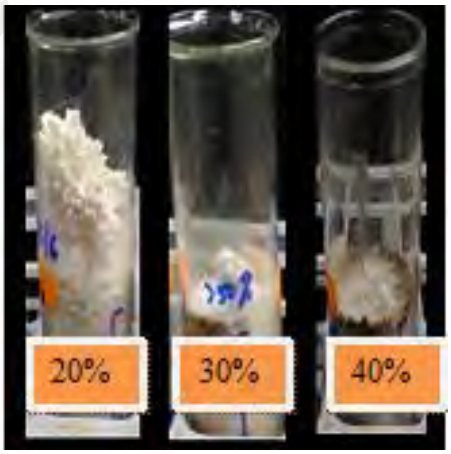


Figure 4.3: Formation of pinhead in 20%, 30% and 40% of FWC

Dense mycelium was observed in the standard substrate supplemented with 30% (w/w) EFBC and also displayed the highest growth rate (0.65 ± 0.02 cm/day) among the other concentrations used (Table 4.2). The lowest growth rate was exhibited by the standard substrate (0.52 ± 0.01 cm/day) and it shows intermediate mycelium density. Since the best concentration is selected based on growth rate and mycelium density, standard substrate supplemented with 30% (w/w) EFBC was chosen for mushroom bag cultivation.

4.2 Bag Culture

4.2.1 Influence of Various Substrate Concentrations on Parameters of Fruiting Bodies

The result displayed the various concentrations used in supplemented substrates to determine the duration of spawn run, pinhead development, days requires from opening to harvesting and measurement of pileus diameter as well as length of stipe of PF1 and PF2 fruiting bodies.

Table 4.3: Influence of various substrate formulations on parameters of fruiting bodies of PF1 and PF2

Strain	Substrates	Days required for spawn run	Days required for primordial development	Days required from opening to harvesting	Cap diameter (cm)	Stipe length (cm)
PF1	Control	20.20 ± 4.44 ^{a}	17.40 ±4.22 ^{a}	24.27 ±5.73 ^{a}	4.82 ± 0.60 ^{b}	3.10 ± 0.73 ^{a}
	10% CW	24.53 ±2.53 ^{ab}	19.00 ±4.47 ^{ab}	22.87 ±5.34 ^{ab}	4.59 ± 0.99 ^{ab}	3.96 ± 1.04 ^{bc}
	10% FWC	21.47 ±2.50 ^{a}	22.20 ±3.66 ^{ab}	26.67 ±6.58 ^{ab}	4.68 ±0.65 ^{ab}	3.60 ± 0.82 ^{ab}
	20% EFBC	18.13 ±1.25 ^{a}	31.67 ±1.86 ^{bc}	36.47 ±2.77 ^{abc}	4.77 ±0.69 ^{ab}	4.06 ± 1.36 ^{bcd}
PF2	Control	29.67 ± 13.16 ^{bc}	15.20 ± 19.43 ^{ab}	19.70 ± 20.81 ^{ab}	3.99 ± 1.82 ^{a}	3.80 ± 1.83 ^{ab}
	10% CW	28.53 ± 11.67 ^{bc}	24.20 ± 16.74 ^{bcd}	28.87 ± 17.45 ^{bc}	4.49 ± 0.67 ^{ab}	4.24 ± 0.72 ^{bcd}
	20% FWC	24.60 ±4.63 ^{ab}	33.13 ±3.88 ^{cd}	36.67 ±4.27 ^{c}	4.38 ± 0.88 ^{ab}	4.95 ± 0.86 ^{d}
	30% EFBC	32.87 ± 16.16 ^{c}	28.07 ± 25.23 ^{d}	32.00 ± 26.81 ^{c}	4.79 ± 0.61 ^{b}	4.78 ± 0.90 ^{cd}

Values are means of 15 replicates ± standard deviation. Values with different small alphabets are significantly different at $p<0.05$.

From Table 4.3, days taken for spawn run in mushroom bag ranged from 18.13±1.25 to 32.87 ± 16.16 days on various wastes used. The shortest time taken to complete the mycelia growth was recorded on standard substrate supplemented with 20% (w/w) EFBC (18.1 days) by PF1 while the longest time taken was by PF2 strain grown in standard substrate supplemented with 30% (w/w) EFBC (32.9 days).

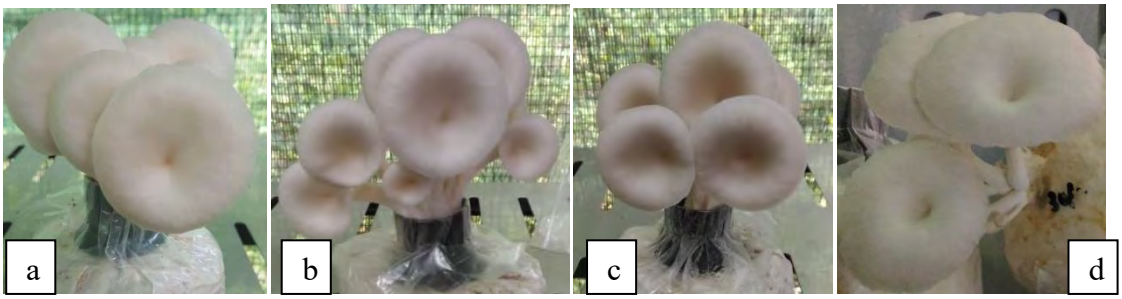
Days required for pinhead or primordial initiation ranged from 17.40 ±4.22 to 33.13± 3.88 days. Both of the strains grown in standard substrate recorded the shortest number of days for primordial initiation, which was approximately 18 days. Meanwhile the most number of days taken from opening to pinhead formation was at 33 days on substrate supplemented with 20% (w/w) of FWC by PF2.

Number of days needed from opening to the first harvest ranged from 19.70 ± 20.81daysto 36.67±4.27 days. The minimum number of days for first harvest was recorded in standard substrate by PF2 strain and the maximum days required (36 days) shared by 20% (w/w) EFBC and 20% (w/w) FWC supplemented substrates by both the oyster mushroom PF1 and PF2 respectively (Table 4.3).

Pileus diameter differed in the substrates used. The diameter ranged from 3.99 cm to 4.82cm on various wastes used. Oyster mushroom PF1 grown on standard substrate gave significantly ($p>0.05$) larger cap diameter at 4.82 ± 0.60 cm while PF2 gave the significantly smaller cap diameter (3.99 ± 1.82cm) when grown in the standard substrate (Table 4.3, Figure 4.4).

Length of stipe measured ranged from 3.10cm to 4.95cm. The shortest stipe length at 3.10 ± 0.73cm was recorded in PF1 cultivated in the standard substrate while the longest stipe was observed in substrates supplemented with 20% (w/w) FWC by PF2 (4.95 ± 0.90cm) (Table 4.3, Figure 4.4).

PF1



PF2

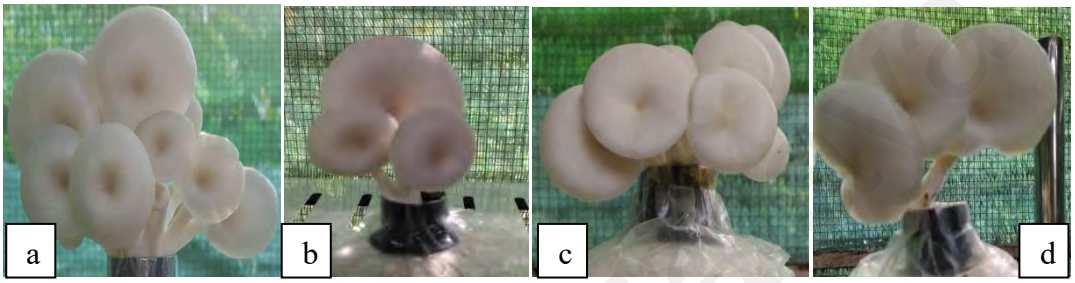


Figure 4.4. Fruiting bodies of white oyster mushroom PF1 and PF2 grown on a); standard substrate b); CW c); FWC d); and EFBC

4.2.2 Influence of Various Substrate Formulations in Yield and Biological Efficiency

According to Table 4.4, PF1 fruiting bodies grown in all the substrates were harvested until 4th harvest. Most of the cultivation of PF2 in the standard substrates with supplements, produced fruiting bodies until three harvests except PF2 grown in standard substrate supplemented with FWC which had 4 harvests. The highest fruiting bodies production was observed in the 1st harvest for both the PF1 and PF2. The yield decreased in the following harvests. The total yield of fruiting bodies ranged from 52.93 ± 25.23 g to 83.37 ± 24.25 g. PF1 strain that was cultivated in standard substrate supplemented with 10% (w/w) FWC gave the highest total fresh weight yield per bag whereas the lowest yield per bag was achieved by PF2 strain grown in standard substrate supplemented with 30% (w/w) EFBC. There was no significant difference in terms of total yield for PF1 mushroom. The study also displayed that there was no contamination of bags recorded for PF1 strain while a low percentage of contaminated bags were found in PF2 strain cultivated in standard substrate (13.33%) and EFBC supplemented substrate (6.67%). However, it was found that the bags shrunk at the end of harvesting cycle due to the insufficient of nutrients and insects were found in the bags.

On the other hand, the BE varied from 88.2 % to 138.9 % in different formulations. The highest BE of PF1 was recorded in standard substrate supplemented with 10% (w/w) FWC. The lowest BE was recorded on standard substrate supplemented with 30% (w/w) EBFC for PF2 mushroom.

Among the two strains used, fruiting bodies of PF1 strain were selected and analysed for nutrient profile as the strain colonised in shorter time, produced fairly larger pileus, shorter stipe, produce more harvest cycles, obtained the highest yield and BE as well as no contamination of bags were recorded.

Table 4.4 : Influence of various substrates formulations in terms of harvests, total yield and BE of mushroom PF1 and PF2

Strain	Substrates	1st harvest (g/bag)	2nd harvest (g/bag)	3rd harvest (g/bag)	4th harvest (g/bag)	No. of contaminated bags (%)	Total Yield (g/bag)	BE (%)
PF1	Control	39.19 ± 7.88^{a}	25.74 ± 11.24^{c}	8.13 ± 9.58^{a}	6.20 ± 7.06^{b}	-	79.26 ± 14.16^{b}	132.1^{b}
	10% CW	35.36 ± 10.33^{a}	27.33 ± 15.03^{c}	8.33 ± 9.77^{a}	8.80 ± 10.54^{b}	-	80.03 ± 18.11^{b}	133.4^{b}
	10% FWC	39.70 ± 8.29^{a}	22.80 ± 8.24^{bc}	14.60 ± 15.81^{a}	6.27 ± 6.08^{b}	-	83.37 ± 24.25^{b}	138.9^{b}
	20% EFBC	44.53 ± 9.24^{a}	21.80 ± 13.56^{bc}	12.60 ± 9.71^{a}	3.60 ± 5.21^{ab}	-	82.53 ± 25.16^{b}	137.6^{b}
PF2	Control	36.69 ± 16.82^{a}	28.67 ± 16.71^{c}	8.27 ± 10.31^{a}	4.00 ± 8.87^{ab}	13.33	77.62 ± 34.23^{b}	129.4^{b}
	10% CW	36.93 ± 14.74^{a}	13.27 ± 14.26^{ab}	8.93 ± 7.55^{a}	-	-	59.13 ± 24.99^{a}	98.6^{a}
	20% FWC	33.67 ± 11.06^{a}	14.87 ± 8.25^{ab}	12.20 ± 12.21^{a}	8.27 ± 6.96^{b}	-	69.00 ± 18.83^{ab}	115.0^{ab}
	30% EFBC	42.93 ± 22.97^{a}	10.00 ± 9.11^{a}	-	-	6.67	52.93 ± 25.53^{a}	88.2^{a}

Values are means of 15 replicates ± standard deviation. Values with different small alphabets are significantly different at $p<0.05$.

4.3 Nutrient Profile

The nutritional contents such as total sugar, total protein, total phenolic content and beta glucan contents of fruiting bodies of *P .floridanus* strain PF1 cultivated on different wastes are presented in Table 4.5. Fruiting bodies grown on all the supplemented substrates were seen to possess at least one nutrient parameters as the highest nutrient content.

Fruiting bodies of PF1 that were cultivated in standard substrate contained the highest total sugar content of 75.12 ± 0.03 mg/g followed by fruiting bodies cultivated in standard substrate supplemented with EFBC (71.22 ± 0.05 mg/g). The least total sugar content was recorded in fruiting bodies cultivated in FWC supplemented substrate at 50.00 ± 0.02 mg/g.

According to table 4.5, the total protein content of PF1 was 200 ± 0.01 mg/g grown on standard substrate supplemented with FWC being the highest followed by substrate supplemented with CW (179.51 ± 0.01 mg/g), while the lowest total protein was detected in fruiting bodies grown on standard substrate at 110.86 ± 0.01 mg/g. Fruiting bodies PF1 that were cultivated in all the substrates showed significant difference ($p<0.05$) on the protein content.

Total phenolic content, on the other hand, shown maximum in fruiting bodies PF1 strain grown on standard substrate supplemented with CW at21.99 ± 0.01 mg/g whereas the lowest total phenolic content was reduced by 16% when compared to CW supplemented substrate, was observed in fruiting bodies PF1 grown on standard substrate (18.45 ± 0.03 mg/g).

As shown in table 4.5, the highest beta-glucan content in fruiting bodies cultivated in standard substrate was 33.19 %, followed closely by fruiting bodies PF1 grown on EFBC supplemented substrate at 31.69% while the lowest was found in CW supplemented substrate (17.62 %).

Table 4.5: Influence of various substrate formulations on total sugar, total protein, total phenolic and beta glucan contents of fruiting bodies of PF1

Strain	Substrates	Total sugar content[1](mg/g of mushroom)	Total protein content[1] (mg/g of mushroom)	Total phenolic content[1] (mg/g of mushroom)	Beta-glucan content [1,2](%)
PF1	Control	75.12 ± 0.03^{b}	110.86 ± 0.01^{a}	18.45 ± 0.03^{a}	33.19^{c}
	CW	55.37 ± 0.07^{a}	179.51 ± 0.01^{c}	21.99 ± 0.01^{d}	17.62^{a}
	FWC	50.00 ± 0.02^{a}	200.00 ± 0.01^{d}	19.95 ± 0.02^{c}	28.29^{b}
	EFBC	71.22 ± 0.05^{b}	133.33 ± 0.01^{b}	19.16 ± 0.02^{b}	31.69^{c}
RDI[3](per day)		<50g[4]	56g[5] (male) 46g (female)	1g[6]	3g[7]

[1] Values are means of 3 replicates ± standard deviation. Values with different small alphabets are significantly different at $p<0.05$.
[2]Calculated using kit protocol.
[3]Recommended Daily Intake.
[4]World Health Organization, 2015 (less than 10% of the total energy intake)
[5]Busch (2018), approximately (0.8 g/kg of body weight).
[6]Perez-Jimenez et al., 2008.
[7]Malaysian Dietary Guidelines, 2010

CHAPTER 5 : DISCUSSION

5.1 Effect of Supplemented Substrates on Linear Growth Rate

Both the strains were inoculated in substrate supplemented with CW showed decrease in growth with increasing concentrations. Coffee waste contain anti nutritional compounds like caffeine and tannins which maybe toxic to mushroom (Pandey et al., 2000) and thus reduced the mycelium growth (Nunes et al., 2017). Therefore, higher concentration of coffee waste added in the substrate, could contain higher amounts of toxic compounds which reduced the mycelium growth. Coffee industry produces residues such as spent coffee grounds, coffee cherry wastes, coffee dried leaves and coffee husk (Murthy & Manonmani, 2008). Murthy & Manonmani (2008) stated that substrate supplemented with coffee grounds alone recorded lower growth and yield compared to the substrate supplemented with various combinations of coffee wastes.

Eventhough the highest mycelia growth for both of the strains in standard substrates supplemented with FWC were recorded at 40%, it was inversely proportional to the mycelium density. Mycelia growth at faster rate is often described as progression of hyphal on unpreferable medium or substrate (Zervakis et al., 2001). In other words, mycelia growth at slower rate with dense mycelium in media or substrate can be regarded as nutrient of the medium or substrate was fully utilised by the fungal strain. On that note, it can be assumed that substrates supplemented with higher composition of FWC are not favourable for mushroom mycelium growth. In this study, 10% and 20% (w/w) of FWC supplementation in standard substrate were chosen for PF1 and PF2, respectively for bag cultivation. This result was more or less similar to the study by Chae & Ahn (2013) where optimum FWC supplementation needed for maximum *Pleurotus ostreatus* fruiting body production was at 25%. Jo et al. (2013b) recorded that substrate supplemented with 10% and 13% of FWC produced maximum *Ganoderma lucidum* and *Pholiota adipose* fruiting bodies, respectively. These findings showed that FWC could be used as substrate

supplement for growing oyster mushroom as well as increasing profits and reducing the cost production (Chae & Ahn, 2013).

The concentration of empty fruit bunch compost (EFBC) supplementation at the highest colonization rate was selected for both of the strains for cultivation of bags. This is because colonization rate is directly proportional to the density of mycelium. Increasing supplementation amount resulted in increasing mycelia growth rate up to 20% and 30% (w/w) for PF1 and PF2 strain, respectively compare to the standard substrate formulation (0.54 ± 0.01 cm and 0.52 ± 0.01cm, respectively). Supplemented substrate can change the physical properties of the whole substrate (Abd Razak, 2013). Mycelium tend to face hardship while growing if the substrate used is very compressed or very loose (Fanadzo et al., 2010). Therefore, particle size of supplemented substrate can affect the fungal growth. The particle size in EFBC was observed to be much finer than the standard ingredients and the substrate with EFBC were dense when water was added. This could reduce spaces between the substrate particle for gaseous exchange and aeration to occur. Space reduction can lead to fungal growth restriction (Amal Nafissa et al., 2008; Bellettini et al., 2019). Other than that, difference in variations of mycelia growth rate in different concentrations can be due to difference in nutrient content (Kavitha et al., 2013). Nitrogen source is vital in promoting mycelia growth. Lack of nitrogen or excessive of nitrogen can inhibit the fungal growth (Fanadzo et al., 2010). According to Harith et al. (2014), the nitrogen content in EFB is 0.36% which is in low quantity, could restrict the growth of mycelium. However, combination of nitrogen content in sawdust and rice bran with EFBC can promote mycelia growth.

5.2 Effects of Supplemented Substrates on Production of Fruiting Bodies

*Pleurotus floridanus*PF1 and PF2 successfully grown in all the agricultural wastes and food waste compost used. Substrate supplementation has become one of the crucial ingredients in mushroom cultivation where it can boost the mushroom production (Tripathy, 2010). In the present study, the range of days for spawn run of *P. floridanus* on different formulations were from 18 to 32 days. Subbu Lakshmi & Sornaraj (2014) reported the range of days of spawn run for *P. flabellatus* were from 16 to 39 days using seafood processing waste together with selected agro-industrial wastes (coir pith, woodchip and sugarcane bagasse).The difference in days for full mycelial spawn running on different substrates could be due to the lignocellulosic materials, chemical composition and C/N ratio (Naraian et al., 2008; Iqbal et al., 2016).In this study, pinhead formation took around 17 to 33 days. The result obtained were similar with the study conducted by Girmay et al. (2016), where the duration taken for the pinhead formation of *P. ostreatus* grown on various agro wastes (cotton seed, paper waste, wheat straw and sawdust)were ranged from 17 to 33 days and number of days needed from bag opening to the first harvest ranged from 22 to 36 days.

According to Yang et al. (2013), mushroom appearance is for commercial purposes, mushrooms with bigger cap diameter and shorter stipe length are considered more favourable characteristics than smaller cap diameter and longer stipe. In this study, there were no significant differences in terms of cap diameter in both the mushrooms PF1 and PF2 to standard substrate except for PF2 in 30% (w/w) EFBC supplemented substrate. Stipe length on the other hand, mushroom PF1and PF2 showed significant difference in 10% (w/w) CW, 20% (w/w) EFBC and 20% (w/w), 30% (w/w) supplemented substrates, respectively in reference to control ($p<0.05$). However, the result from this study were similar to the result reported by Mondal et al. (2010). Mondal et al. (2010) reported that *Pleurotus florida* cultivated on substrates such as banana leaves, rice straw and sawdust

produced the mushroom cap diameter ranged from 4.13 cm to 7.79 cm and 2.47 cm to 3.80 cm for stipe length. Besides that, the result from this study was also similar to the study conducted by Islam et al. (2017). The authors reported that cap diameter and stipe length of *Pleurotus florida* in indoor controlled environment were 6.2 cm and 4.5 cm, respectively. Onyango et al. (2011) stated that, factors such as supplemented substrates, environmental conditions and sources of nitrogen should be considered to enhance the quality, growth and production of mushrooms. Besides that, both strains PF1 and PF2 grown in standard substrates supplemented with 20% and 30% (w/w) EFBC produced large fruiting bodies (4.77 and 4.79 cm, respectively) as well. The addition of EFBC to the standard substrate can enlarge the cap diameter and reduce the stipe length. In addition, producing fruiting bodies in bigger size is categorised as good quality for marketing (Onyango et al., 2011). However, their statement was contradicted to Shen & Royse (2001) where the authors stated that larger fruiting bodies were poor in quality as prone to breaking during packaging.

The production of fruiting bodies in this study were able to be harvested until three to four harvests. However, fruiting bodies production decreased as the number of harvests increased which due to the decreasing nutrient and minerals in the substrate (Amal Nafissa et al., 2008). Marlina et al. (2015) reported that *P.ostreatus* which cultivated on EFB able to produce fruiting bodies until three to four harvests. Similarly, Iqbal et al. (2016) reported that *P. florida* cultivated on wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw able to produce fruiting bodies up to three harvests. Total yield per bag for mushroom PF1 was the highest in standard substrate supplemented with FWC followed closely by standard substrate supplemented with EFBC. However, there is no significant differences in total production of fruiting bodies for PF1 grown in standard substrate and the three concentrations used. Fruiting bodies of mushroom grown on substrates supplemented with the three wastes were lower compared to standard

substrate especially standard substrates supplemented with 10% (w/w) CW and 30% (w/w) EFBC. The latter two substrates stopped at 3rd and 2nd harvests, respectively. Difference in mushroom production on various substrates may due to the difference in nutrient content, C/N ratio, phenolic compounds, and nature of lingocellulose complex (Kavitha et al., 2013).

In this study the BE varied from 88.2% to 138.9% on different wastes. Ahmed et al. (2013) reported that BE of newly introduced oyster mushroom strains, *P. high-king* and *P. geesteranus* cultivated on wheat bran supplemented with sawdust were in the range between 56.4% and 95.8%. Das et al. (2012), also reported that the BE of *P. sajor-caju* and *P. floridanus* cultivated on rice straw were in the range of 65% to 92%. Nevertheless, the result from this study fall within the range of the study conducted by Iqbal et al. (2016), where the BE of *P. floridanus* were between 96% to 136% when cultivated on various agro wastes (wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw). The variations in BE might be affected by the mushroom species, spawn strain, spawn rate and the use of wastes as supplements in the standard substrate (Ahmed et al., 2013; Iqbal et al., 2016).

5.3 Effect of Supplemented Substrates on Nutrient Composition

Nutrient composition in a mushroom may differs based on the substrate type and method of cultivation (Kalaras et al., 2017).

Total protein content found in *P. floridanus* cultivated on the supplemented substrates varies from 110.86 ± 0.01 mg/g to 200.00 ± 0.01mg/g. Alam et al. (2007) and Khan et al. (2008) reported that dried *P. florida* contained 20.56 ± 1.45 g/100g and 20.6 ± 2.6 g/100g, respectively. Moreover, Deepalakshmi & Mirunalini (2014) reported the protein contents of *P. ostreatus* were in the range of 17 to 42 g/100g dried weight. The total protein content depends on nitrogen level in the substrate, development stages of harvested fruiting bodies and geography location (Colak et al., 2009). According to Busch (2018), USDA guidelines recommended daily intake for protein is approximately 0.8 g/kg of body weight. In other words, 56g and 46g of protein are needed daily by an average man and woman, respectively. Hence, an average person can take around 200g - 300g of mushroom grown in FWC supplemented substrate to achieve the suggested daily dosage. Mushrooms contained good source of protein and essential amino acids especially to vegans (Wani et al., 2010).

Total sugar content in *P. floridanus* cultivated on the supplemented substrates ranged from 50.00 ± 0.02mg/g to 75.12 ± 0.03 mg/g. The result from this study is similar to the study conducted by Lin et al. (2016) where the total sugar of *P. ostreatus* fruiting bodies and mycelia were from 47.43 ± 0.37mg/g to 92.17 ± 1.64 mg/g dried weight, respectively. In addition, Reis et al. (2012) also reported the total sugar content of *P. ostreatus* and *P. eryngii* were 4970 ± mg/100g and 8670 ± mg/100g, respectively. The new guideline on daily intake of sugar recommended by World Health Organization (2015) should be less than 10% of the total energy intake, which is equivalent to 50g per day. Therefore, an

average adult can consume around 300g of mushroom to reach the recommended daily intake.

Polyphenolic compounds such as total phenolic content is regarded as the bigger contributors to the antioxidant capacity of plants (Gursoy et al., 2009). Polyphenolic compounds consist of redox properties where polyphenols act as antioxidants that donate hydrogen as well as singlet oxygen quenchers (Rice-Evans et al., 1996). In the present study, total phenolic content of the fruiting bodies grown on agricultural wastes and food waste compost ranged from 18.45 mg/g to 21.99 mg/g dried mushroom. The highest total phenolic content was found in the mushroom grown in CW supplemented substrate. The differences may be due to type of substrate used, management techniques and harvesting time (Heleno et al., 2010). Total phenolic content analysis conducted by Orhan & Ustun (2011) on *Polyporus* sp., *Lactarius deliciosus, Trametes versicolor* and *Cantharellus cibarius*, ranged from 2.50 ± 0.52 mg/g to 51.27 ± 1.44 mg/g. The highest total phenolic content was found in *Lactarius* species. On the other hand, Dubost et al. (2007) and Khatun et al. (2015) reported the total phenolic content of *Pleurotus ostreatus* and *P. floridanus* were recorded at 4.27 ± 0.69 mg/g and 119 μg^{-1} dry weight, respectively. According to Correa et al. (2015), daily dosage of phenolic compounds are recommended to be more than other antioxidants' daily dosage like vitamin C. The recommended daily intake for polyphenols is approximately 1g/day (Perez-Jimenez et al., 2008), where phenol content comprises of the 30% and flavanoid contents cover the 60% of the total polyphenol intake (Scalbert & Williamson, 2000). Therefore, an average adult needed around 333mg of phenol content and approximately 15g to 18g of mushroom can be consumed to achieve the recommended daily dosage.

Beta-glucan is a soluble fiber that easily can be found in barley grains and oats. In this study, total beta-glucan content of mushroom grown on agricultural wastes and food

waste compost ranged from 17.62% to 33.19%. According to Malaysian Dietary Guidelines (2010), recommended daily dosage for beta-glucan is 3g per day to lower the cholesterol and low density lipoprotein (LPL) level. The results obtained in this study except the mushroom grown on CW supplemented substrate, showed high beta-glucan content than the oat products available in market Oat BG22. Mushrooms could be an alternative choice to obtain the beta-glucan for those who are not fond of eating oats.

CHAPTER 6 : CONCLUSION

The potential use of coffee waste, food waste compost and empty fruit bunch compost was studied to explore their usage as alternative substrate component for *Pleurotus floridanus* cultivation. The findings showed that usage of these wastes as supplementation to standard formulation induce mycelia growth and further produce *P. floridanus* fruiting bodies. The first part of this study was linear growth test which was conducted to investigate the most suitable substrate formulations with the fastest growth rate and dense mycelium. The findings revealed the most suitable CW, FWC and EFBC concentrations supplemented in standard substrate for PF1 strain were 10% (w/w) at 0.37 ± 0.02cm/day, 10% (w/w) (0.52 ± 0.02 cm/day) and 20% (w/w) (0.61 ± 0.02 cm/day), respectively. Meanwhile for PF2 strain, the most suitable concentrations of standard substrate supplemented with CW, FWC and EFBC were 10% (0.38 ± 0.02 cm/day), 20% (0.52 ± 0.02 cm/day) and 30% (0.65 ± 0.02 cm/day), respectively. The results showed that supplementing wastes in low concentration in standard substrate provide higher growth rate and dense mycelium.

The second part of this study was to use the selected substrate formulations for bag cultivation.PF1 strain grown on standard substrate supplemented with 10% (w/w) FWC obtained the highest fruiting bodies yield at 83.37 ± 24.25g with BE of 138.9%. The fruiting bodies of strain PF1 is not significantly difference compared to standard substrate on mushroom yield and BE. PF2 strain produced the highest mushroom yield and BE at 77.62 ± 34.23g and 129.4% respectively, when grown on standard substrate. The first harvest was found to produce maximum yield for PF1 and PF2 strains in all the substrate formulations used and it was noted that the yield gradually reduce in the next harvesting cycles. In terms of number of harvesting cycle, strain PF1 were able to produce fruiting bodies until 4 harvests compared strain PF2. Producing fruiting bodies in bigger

size and shorter stipe length are considered as good quality for marketing. Hence, these criteria can be seen in fruiting bodies of strain PF1 grown on all the substrate formulations.

Fruiting bodies of strain PF1 was subjected for nutrient profile as the strain colonised in shorter time, produced fairly larger pileus, shorter stipe, produce more harvest cycles, obtained the highest yield and BE as well as no contamination of bags were recorded. Fruiting bodies of strain PF1 that grown on standard substrate contained the highest total sugar content (75.12 ± 0.03 mg/g) and beta-glucan content (33.19%). Meanwhile, strain PF1 grown on standard substrate supplemented with CW and FWC produced fruiting bodies rich in total phenolic (21.99 ± 0.01 mg/g) and protein content (200 ± 0.01 mg/g). White oyster mushroom is a good source of nutrients especially protein where protein deficiency is very serious nutritional hazard globally. These data shows that mushrooms can provide balance dietary in everyday lives.

In conclusion, these wastes can be utilised as supplements, in the production of mushrooms and reduce the wastes in landfills as well as contributes to good source of nutrition. It is recommended to use composted CW instead of raw CW in future studies. Besides that, it is also suggested that FWC be used extensively in mushroom cultivation, as it showed promising result in terms of production and nutrient content. It is also advisable to utilise more EFBC, as the waste is almost free of charge and the only expense will be on the delivering the waste. Recycling these wastes as supplements can be helpful in eradicating waste disposal problems as well as solving the problem of sawdust scarcity.

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