ORGANIC WASTE SUPPLEMENTATIONS AS ALTERNATIVE SUBSTRATE FOR CULTIVATION OF THE GREY OYSTER MUSHROOM *Pleurotus pulmonarius* (FR.) QUELET

TANG CHEN SEOK

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2019

ORGANIC WASTE SUPPLEMENTATIONS AS ALTERNATIVE SUBSTRATE FOR CULTIVATION OF THE GREY OYSTER MUSHROOM *Pleurotus pulmonarius* (FR.) QUELET

TANG CHEN SEOK

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF BIOTECHNOLOGY

INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2019

UNIVERSITY OF MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **TANG CHEN SEOK**

Matric No: **SGF 160025**

Name of Degree: **MASTER OF BIOTECHNOLOGY**

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

ORGANIC WASTE SUPPLEMENTATIONS AS ALTERNATIVE SUBSTRATE FOR CULTIVATION OF THE GREY OYSTER MUSHROOM *Pleurotus pulmonarius* (FR.) QUELET

Field of Study: **FUNGAL BIOTECHNOLOGY**

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date:

Subscribed and solemnly declared before,

Witness's Signature Date:

Name:

Designation:

ORGANIC WASTES SUPPLEMENTATIONS AS ALTERNATIVE SUBSTRATE FOR CULTIVATION OF THE GREY OYSTER MUSHROOM *Pleurotus pulmonarius* (FR.) QUELET

ABSTRACT

Mushroom industry plays an important role in health and nutrition as well as economic resources in both urban and rural areas. Due to the limitation of raw materials supply and the increasing cost of substrate especially sawdust for mushroom cultivation, coffee waste (CW), food waste compost (FWC) and empty fruit bunch compost (EFBC) were selected as alternative supplemented substrates to cultivate grey oyster mushroom, *Pleurotus pulmonarius*. The objectives of this study were to optimise the substrate formulations and to evaluate the growth performance, production yield of *P. pulmonarius* grown on the standard substrate supplemented with varying concentrations of CW, FWC and EFBC along with analysing the nutrient profile of selected *P. pulmonarius* fruiting bodies. In this study, *P. pulmonarius* strains (S01 and S02) were grown and the linear growth study for *P. pulmonarius* strains (S01 and S02) grown on standard substrate supplemented with different concentrations (10 %, 20 %, 30 % and 40 % (w/w)) of CW, FWC and EFBC were carried out. The strains tested showed variable growth rates and mycelium compactness on each supplemented wastes at different concentrations. Based on the linear growth study, a total of eight substrate formulations for both strains initially chosen for the bag preparations, included: control substrate and 10 % (w/w) of CW supplementation for both strains respectively, 10 % (w/w) of FWC supplementation for strain S01, 20 % (w/w) of FWC supplementation for strain S02 and 30 % (w/w) of EFBC supplementation for both strains. It was observed that the supplementation of various wastes at their lower concentrations had enhanced mycelium growth and only fast growth rate with dense mycelium may reflect the utilisation of nutrient resources is optimum. In order to select

a comparatively more suitable strain to be used in nutrient analysis for *P. pulmonarius*, an evaluation and comparison of growth performance, yield and biological efficiency (BE) among the strain was conducted. Strain S02 has proved to have fast colonisation, short pinheads development, strong pathogenicity, higher percentages of successfully fruiting bags, better yield and BE than strain S01. Thus, strain S02 were selected and analysed for nutritional attributes. There were high protein content in *P. pulmonarius* compared to sugar, phenolic and β-glucan. The protein content was significantly higher ($p < 0.05$) in all substrate supplemented with wastes compared to the control substrate. Hence, the efficiency of mycelium in transforming the chemical compositions of substrate and different wastes into fruiting bodies itself can cause the variations among the nutritional attributes of *P. pulmonarius*. Strain S02 grown on standard substrate supplemented with 20 % (w/w) of FWC recorded the highest biological efficiency (101.40±29.92 %) and the fruiting bodies contained the highest β-glucan content (29.58 ± 0.01 %). The days required for mycelium running was 24.87±3.10 and the pinheads formation was 14.13±3.10. In conclusion, CW, FWC and EFBC can be utilized as supplement in the cultivation of grey oyster mushrooms and an alternative way for solid waste management.

Keywords : grey oyster mushroom, substrates, yield, biological efficiency, nutrient analysis

PENAMBAHAN SISA ORGANIK SEBAGAI SUBSTRAT ALTERNATIF UNTUK PENANAMAN CENDAWAN TIRAM KELABU *Pleurotus pulmonarius* (FR.) QUELET

ABSTRAK

Industri penanaman cendawan memainkan peranan penting dalam bidang kesihatan, pemakanan, dan juga sumber ekonomi di kawasan bandar dan luar bandar. Disebabkan oleh bekalan bahan mentah yang terhad dan peningkatan kos substrat terutamanya habuk gergaji bagi industri penanaman cendawan, sisa kopi (CW), kompos sisa makanan (FWC), dan kompos buah tandan kosong (EFBC) telah dipilih sebagai alternatif substrat tambahan untuk menanam cendawan tiram kelabu, *Pleurotus pulmonarius*. Objektif kajian ini adalah untuk mengoptimumkan formulasi substrat dan menilai kadar pertumbuhan, penghasilan cendawan bagi pertumbuhan *P. pulmonarius* dalam standard substrat dengan konsentrasi yang berbeza di kalangan CW, FWC and EFBC, serta menganalisa kandungan nutrien *P. pulmonarius* dalam janabuah. Dalam kajian ini, strain *P. pulmonarius* (strain S01 dan S02) telah ditumbuh dan kajian pertumbuhan linear bagi *P. pulmonarius* (strain S01 dan S02) dalam standard substrat dengan konsentrasi yang berbeza (10 %, 20 %, 30 % dan 40 % (w/w)) telah dijalankan. Jenis strain yang dikaji menunjukkan pelbagai kadar lanjutan miselium dan kepadatan berdasarkan konsentrasi yang berbeza di kalangan sisa masing-masing. Berdasarkan kriteria pemilihan, sejumlah lapan substrat formulasi dari kedua-dua strain telah dipilih untuk persiapan beg, iaitu kawalan substrat dan formulasi tambahan dengan 10 % (w/w) CW bagi kedua-dua jenis strain, formulasi tambahan dengan 10 % (w/w) FWC bagi strain S01 dan formulasi tambahan dengan 20 % (w/w) FWC bagi strain S01, serta formulasi tambahan dengan 30 % (w/w) CW bagi kedua-dua jenis strain. Dalam kajian ini, konsentrasi yang rendah dengan tambahan sisa yang berbeza dapat mempertingkatkan pertumbuhan miselium dan

hanya kadar pertumbuhan miselium yang cepat dengan padat dapat dilihat dari penggunaan sumber nutrien yang dioptimumkan oleh kulat. Penilaian dan perbandingan prestasi pertumbuhan, hasil dan BE antara kedua -dua strain telah dijalankan untuk memilih strain yang lebih sesuai digunakan dalam analisis nutrient bagi *P. pulmonarius*. Strain S02 terbukti mempunyai kolonisasi substrat yang cepat, pembetukan pinheads yang pantas, patogenik yang kuat, peratusan yang lebih tinggi bagi beg yang berjaya berbuah, hasil dan juga BE yang lebih baik berbanding dengan strain S01 dalam keseluruhan. Strain S02 telah dipilih dan dianalisa bagi segi komposisi nutrisi. Komposisi nutrisi cendawan ini didapati mempunyai kandungan protein yang tinggi berbanding dengan gula, fenolik β-glucan, Sementara itu, kandungan protein adalah lebih tinggi ($p < 0.05$) di antara semua substrat tambahan dengan sisa berbanding dengan kawalan substrat. Oleh itu, kecekapan miselium dalam mengubah komposisi kimia substrat dan sisa yang berbeza ke dalam badan-badan berbuah itu sendiri boleh menyebabkan variasi antara *P.pulmonarius*. Dalam kajian ini, formulasi tambahan dengan 20 % (w/w) FWC bagi strain S02 menunjukkan kecekapan biologi yang tertinggi (101.40±29.92%) dan juga kandungan β-glucan (29.58± 0.01 %). Tempoh hari yang diperlukan untuk miselium kolonisasi sehingga memenuhi bag adalah 24.87 ± 3.10 dan pembentukan pinhead adalah 14.13 ± 3.10. Kesimpulannya, CW, FWC dan EFBC boleh digunakan sebagai substrat tambahan untuk penanaman cendawan tiram kelabu dan sebagai cara alternatif bagi pengurusan sisa pepejal.

Kata kunci : cendawan tiram kelabu, substrat, hasil, keefisienan biologi, analisis nutrien

ACKNOWLEDGEMENT

First and foremost, I would like to express my special thanks of gratitude to my supervisors, Associate Prof. Dr. Tan Yee Shin and co-supervisor Prof. Dr. Vikineswary Sabaratnam who gave me the golden opportunity as the unceasing support to my master research. I am extremely thankful and indebted to them for sharing professionalism, sincere and valuable guidance and encouragement extended to me. Their patience, motivation, immense knowledge and financial support helped me throughout this research and writing of this thesis.

My sincere thanks also go to Faculty of Science, Institute of Graduate Studies (IPS) and Mushroom Research Centre (MRC) of Universiti Malaya (UM) which gave admission to the laboratory and research facilities. I am also grateful to Miss Azliza Anuar and Mr. Khairul Nizam for providing me with all the necessary facilities for the research. Without their valuable support it would not be possible to conduct this research.

Thank you for my coursemates Thian Sing Kit, Zuhaili Bt Yusof, Fatin Fatihah Bt Abd Aziz and Nur Farahin Bt Mohd Isa in the stimulating discussions and solving problems in whatever I encountered with this research. Also, many thanks to my wonderful lab mates Umahsreerekah a/p Gopala Krishnan, who teaching me to improve my weaknesses on lab works and enlightening me on doing research.

Last but not the least, I would like to thank my parents and siblings for supporting me spiritually during studying master, writing this thesis and my life in general. I also place on record, my sense of gratefulness to one and all, who directly or indirectly, have lent their hand in this research. Thanks a million!

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA	:	Analysis of variance
BE	:	Biological efficiency
BSA	:	Bovine Serum Albumin
Ca	:	Calcium
$CaCO_3$	:	Calcium carbonate
Cd	:	Cadmium
Co	:	Cobalt
CS	:	Coffee silver skin
Cu	:	Copper
CW	:	Coffee waste
DMRT	:	Duncan's Multiple Range Test
EFBC	:	Empty fruit bunch compost
U/m	:	Enzyme unit per
FWC	:	Food waste compost
GAE	:	Gallic acid equivalents
GOPOD	:	Glucose-oxidase-peroxidase-reagent
k cal	:	Kilocalories
Pb	:	Lead
Mn	:	Manganese
Mg	:	Magnesium
mM	:	MilliMolar
M	:	Molar
mol	:	Moles
Mo	:	Molybdenum
NaH_2PO_4	:	Monosodium phosphate

Ni	:	Nickel
ppm	:	Parts per million
P	:	Phosphorus
psi	:	Pounds per square inch
K	:	Potassium
KOH	:	Potassium hydroxide
SD	:	Sawdust
Na	:	Sodium
Na_2CO_3	:	Sodium carbonate
SCG	:	Spent coffee grounds
SPSS	:	Statistical Package for the Social Sciences
H_2SO_4	:	Sulfuric acid
TPC	:	Total phenolic content
TCA	:	Trichloroacetic acid
v/v	:	Volume per volume
w/v	:	Weight per volume
w/w	:	Weight per weight
Zn	:	Zinc

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Background and Problem Statement

Mushroom research and cultivation have been receiving increased attention over recent times because mushrooms are nutritious food with medicinal effects in the prevention and treatment of various diseases (Yang *et al.*, 2003). Medicinal and culinary mushrooms are reported to have antibacterial, anti-inflammatory, antioxidant, antiviral, antitumor, immunomodulating, antioxidant, cardiovascular, anti-hypercholesterolemia, anti-parasitic, antifungal, detoxification, hepatoprotective, and anti-diabetic properties (Chang & Wasser, 2012). Moreover, mushroom cultivation can play a major role in the livelihood of households as a source of economic empowerment for women in both urban and rural areas and even small holder farmers (Narayanasamy *et al.*, 2008).

In Malaysia, mushrooms are reported to be one of the seven high-value crops that are intensively cultivated (Ministry of Agriculture Malaysia, 2011). From the sources from Department of Agriculture Malaysia (2015) the total value of mushroom production increased from RM 49.1 million in 2007 to RM 110 million in 2014 which further increased the number of growers, land area and productivity in Malaysia (Rosmiza *et al.*, 2016). Even though the mushroom production provides valuable additional business options to growers, several issues and challenges were identified to obstruct successful mushrooms industry development in Malaysia. The main problem is the limitation of raw materials supply and the increasing cost of substrate especially sawdust (Rosmiza *et al.*, 2016). Since raw materials and preparation of selective formulations are the main cost inputs in mushroom production, growers are looking for alternative ways to reduce the amount of raw materials while lowering their production cost by maximizing mushroom

yield (Royse, 2010). Therefore, the addition of organic agricultural and food waste to the mushroom substrate medium could be a way to reduce the cost (Jo *et al.*, 2013).

The large amounts of solid wastes, residues and by-products discharged from agriculture sector and food processing industries have been continuously increased and cause environmental concerns around the world. New Straits Times reported that Malaysia disposes 3,000 tonnes of avoidable food waste which is the largest contributor of solid waste and source of harmful greenhouse gases in the country (Daim, 2016).The current solutions for food waste disposal was in landfills or incineration, or by dumping into the sea which are not practical and eco-friendly as it creates environmental pollutions (Subbu Lakshmi & Sornaraj, 2014). Therefore, food waste recycling programmes are presently implemented by the government in order to reduce the discharge of organic wastes and turn it into nutrient-rich fertiliser or compost. Since food waste compost (FWC) is a nutritious complex product which can be utilised by heterotrophic organisms such as mushrooms (Stoknes *et al.*, 2008).

Coffee is one of the most important crops due to the high popularity of the beverage worldwide (Murthy & Naidu, 2012). Currently, the coffee grounds that have been used only once by restaurants and cafes are then immediately discarded. Eventually, those wastes of coffee grounds are sent to landfill for disposal with food wastes which also contributed towards greenhouse gases of global warming. However, coffee wastes can be reused to make good soil amendments. According the information developed by Soil and Plant Laboratory Incorporation, the use of Starbucks coffee grounds in amending mineral soils indicated that 25-35 % (v/v) coffee grounds mixed with minerals soils to enhance soil structure (Sunset, 2017). Coffee waste is a valuable organic matters and its nutrients can be recaptured for use as soil fertilisers. The waste

was collected by specialized agencies for different purposes such as composting, bioenergy production and mushroom growth (Pujol *et al.*, 2013).

Apart the wastes from food industries, Malaysia produces an abundant supply of empty fruit bunches from oil palm industry which are considered as wastes and have not been utilized satisfactorily (Aljuboori, 2013). This waste is available throughout the year when the palms are pruned during the harvesting of fresh fruit for the palm oil production. These wastes can become critical environmental issues to our country if proper management is not undertaken. Alternatively, these wastes can be converted to higher value and useful products by chemical and biological process (Wang, 1999). The composting of empty fruit bunches resulting in the production of a stable compost that is suitable for crop production (Thambirajah *et al.*, 1995). Besides, empty fruit bunches and shredded palm press fibres have been used to produce oyster mushroom in order to recycle low quality biomasses into a valuable high protein food (Tabi *et al.*, 2008).

It is believed that oyster mushroom cultivation play an important role in waste disposal management (Das & Mukherjee, 2007). Generally, oyster mushroom grow under a wide range of crop residues and has short growth time (Sánchez, 2010). Their fruiting bodies are less vulnerable to attack by diseases and pest as compared to other edible mushrooms (Hoa *et al.*, 2015). Hence, the combination of crop production and waste mitigation signifies the economic potential for both mushroom farmers and waste handlers (Stoknes *et al.*, 2008).

Pleurotus pulmonarius (Fr.) Quel from the Pleurotaceae family, is a common edible grey oyster mushroom initially cultivated in India after the late of 1940s and are widely grown in warm tropical regions such as Asia (Maftoun *et al.*, 2015). It is a white-rot

fungus which plays an important role in recycling carbohydrates through lignin degradation (Narayanasamy *et al.*, 2008). White rot fungi are able to grow on lignocellulose waste by secreting a battery of extracellular enzymes such as cellulases, xylanases and lignin-modifying enzymes (Adebayo & Martinez-Carrera, 2015). This unique ability has allowed the study of a wide range of substrate materials of lignocellulose waste in grey oyster mushroom cultivation (Pathmashini *et al.*, 2009). Therefore, the cultivation of edible grey oyster mushrooms using coffee waste (CW), food waste compost (FWC) and empty fruit bunches is environmentally friendly and feasible to recycle organic agricultural and food by products into high nutritional and medicinal quality food and also help in solid waste management.

An experiment to assess the standard substrate supplemented with CW, FWC and empty fruit bunch compost (EFBC) at different concentrations (10-40 % w/w) were evaluated in this study in order to expand the growth medium for *P. pulmonarius* cultivation. The linear growth method was adopted to choose the best substrate formulations of the wastes. Based on the results from the linear growth tests, the growth performances, production yield and nutrient profiles of the selected strain was evaluated.

1.2 Significance of the Research

Utilisation of CW, FWC and EFBC in the standard substrate enable to contribute to the mushroom research and development to gain knowledge into improvement for media formulations of mushroom production. On the other hand, the successful cultivation of *P. pulmonarius* on agricultural and food wastes no doubt will help in solving solid waste disposal issues in Malaysia, where solid wastes being consistently produced in large quantities.

1.3 Objectives of the Research

The objectives of this study were to:

i. investigate the substrate formulations of standard substrate supplemented with varying concentrations of CW, FWC and EFBC for the growth of *P. pulmonarius*.

ii. evaluate the growth performance and production yield of *P. pulmonarius* grown on the standard substrate supplemented with CW, FWC and EFBC.

iii. analyze the nutrient profile of *P. pulmonarius* fruiting bodies grown on standard substrate supplemented with CW, FWC and EFBC

CHAPTER 2: LITERATURE REVIEW

2.1 Grey Oyster Mushroom

2.1.1 Taxonomy

Pleurotus spp. belongs to the fungi kingdom and is grouped within Basidiomycota division, in Agaricomycetes class, Agaricales order and in the family of Pleurotaceae. The genus name*, Pleurotus* is believed to be derived from Greek word *pleurē*, meaning side ear (Miles & Chang, 2004). It is reported that there are 40 species of *Pleurotus* mushrooms (Jeena *et al.*, 2014) but only 25 species are commercially cultivated in different parts of the world. *Pleurotus ostreatus* (oyster mushroom), *P. eryngii* (king oyster), *P. pulmonarius* (phoenix oyster mushroom), *P. djamor* (pink oyster mushroom), *P. sajor-caju* (indian oyster), *P. cystidiosus* (abalone oyster), *P. citrinopileatus* (golden oyster mushroom) and *P. cornucopiae* (branched oyster mushroom) are the most popular commercially cultivated edible oyster mushrooms around the world with great economic value (Pérez-Martínez *et al.*, 2015; Knop *et al.*, 2015; Zhang *et al.*, 2017).

Pleurotus pulmonarius (Fr.) Quel. has been known as lung oyster, Indian oyster, Italian oyster and phoenix oyster mushroom (Maftoun *et al*., 2015). In Malaysia, it is locally known as grey oyster or 'Cendawan Tiram Kelabu'. It is typically characterized by a decurrent gills and short stipe as well as white spore print (Miles & Chang, 1997). The grey oyster mushroom develops in shelf-like clusters and its cap grows broadly convex to flat, ranging from 4 to 15cm in width. The colour of the cap appears pale brown to dark brown, depending on the season (Kuo, 2005). The grey oyster mushrooms are soft and have a slightly chewy texture, and can develop a slight seafood aroma and taste.

Figure 2.1: Grey oyster mushrooms*, P. pulmonarius* grown on standard substrate in mushroom house, Fungal Biotechnology Laboratory. September 19, 2017.

2.1.2 Distribution

Pleurotus pulmonarius is pantropical distribution where there is a perfect balance of rain and moderate temperature depending on the varieties. Its natural habitat include trunks and stumps of deciduous trees, and are even artificially cultivated on a large variety of agricultural residues (Ng *et al.*, 2015). It is a saprotrophic fungus and a primary decomposer on wood, it can be used for mycoremediation (Hearst *et al.*, 2009). Generally, this mushroom is whole year round and favors blooming during peak season of late fall. It is extremely famous and highly recognized in Asia, especially in Japan and China where it is served it as a delicacy in stir-fry or soup forms. They are sold fresh in great volumes in the market and often appear in vegetarian cuisine.

2.1.3 Nutritional and Medicinal Properties

Grey oyster mushroom is considered as a functional food which can be used for both culinary and medicinal aspects due to its pleasant taste and desirable pharmacological properties (Mohamed Imran *et al.*, 2011). Fresh grey oyster mushroom can be sold in the marketplace or mushroom industries in large packed at a low price. Besides, it is rich in protein, nonstarchy carbohydrates, dietary fiber, minerals as well as low in fat content

and calorific value (Nutritional values of grey oyster mushroom is given in Table 2.1.). Therefore it could be an excellent inclusion in the diet of individuals with hyperlipidemia and diabetes (Manzi *et al.*, 1999; Valverde *et al.*, 2015). Furthermore, it is claimed to have various medicinal properties (Ng *et al.*, 2015) and a series of compounds have already been precisely defined including several polysaccharides, phenolics, terpenes and sterols (Morais *et al.*, 2000; Sanchez, 2004). Smiderle *et al* (2011) suggested that β-glucans extracted from the polysaccharides of *P. pulmonarius* has analgesic (anti-nociceptive) and anti-inflammatory effects. It also contains anti-proliferative effects based on the test conducted by Lavi *et al* (2010).

Table 2.1: Nutritional components of several edible mushrooms (g/100 g dry matter) (Adapted from: Singh *et al.*, 2011).

Mushroom Type	Carbohydrate	Fiber	Protein	Fat	Ash	Energy (k cal)
Volvariella volvaceae (Paddy Straw)	54.80	5.50	37.50	2.60	1.10	305
Pleurotus sajor-caju (Grey Oyster)	63.40	48.60	19.23	2.70	6.32	412
Pleurotus ostreatus (Tree or Pearl Oyster)	57.60	8.70	30.40	2.20	9.80	265
Agaricus bisporus (Button)	46.17	20.90	33.48	3.10	5.70	499
Calocybe indica (Milky)	64.26	3.40	17.69	4.10	7.43	391
Lentinula edodes (Shiitake)	47.60	28.80	32.93	3.73	5.20	387
Flammulina velutipes (Winter)	73.10	3.70	17.60	1.90	7.40	378
Auricularia auricular-judae (Wood Ear)	82.80	19.80	4.20	8.30	4.70	351

2.1.4 Cultivation of *Pleurotus pulmonarius*

Pleurotus spp. are described to be the second most cultivated edible mushroom worldwide after *Agaricus* species due to its economic and ecological values, as well as medicinal properties (Sánchez, 2004). It is reported that China has been the major producer and contributes to about 88% of the total world production of *Pleurotus* spp. (Jeena *et al.*, 2014). Besides, the cultivation of oyster mushroom is widespread in many

other Asian countries such as Thailand, Vietnam, India, Japan, Korea and Taiwan. The cultivation of this mushroom is not limited by geography because it can be cultivated in temperate and subtropical forests throughout the world, and can be basically found on a variety of sawdust substrate from many types of woods (woodchips from tropical wood, rubber wood and palm wood).

In Malaysia, grey oyster mushroom is one of the top culinary mushrooms cultivated and is highly demanded by consumers. Interest in *P. pulmonarius* cultivation in Malaysia is increasing gradually as it can offer higher income to local growers, and its ability to grow in tropical regions of Malaysia (Islam *et al.*, 2016). There are a lot of issues that exists about oyster mushroom cultivation and production in Malaysia. Generally, sawdust is the primary substrate used by local industry growers, but there are some problems in the use of sawdust for *P. pulmonarius* cultivation. In past years, rubber wood sawdust was easily available and at cheaper rates, but day by day it is becoming scanty and costly. Thus, the use of costly substrates for growing mushrooms subsequently increases their cost of production. Furthermore, the completion among in use of sawdust between mushroom cultivation with other industries is increasing. Hence, alternative substrates to substitute the rubber wood sawdust and optimised the condition for high yield mushroom growth are both desperately needed by our local growers.

Sawdust is the powdery particles of wood produced by sawing. It is the main substrate medium in grey oyster mushroom cultivation. The market price of SD can range from RM 4 to RM 11 per kg in Malaysia through personal communication with farmer. It composed of cellulose, hemicellulose, and lignin which provide carbon and nitrogen sources for growing mushroom. Thus, the suitable solution to alternative substrates for sawdust is by using food and agricultural wastes. In this study, three wastes, namely

coffee waste (CW), food waste compost (FWC) and empty fruit bunch compost (EFBC) were selected as supplemented substrates. The cellulose, hemicellulose and organic matter found in these food and agro-industrial wastes can be the main source of nutrients for growing mushroom (The proximate compositions are given in Table 2.2.). Since the CW, FWC and EFBC are available in abundance with little or no other use and almost free of charge, thus these can be utilised for better recycling through mushroom production. However utilisation CW, FWC and EFBC as alternative substrate medium for cultivation *P. pulmonarius* remain in doubt.

Table 2.2 Proximate chemical composition of different agro wastes.

Compositions	Wastes			
	CW[1]	SD[2]	FWC[2]	EFBC[6]
Cellulose (g)	12.4±0.8	40-55%[5]	NA	33.9±4.7%
Hemicellulose (g)	39.1±1.9	24-40%[5]	NA	15.9±2.5%
Lignin (g)	23.9±1.7	13-25%[5]	NA	38.1±3.1%
pH	6.1[4]	6.9±0.1	7.4±0.1	8.1±0.8
Water content (%)	NA	15.9±0.2	19.5±0.1	51.8±3.7
OM (%)	98.0±1.5[4]	80.3±0.1	68.3±0.6	NA
T-C (%)	44.0±2.0[4]	46.6	39.6	28.81±3.3
T-N (%)	2.8±0.10	0.1±0.0	3.1±0.2	2.3±0.1
C/N ratio	16.9	615.9	12.8	12.4
P(g/kg)	1.8±0.1	0.3±0.0	2.7±0.0	1.4±0.5%
K (g/kg)	11.7±0.0	0.3±0.0	0.9±0.0	2.8±0.6%
Ca(g/kg)	1.2±0.0	3.3±0.2	25.2±0.3	1.0±0.3%
Mg(g/kg)	1.9±0.0	0.3±0.0	1.6±0.0	0.9±0.1%
Na(g/kg)	33.7±8.8	ND	6.1±0.0	NA
Pb(mg/kg)	<1.6	ND	ND	NA
Zn(mg/kg)	8.4±0.2	12.4±6.9	3.3±3.5	157.4±56.0
Cu(mg/kg)	18.7±0.9	4.6±2.2	11.9±1.5	74.3±10.2
Cd(mg/kg)	<0.2	0.4±0.0	0.4±0.0	NA
Ni(mg/kg)	1.2±0.6	ND	ND	19.3±2.4
Mn(mg/kg)	28.8±0.7	NA	NA	151.2±30.8
Co(mg/kg)	15.2±0.1	NA	NA	NA
Fe(mg/kg)	NA	NA	NA	1.0±0.2

Source: 1: Ballesteros et al., (2014), 2: Jo et al., (2013); 3: Rizki and Tamai, (2011); 4: Hachicha et al., (2012); 5: Deraman, (1993); 6: Baharuddin et al., (2010).

CW, coffee waste; SD, Sawdust; FWC, food waste compost; EFBC ,empty fruit bunch compost; OM, organic matter; T-C, total carbon; T-N, total nitrogen; ND, not detected, NA, not available.

2.2 Food Waste Compost

According to Solid Waste Corporation of Malaysia, Malaysia generates about 38,000 tonnes of waste per day. Around 15,000 tonnes are food waste, and approximately 8000 tonnes (60 %) of waste that is being generated is avoidable food waste (The Star Online, 2016). Large amounts of food waste are produced from schools, restaurants, hotels, farms, and also supermarkets. The cost of managing waste increases and special space in landfills will be needed. As a result, those food wastes mostly end up rotting in landfills. In order to solve this issue, food waste is recycled in forms such as compost and fertilisers.

Currently, the Zero Waste Campaign (ZWC) organized by Universiti Malaya (UM) is a food waste recycling program for collection food waste from food outlets in campuses to decompose them into useful compost (The Star Online, 2015). Since FWC is a nutritious product and contains low toxic concentration (Table 2.2) , it can be utilised by heterotrophic organisms such as fungi (Stoknes *et al.*, 2008). Jo *et al.* (2013) demonstrated the potential usage of FWC as a component of a growth medium for production of *Ganoderma lucidum* because this practice has the dual benefits of increasing yield and disposal of organic wastes (Yang *et al.*, 2003). Nevertheless, information on mushroom cultivation using FWC as the substrate medium is currently limited and its effectiveness in contributing to mushroom growth is still to be confirmed in mushroom research and development (Chae & Ahn, 2013).

2.3 Coffee Wastes

Coffee is one of the most popular and appreciated drinks around the world being consumed for its refreshing and stimulating properties, which are defined by its green bean composition and changes occurring during the roasting process (Mussatto *et al.*, 2011). As a consequence of this high demand, the coffee industry is generating large

quantities of residues which include spent coffee grounds (SCG) and coffee silver skin (CS). SCG is the residual material obtained throughout the treatment of coffee powder with hot water or steam for instant coffee preparation. Nearly 50 % of the world coffee production is processed for soluble coffee preparation, which produces around 6 million tons of SCG per year (Mussatto *et al.*, 2011).

In recent years, SCG and CS have attracted great attention since they are generated in large amounts every year and represent a great pollution hazard if discarded into the environment. However, since these residues are derived from coffee bean, they are expected to have composition and properties similar to these beans (Table 2.2) and thus could be exploited for different industrial applications (Ballesteros *et al.*, 2014). Hence, some alternatives have been proposed to reuse these coffee residues. One of the ways is that SCG can be used as a substrate for fungal cultivation (Machado *et al.*, 2013). In spite of these possible applications, CW are still underutilised as a valuable material; it is necessary to focus on the exploitation of CW in order to add value to these unused materials and decreasing their impact to the environment.

2.4 Oil Palm Wastes

Palm oil has made remarkable and incessant growth in the global market over the past four decades, and it is expected that in the period of 2016 – 2020, the average annual production of palm oil in Malaysia will reach 15.4 million tonnes (Abdullah & Sulaiman, 2013). The largest amounts of oil palm wastes being created are empty fruit bunches (EFB), oil palm fronds (OPF) and palm pressed fibres (PPF). About 60 % of all the produced fibre and shell waste are burnt to generate electricity and the remaining 40 % of the waste is removed by contractors to be put into landfills owing to a ban on open burning of agricultural waste in Malaysia (Abd Razak *et al.*, 2013). These palm oil wastes

are heterogeneous water insoluble materials consisting of cellulose, hemicelluloses and lignin; and to a lesser extent pectin, starch and other polysaccharides (Thomsen, 2005). The problems associated with the disposal of these solid wastes and high cellulosic components of EFBC make these a potential source of substrate for mushroom production (Table 2.2). According to Wang and Yang (2007), expensive treatments or disposal methods are necessary if these wastes are not going to be recycled. Ultimately, adverse effects on the environment would also be a problem if these wastes are not managed well and left in the waste stream.

2.5 General Nutritional Requirement for Mushroom Growth

Mushrooms, unlike plants, cannot undergo photosynthesis because of the absence of chlorophyll and thus it can only get the nutrients from organic materials. Mushrooms need carbon, nitrogen and inorganic compounds as their nutritional sources (Sharma *et al.*, 2013). The main organic sources needed by the mushrooms are carbon sources such as cellulose, hemicelluloses and lignin, along with other compounds which can be easily digested by the extracellular enzymes in order to provide nutrients for mushroom growth. These enzymes present a non-specific biocatalyst mechanism and have been used for bioremediation process due to their ability to degrade azo, heterocyclic, reactive and polymeric dyes (Baldrian & Snajdr, 2006; Forgacs *et al.*, 2004).

The organic compounds can be converted to the various carbohydrates, proteins, lipids, purines, pyrimidines and vitamins once they have moved into the fungal cells, where they are needed for central activities and structural needs of the fungi (Miles & Chang, 2004). For this purpose, types and formulation of substrates chosen are critical in mushroom production where they should contain a balance of nutritional compounds.

2.5.1 Basic Substrate

Substrates used in mushroom cultivation have effects on chemical, functional and sensorial characteristics of mushrooms (Oyetayo & Ariyo, 2013). Since *Pleurotus* spp. is a saprophyte, nutrients from a variety of agricultural residues through mycelium can be obtained for growth and development. Agricultural waste is created in huge amounts, and it becomes an interesting substrate because of its commercial exploitation as well as connected environmental issues (Silva *et al.*, 2007). Many studies have been conducted to investigate the ability of *Pleurotus* spp. to grow on different agro-industrial waste, such as rice straw, wheat straw, cotton waste, pine needles, olive mill waste and so forth. (Hussain *et al.*, 2002; Pant *et al.*, 2006; Kalmis *et al.*, 2008; Al-Momany & Ananbeh, 2011).

A mixture of agro-wastes can be remarkable. According to Owaid *et al.* (2015), productivity and biological efficiency were improved in some combinations when compared with wheat straw. This is due to the differences in the capability of such substrates to aid the nutritional and environmental requirements, and also variance in cellulose, hemicellulose and lignin contents (Kuhad *et al.*, 1997). Beside, Mukhopadhyay *et al.* (2002) and Curvetto *et al.* (2002) reported that both qualitative and quantitative aspects (biological productivity and efficiency) are influenced by the type of nutrient and growth conditions in fungus growth and development. For instance, wheat straw was found to be superior over other types of agricultural residues in colonization and production rates (Philippoussis *et al.*, 2001; Pant *et al.*, 2006; Fanadzo *et al.*, 2010).

Carbon, nitrogen, minerals and vitamins are the four essential chemical compounds required by mushroom for growth. Therefore, to ensure success in mushroom cultivation, all four compounds should be sufficiently present in the basic substrate with emphasis on

a balanced content of carbon and nitrogen. Carbon and nitrogen play an important role in the overall process of mushroom cultivation. The various forms of carbon sources such as monosaccharides, oligosaccharides and polysaccharides are significant for the mycelium growth, especially polysaccharides (cellulose and hemicellulose). Polysaccharides are mostly hydrolysed to produce sugar while nitrogen sources are needed by fungi to synthesise proteins as constituents of their cell wall (Miles & Chang, 1997).

2.5.2 Supplement

Use of supplements in mushroom cultivation can enhance the nutritional content, accelerate the mycelium growth as well as increase the production yield (Philippoussis, 2009; Royse 1997). There are a wide range of protein rich-materials that can be used in mushroom cultivation, for example rice bran, wheat bran, spent grain, spent yeast, molasses, cottons and coffee wastes. According to Sánchez (2004), supplementing may cause overheating of the substrate if growers are not familiar to anticipate and control the temperature and humidity in order to maintain a steady substrate temperature. In addition, there might be risks from contaminants and insects because supplementation changes the number and the types of supported (Stamets, 1993). In other words, contamination can easily occur if the supplementation of the substrate is not added appropriately. Hence, extra caution should be required to prevent any contamination and ensure a successful supplementation. One of the methods to avoid those problems is by prolonging the pasteurization cycle of the substrates.

Although many studies have reported an increase in production yield of mushroom by adding supplements to bulk substrate, it depends on the individual types of mushrooms cultivated, as well as the types and concentration of supplements used. However,

excessive of use of supplements can results in adverse effect of the substrate on mushroom production. According to Royse (1997), the addition of different starch-based supplements such as wheat, rice bran, millet and maize powder to sawdust serve as major nutrients to provide an optimum growth medium for mushroom cultivation. Research conducted by Chang (1996) and Okhuoya *et al.* (2005) exhibited improvement of the production, quality, flavour, and shelf life of cultivated mushroom by adding supplements to the substrate. It was found that the positive effect of the supplementation can be correlated with the nutrients present in those additives.

2.6 Stages in Mushroom Cultivation

Mushroom culture includes several different stages, each of which must be carefully performed. According to Dung *et al.* (2012), the procedure of cultivating oyster mushrooms has three main steps: isolating mushroom from fruiting bodies, preparing spawn, and cultivating mushrooms from these spawns to harvest fruiting bodies. Basically, these stages involved inoculation and incubation of the specific fungus in a suitable substrate, followed by colonisation of the fungal mycelium on the substrate and eventually growth of fruiting bodies for harvesting. The life cycle of oyster mushroom shows the typical growth pattern of basidiomycetes which are divided into vegetative phase (mycelium growth) and reproductive phase (fruiting body) (Adebayo & Martinez-Carrera, 2015). In order to provide good growth of mushroom, substrates chosen for both phases are crucial to provide a suitable environment for mushroom cultivation. Supplementation of substrate should also be taken in account in both phases for enhancing mushroom growth and fruiting.

2.6.1 Inoculation and Tissue Culture

The first stage of mushroom cultivation is to obtain pure mycelium of the specific mushroom strain. The mycelium can be obtained from spores, pieces of the specific mushroom or from mother culture providers such as Mushroom Research Centre (MRC), Universiti Malaya which has been authenticated by experts. Matured, healthy and fresh mushrooms are collected and split in half by hand longitudinally. Some inner tissue is usually taken from the upper part of the mushroom stripe and placed on PDA plates which can be kept at room temperature (28 ± 2ºC) for 8 days. Within 2 or 3 days, some cotton white and delicate mycelia are produced from that small piece of tissue (Subbu Lakshmi & Sornaraj, 2014). The mycelium are grown rapidly until the surface of the agar medium is completely shielded. The growth of mycelium around the area where tissue is introduced on agar without any contamination is considered a positive growth. Then, it is considered ready to be transferred to spawn substrate to make spawn.

2.6.2 Spawn Production

Zadrazil *et al.* (2004) reported that spawn in mycelium running phase involves the growth of mycelium through the substrate; as well as inoculation and mycelium biodegradation of the specific bulk substrate. Together with the mycelia itself, this supports the formation of fruiting bodies. To obtain an inoculum, the mycelium is grown on cereal grain, for example wheat, millet, corn or rye, which is frequently called the "spawn" The mycelium-coated grain act as intermediate medium or "seed" to allow the chosen mycelium to multiply before letting it speedily colonise the specific bulk substrate (Miles & Chang, 2004). The success of mushroom production depends on the quality of spawn, which must be prepared under sterile conditions to diminish contamination of the substrates. During this phase, it is important to maintain moderate humidity and temperature in order to achieve optimum growth of spawn, so the mycelia can fill over

the compost a period of weeks. The phase in which the spawn was produced is called as vegetative phase in the life cycle of mushroom.

The substrate used in inoculum or spawn production may be different from materials used in the cultivation of mushroom. Several studies have been conducted to improve the quality and techniques for mushroom production. For instance, the spawn for cultivation of *P. pulmonarius* has been prepared in different ways: on grains, such as wheat, sorghum and oat (Asghar *et al.*, 2007). Production of spawn is considered as a difficult and fastidious task and is often regarded as non-practical for the common mushroom grower. Hence, it is usually produced by spawn manufacturers using microbiological sterile techniques (Philippoussis, 2009). A more rapid spawn run would reduce the amount of time where non-colonised substrate is exposed to competitors such as molds and bacteria.

2.6.3 Mushroom Growth

When spawn running phases are completed, pinheads begin to form under suitable environmental conditions. This is followed by the production of fruiting bodies. Normally, the bags are maintained under optimal temperature and moisture content as well as growth conditions that favour fruiting. The maximum yield of *P. sajor-caju* was found during rainy seasons, when the temperature was nearly 20-26 ºC and the relative humidity was around 70-90%. The humidity during formation of pinheads were at 80-90 %, while the relative humidity for development of fruiting bodies were at 75-80% (Iwade & Mizuno, 1997). Studies on mushroom cultivation found that the use of different strains, different lignocellulosic substrates, types of spawn, and physiochemical conditions have an influence on yield and growth of each particular mushroom (Mandeel *et al.*, 2005; Savedra *et al.*, 2006; Kirbag & Akyuz, 2008; Onuoha, 2009).

Figure 2.2: Baby grey oyster mushrooms emerging from substrate bag in mushroom house, Fungal Biotechnology Laboratory. November 11, 2017.

2.7 Environmental Conditions

A number of parameters influence the survival and activity of mycelium in any given environment. Occurrence and abundance of fungus in an environment are determined not only by the available carbon sources, but also various physical and chemical factors. These include oxygen availability (aeration), nutrient availability, temperature, and water activity (humidity). Inhibition of mycelium growth can be caused by a limitation imposed by any one of these factors, but the cause of contaminant are sometimes difficult to control.

2.7.1 Temperature

Australian Mushroom Growers Association (AMGA) reported (cited in Bellettini *et al.*, 2019) the major extrinsic factors that affect stipe height, stipe and cap diameters in mushroom are temperature, humidity, clean air and compact material. Oyster mushroom can grow at adequate temperatures ranging from 18 to 30 ºC (Meijia & Alberto, 2013). Generally, the substrate-containing inoculum was successively kept in a dark room at 23º C for spawn running (Li *et al*., 2015). According to Oei and Nieuwenhuijzen (2005), the optimal incubation temperature ranges from 5 to 30º C for the cultivation of *P. pulmonarius*; this means that at that temperature range, mycelium can stay viable but the growth rate can decline at both high and low ends of this range. Besides, Oei and

Nieuwenhuijzen (2005) reported that the optimal temperature required for fruiting ranges from 20 to 25 ºC. Moreover, the optimal environment for mycelium growth and their subsequent fruiting is usually very diverse for cultivation of *Pleurotus* spp. (Bellettini *et al.*, 2019). Fruiting body development is often stimulated after significantly altering environmental circumstances (Thomas *et al.*, 2013). According to Oei (2003) and Owaid *et al.* (2015), the temperature can be changed to 10-16 ºC to induce fructification when substrates were fully colonised. It was found that stipe height and cap size of mushrooms can be affected by lower temperatures and dry condition (Sher *et al.*, 2010).

2.7.2 Humidity

The humidity range is fairly wide for most fungi - from 20 to 70 % (Pandey *et al.*, 2001). In order to enable satisfactory growth of *Pleurotus* spp., a suitable humidity for the duration of darkened spawn-running and mycelia induction should cover a range between 60–75 % and 85–97 % respectively in the environment (Miles & Chang, 2004 ; Li *et al.*, 2015). High humidity is favourable for pinheads and fruiting bodies formation naturally. During the growth of *P. flabellatus* on sawdust supplemented with fishery wastes, the relative humidity of the culture room was maintained at 85–90 % by pouring 25 L of water on the floor and wall per day (Subbu Lakshmi & Sornaraj, 2014). Similarly, Kim *et al.* (2013) also cultivated *P. eryngii* where the humidity of the incubation room was maintained at 85–95 %.

2.7.3 Aeration

Oxygen is crucial for mushroom during fructification. Bellettini *et al.* (2019) reported that the development of microorganisms were dependant on oxygen flow speed through the substrate and the speed of oxygen consumption by microorganisms through gaseous exchange with the environment. The study also revealed that aeration has various

functions, which include oxygen provision for aerobic growth and metabolism, moisture adjustment, temperature regulation, as well as metabolite elimination of water vapour, carbon dioxide and volatile compounds.

Aerobic mushrooms require oxygen for their survival and development through lignin degradation. During the spawn running stage, it is important to keep carbon dioxide concentration at 2000–2500 mgL^{-1}. After the mycelium run is complete and pinhead growth is stimulated, fruiting bodies were allowed to develop in an environment where carbon dioxide level is around 1500–2000mgL^{-1} (Li *et al.*, 2015). According to Kalmis and Sargin (2004), the carbon dioxide level was maintained around 1000 ppm by aeration after pinhead formation. As air holds high carbon dioxide levels, it will yield mushrooms with thick and short stipe pileus. Hence, a decrease in carbon dioxide level is required during the fruiting stage, as well as an increase in oxygen level.

On the other hand, carbon dioxide and oxygen concentration inside plastic mushroom growing bags can affect the speed of colonization and growth of mushrooms. If the substrate bag possesses good porosity and sustains oxygen supply within the matrix of solid particles, gaseous exchange efficiency can contribute to the normal growth and development of mushroom (Gregori *et al.*, 2007).

2.7.4 Luminosity

Light is not necessary to be induced at the beginning of the mycelium growth phase, but it is required for fruiting body production. Roshita *et al.* (2018) studied the effect of different colours of light that produce the highest yield with good physical quality of oyster mushrooms. The colours used include fluorescent light (185-254 nm). T, blue LED (450 to 475 nm), green LED (520-530 nm) and red LED (564-580 nm) for fruiting

induction after mycelia growth. The result from the study showed that blue LED appeared to be the most effective in enhancing the production of oyster mushrooms. In a study conducted by Kues and Liu (2000), the active wavelengths that control fruiting body induction and maturation were found to be in the range of blue light (520nm).

According to Oei and Nieuwenhuijzen (2005), oyster mushrooms do not produce cap, but a mushroom stipe-like structure of coral in the complete absence of light. High density of light in the environment can yield mushrooms with paleness, deformations, decoloration, elongated stipes and reduction of cap diameter (Marino *et al.*, 2003).

CHAPTER 3: MATERIALS AND METHODS

3.1 Mushroom Strain

The locally grown *P. pulmonarius* (strains S01 and S02) were subcultured from the mother cultures obtained from MRC (Mushroom Research Centre), UM. The identification were confirmed by mycologist, Associate Prof. Dr. Tan Yee Shin at MRC The code and origin of *P. pulmonarius* used in current study was showed in Table 3.1. The tissue culture were maintained on potato dextrose agar (PDA, Difco, Maryland, USA) and pH 6.0 ± 0.2 in room temperature, 28 ± 2 ºC for 10 to 14 days.

Table 3.1: The code and origin of *P. pulmonarius* used in current study.

Strains	Code[1]	Origin
S01	UMP001 (Thai)	Jenjarom, Selangor
S02	UMP002 (PL27)	Semenyih Selangor

[1] Avin *et al.*, 2016

3.1.1 Master Stock Preparation

Master stock of two strains of *P. pulmonarius* were prepared as back up prior to protection of cultures loss through contamination on the subcultured petri plates. Master stock cultures were prepared by storing the mycelium plugs of strains and preserved in sterile distilled water for long term preservation (Diamantopoulou & Philippoussis, 2015). The Bijou bottles were half filled with distilled water and moderately tighten with its caps before sterilised in an autoclave at 121 ºC (15 psi) for 15 minutes. After the sterilisation, the Bijou bottles were allowed to cool down and ready to be used. In laminar flow, the mouth of Bijou bottles were flamed and filled with 5mm disc mycelium plugs under sterilised condition. One bottles was filled with 5 to 6 mycelium plugs from 10 to 14 days old mycelium culture. The cap were closed tightly and the mouth of the Bijou bottles were flamed lightly again before parafilm were sealed to avoid any contamination. For

one strain, five Bijou bottles of master were prepared and stored at 4 ºC under refrigerator for a long term period (Paul *et al.*, 2015).

3.2 Inoculum Spawn Production

The sorghum grains were cleaned manually and soaked overnight until moisture level up to 60-70 % (Asghar *et al.*, 2007). The sorghum grains (80g per flask) were supplemented with 1 % (w/w) calcium carbonate $CaCO_3$ and put into 250 ml conical flasks, closed with cotton plug and covered aluminium foil before sterilised in an autoclave at 121 ºC (15psi) for 15 minutes. After the sterilisation, flasks were allowed to cool down for 24 hours before inoculating with mycelium culture of *P. pulmonarius*. One flask was filled with 5 to 7 mycelium plugs which age ranged 10 to 14 days. The flasks were incubated at 25± 2 ºC in dark condition for 14 to 21 days (Bonatti *et al.*, 2004; Pathmashini *et al.*, 2009) until the mycelium colonised the entire grains.

3.3 Substrate Preparations for Race Tubes

Food waste compost (FWC) was obtained from Zero Waste site in UM, Kuala Lumpur, Malaysia. The coffee waste (CW) was collected from Starbucks Coffee shops and empty fruit bunch compost (EFBC) was obtained from Dr Fauziah binti Shahul Hamid, Solid Waste Management Lab in UM.

In order to choose the best supplementation level in the standard formulation for each waste for each strain, linear growth study was carried out in glass race tubes (150 mm x 25 mm). The formulation of substrate consisted of standard growth medium supplemented with different concentrations of FWC or CW or EFBC. The standard growth medium (S) consisted of 89 % (w/w) sawdust (SD), 10 % (w/w) rice bran (RB) and 1 % (w/w) calcium carbonate ($CaCO_3$). For substrate supplemented with CW or FWC

or EFBC formulations, their contents were 10, 20, 30 and 40 % (w/w) respectively (Jo *et al.*, 2013); the reminder of the medium consisted of standard growth medium (Table 3.2). The moisture content of the substrate was maintained around 75 to 85 % (Das and Mukherjee, 2007) and the pH was stabilised by $CaCO_3$ at 5.5-6.5 (Smith *et al.*, 2002). The race tubes were stopped with non-absorbent cotton plugs and sterilised in an autoclave at 121 ºC (15psi) for 15 minutes. After the sterilisation, each tubes were cooled down to room temperature for 24 hours and then inoculated with mycelium plug. The race tubes were incubated 25± 2 ºC in dark condition and visible mycelium extension was measured every 3 days. A total of five replicates of race tubes was performed for each composition.

Table 3.2: The substrate formulations used in current study.

No.	Substrate formulations (percentage) and moisture content	C:N ratio[1]
1.	S= SD+RB (90:10), 80%	396:1
2.	S+CW(90:10), 80%	358:1
3.	S+CW(80:20), 80%	321:1
4.	S+CW(70:30), 80%	284:1
5.	S+CW(60:40), 80%	286:1
6.	S+FWC(90:10), 80%	357:1
7.	S+FWC(80:20), 80%	318:1
8.	S+FWC(70:30), 80%	279:1
9.	S+FWC(60:40), 80%	240:1
10.	S+EFBC(90:10), 80%	357:1
11.	S+ EFBC (80:20), 80%	319:1
12.	S+ EFBC (70:30), 80%	281:1
13.	S+ EFBC (60:40), 80%	242:1

[1]Mathematical calculation of carbon to nitrogen ratio of the substrate formulations have shown in Appendix B.

3.4 Bag Cultures

After confirmation with the best substrate formulation from each wastes and strains in linear growth study, the best formulations of the substrate from the wastes and strains were prepared for bag cultures (with a replicate of fifteen for each category, n = 15). The moisture content of the mixture of substrate was maintained at 75-85 % (Das &

Mukherjee, 2007). The pH was stabilised by $CaCO_3$ at 5.5-6.5 (Smith *et al.*, 2002). Mixture of substrates (300 g) were placed into transparent polyethylene bags and covered with closed caps and neck before sterilised in the autoclave for 15 minutes at 121 ºC (15psi). After sterilization, the bags were cooled down to room temperature for 24 hours. The sterilized bags were inoculated with wholly colonized spawn. All the bags were kept in a darkened spawn running room at 25 ± 2 ºC until spawn run completed (Jo *et al.*, 2013; Pathmashini *et al.*, 2009).

3.5 Cropping and Harvesting

Mushroom bags were transferred and kept open in the mushroom house for fruiting after completed the spawn run process. Mushrooms house condition were maintained at 28 ± 2 ºC with 80 % relative humidity (Jo *et al.*, 2013).

Figure 3.1: Mushroom house at Fungal Biotechnology Laboratory. November 5, 2018. **(a)** Outside view of mushroom house **(b)** Internal view of mushroom house.

The mushrooms were harvested manually and weighed for further quantitative and chemical analysis. The following measurements were recorded: days required for the completion of spawn run, pinheads formation and duration from opening to first harvesting in the substrate bag, total weight of harvest fruiting bodies, diameter and stipe length of mushroom, the percentage of bags contaminated and successfully fruiting.

Furthermore, biological efficiency (BE) based on four fruiting (first to fourth harvest) was calculated using the formula below:

$$\text{Biological efficiency} = \frac{\text{Total fresh weight of harvested mushrooms}}{\text{Dry weight of substrate}} \times 100$$

(Adapted from Subbu Lakshmi and Sornaraj, 2014)

3.6 Statistical Analysis

Data obtained were analysed using ANOVA through SPSS software and Ducan's Multiple Range Test (DMRT) at 95 % least significant difference (LSD). Table were constructed for evaluation and comparison of their linear growth rate, growth performance, yield and BE of *P. pulmonarius* in order to select the best strain with their substrate formulations for nutrient analysis.

3.7 Nutrient Analysis

At least 2 kg of fresh *P. pulmonarius* was used for freeze-drying and a total of 200 g freeze dried was outsourced to analyse the nutrient profile (total sugar content, total protein content, total phenolic content, and β-glucan content). The methodology for determination of nutrient profile were shown in Appendix F.

CHAPTER 4: RESULTS

4.1 Linear Growth Study

The selection of best supplemented substrates were based on the mycelium growth rate and compactness (Figure 4.1).

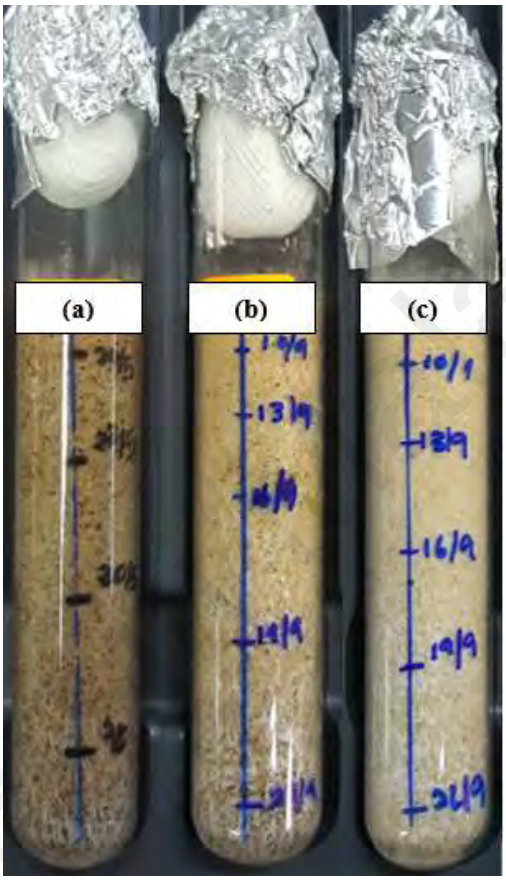

Figure 4.1: Mycelium compactness of *P. pulmonarius* on control substrate in race tubes. **(a)** Sparsely mycelia growth marked as"+" **(b)** Less compact mycelia growth marked as"++"**(c)** Compact mycelia growth marked as"+++". (Abd Razak *et al.*, 2013).

4.1.1 Effect of Coffee Waste on Linear Growth

Based on Table 4.1, both strains grown on control substrate gave the fastest growth rate (0.53±0.02 cm d^{-1} and 0.46±0.02 cm d^{-1}), whereas both strains grown on standard substrate supplemented with 40 % (w/w) of CW showed the lowest growth rate, (0.33±0.01 cm d^{-1} and 0.26±0.00 cm d^{-1}). However, both strains grown in standard substrate supplemented with 10 % (w/w) of CW did not show a significant difference (p

< 0.05) in mycelium growth rate when compared to the control substrate (Table 4.1) (see Appendix C). Besides, both strains grown on 10 % (w/w) of CW supplementation showed less compact mycelia growth as compared to the control substrate. Therefore, for both strains a 10 % (w/w) of CW supplementation was selected for bag preparation.

Table 4.1: Linear growth rate of mycelium grown on standard substrate supplemented with varying concentrations of CW.

Strains	S01		S02	
Substrate formulations/ Criterias	Linear Growth Rate (cm/day)	Mycelium thickness	Linear Growth Rate (cm/day)	Mycelium thickness
Control	0.53±0.02^{d}	++	0.46±0.02^{d}	++
10 % CW	0.52±0.03^{d}	++	0.45±0.01^{d}	++
20 % CW	0.43±0.02^{c}	+	0.37±0.02^{c}	+
30 % CW	0.38±0.05^{b}	+	0.32±0.01^{b}	+
40 % CW	0.33±0.01^{a}	+	0.26±0.00^{a}	+

Values are means of 5 replicates ±; standard deviation. Values in a column followed by the same alphabet indicate no significant different ($p < 0.05$) in linear growth rate.

4.1.2 Effect of Food Waste Compost on Linear Growth

The mycelium growth rate for strain S01 obtained in standard substrate supplemented with different concentrations of FWC were significantly different ($p < 0.05$) when compared to the control substrate (Table 4.2) (see Appendix C). Strain S01 grown on control substrate showed the lowest growth rate (0.60±0.02 cm d^{-1}) whereas when grown on 30 % (w/w) of FWC supplementation showed the fastest growth rate (0.69±0.04 cm d^{-1}). However, compact mycelium growth was observed at 10 % (w/w) of FWC supplementation while loose mycelium growth was observed at 40 % (w/w) of FWC supplementation for strain S01. Besides, strain S01 grown on 10 % (w/w) of FWC supplementation showed the mycelium grew gradually beyond the medium on race tubes and thus chosen for bag cultivation (Figure 4.2).

Meanwhile, the mycelium growth rate for strain S02 obtained in standard substrate supplemented with different concentrations of FWC showed a significant difference (p <

0.05) when compared to the control substrate (Table 4.2) (see Appendix C). Strain S02 grown on control substrate showed the lowest growth rate (0.57±0.02 cm d^{-1}) whereas when grown on 30 % and 40 % (w/w) of FWC supplementation showed the fastest growth rate (0.71±0.0 cm d^{-1}). However, strain S02 grown on 10 % and 20 % (w/w) of FWC supplementations supported compact mycelium growth. Hence, strain S02 grown on 20 % (w/w) of FWC supplementation was selected due to ability of mycelium to degrade at higher concentration of waste which could contribute to lesser waste disposal.

Table 4.2: Linear growth rate of mycelium grown on standard substrate supplemented with varying concentrations of FWC.

Strains	S01		S02	
Substrate formulations/ Criterias	Linear Growth Rate (cm/day)	Mycelium thickness	Linear Growth Rate (cm/day)	Mycelium thickness
Control	0.60±0.02[a]	++	0.57±0.02[a]	++
10 % FWC	0.68±0.02[b]	+++	0.70±0.03[b]	+++
20 % FWC	0.67±0.00[b]	+	0.70±0.03[b]	+++
30 % FWC	0.69±0.04[b]	+	0.71±0.00[b]	++
40 % FWC	0.67±0.00[b]	+	0.71±0.00[b]	+

Values are means of 5 replicates ±; standard deviation. Values in a column followed by the same alphabet indicate no significant different ($p < 0.05$) in linear growth rate.

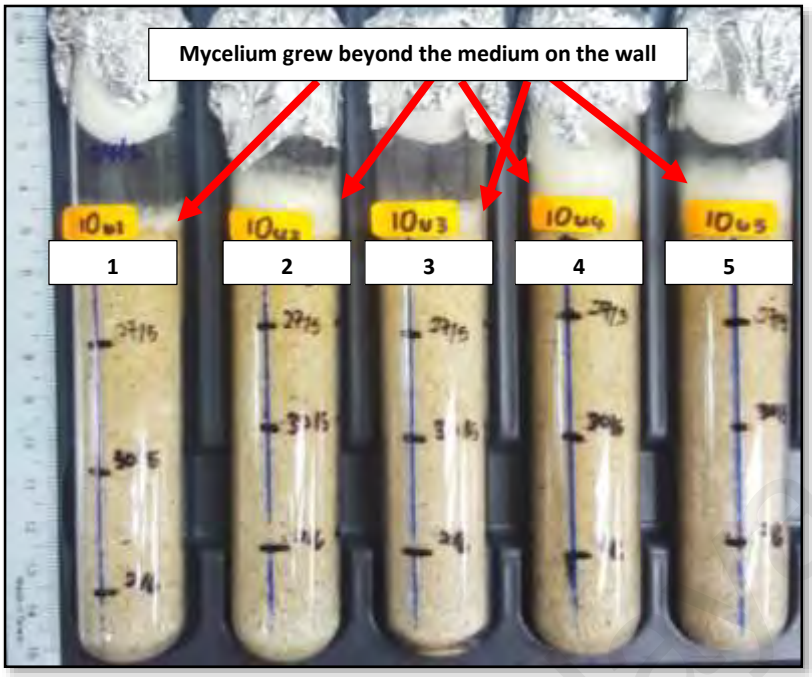

Figure 4.2: Mycelium grew gradually beyond the medium on race tubes on the 18th day after the inoculation. Number stated on the race tubes presented are replicate numbers. The mycelium grew above the medium in the race tubes in 10 % (w/w) of FWC supplementation.

4.1.3 Effect of Empty Fruit Bunch Compost on Linear Growth

Both strains grown on standard substrate supplemented with 30 % (w/w) of EFBC showed the most rapid mycelium growth rate, which was 0.75±0.03 cm d^{-1} (Table 4.3). Both strains grown on 20 % and 30 % (w/w) of EFBC supplementations supported compact mycelium growth, while grown on 40 % (w/w) of EFBC supplementation had sparse mycelium growth. Since the mycelium growth rate for strain S01 grown on 30 % (w/w) of EFBC supplementation displayed a significant different ($p < 0.05$) compared to grown on the control substrate (Table 4.3) (see Appendix C) and the pinheads formation was observed after the spawn run completed (Figure 4.3), thus, it was selected for bag preparations. The mycelium growth rate for strain S02 grown on 30 % (w/w) of EFBC supplementation was significantly higher ($p < 0.05$) compared to control substrate and 20 % (w/w) of EFBC supplementation (Table 4.3) (see Appendix C). Hence, it was selected for bag preparation.

Table 4.3: Linear growth rate of mycelium grown on standard substrate supplemented with varying concentrations of EFBC.

Strains	S01		S02	
Substrate formulations/ Criterias	Linear Growth Rate (cm/day)	Mycelium thickness	Linear Growth Rate (cm/day)	Mycelium thickness
Control	0.63±0.03^{a,b}	++	0.58±0.03^{a}	++
10 % EFBC	0.55±0.16^{a}	++	0.58±0.04^{a}	++
20 % EFBC	0.67±0.00^{b,c}	+++	0.67±0.00^{b}	+++
30 % EFBC	0.75±0.03^{c}	+++	0.75±0.03^{d}	+++
40 % EFBC	0.62±0.06^{a,b}	+	0.71±0.00^{c}	+

Values are means of 5 replicates ±; standard deviation. Values in a column followed by the same alphabet indicate no significant different ($p < 0.05$) in linear growth rate.

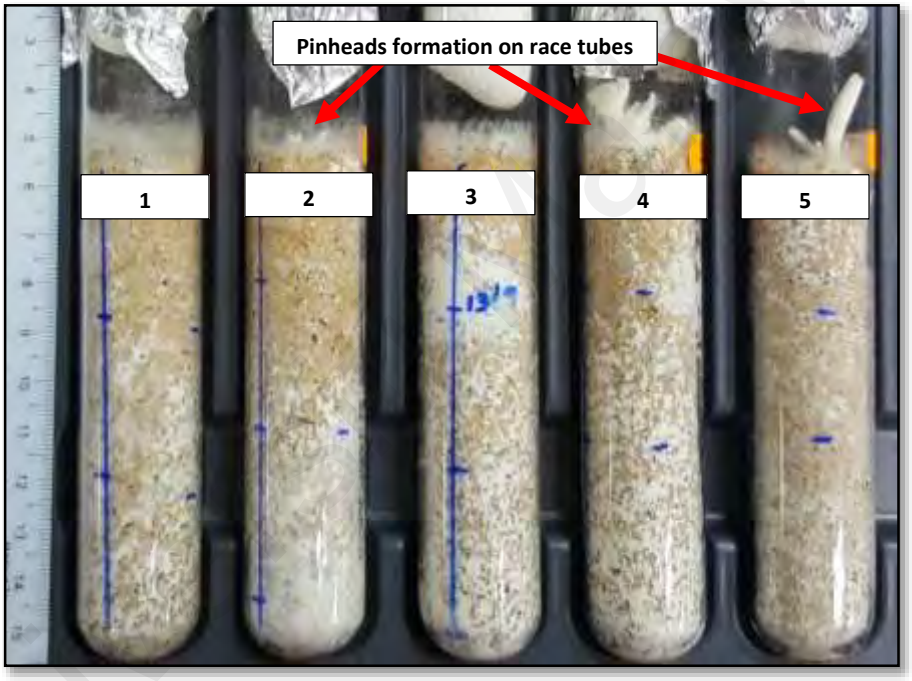

Figure 4.3: Pinheads were visible in 30 % (w/w) of EFBC supplementation on the 18th day after the inoculation. Number stated on the race tubes presented are replicate numbers. Replicate number 2, 4 and 5 even showed pinheads formation after fully grown.

4.2 Fruiting Bodies Production of *Pleurotus pulmonarius*

Based on the selection criterias, a total of eight substrate formulations from both strains initially chosen for the bag preparations, included: control substrate and 10 % (w/w) of CW substrate supplementation for both strains respectively, 10 % (w/w) of FWC supplementation for strain S01, 20 % (w/w) of FWC supplementation for strain S02 and 30 % (w/w) of EFBC supplementation for both strains. The eight substrate formulations

from both strains were cultured in mushrooms house with 15 individual bags. The final decision to choose the best strain for nutritional analysis was made by an evaluation and comparison of their growth performance, yield and BE of *P. pulmonarius*.

4.2.1 Growth Performance of of *Pleurotus pulmonarius*

The evaluation and comparison of growth performance (days required for mycelium running, pinheads formation and from opened to first harvest as well as measurement of cap diameter and stipe length of two strains of *P. pulmonarius* grown on selected substrate formulations was shown in Table 4.4.

The result showed that strain S02 grown on control substrate was recorded the fastest (14.64±4.67 days) in complete colonizing the substrates, whereas strain S01 grown on control substrate was recorded the slowest (31.20±2.78 days) in complete colonizing the substrates. Strain S02 grown on control substrate and 10 % (w/w) of CW supplementation were significantly higher ($p < 0.05$) in the days required for mycelium running compared to all the formulations grown with strain S01 (Table 4.4) (see Appendix D). Days required for pinheads formation ranging from 13 to 25 days on various wastes used. Strain S02 grown on 10 % (w/w) of CW supplementation was recorded the shortest number of days for pinheads formation (13.60±1.60 days), while when grown on 30 % (w/w) of EFBC supplementation was recorded the longest (25.13±3.94 days).

Strain S02 grown on control substrate showed the shortest number of days (16.20±2.31 days) required from opened to first harvest and strain S01 grown on 10 % (w/w) of CW supplementation showed the longest number of days (27.20±8.99 days). Apparently, most of the substrate formulations (control substrate, 10 % (w/w) of CW supplementation and 20 % (w/w) of FWC supplementation) grown with strain S02 was significantly higher in

the days required from opened to first harvested compared to all the formulations grown with strain S01 (Table 4.4) (see Appendix D).

Both strains grown on different formulations were significantly difference ($p<0.05$) in cap diameter and stipe length of (Table 4.4) (see Appendix D). Strain S02 grown on 30 % (w/w) of EFBC supplementation showed the larger (8.44±2.25 cm) cap diameter, while strain S01 grown on 10 % (w/w) of FWC supplementation showed the smallest (5.43±2.82 cm) cap diameter. Besides, the result showed that strain S02 grown on standard substrate was recorded the longest (4.76±1.11 cm) in stipe length and strain S01 grown on 30 % (w/w) of EFBC supplementation was recorded the shortest (3.15±1.49 cm). Interestingly, most of the formulations grown with strain S02 had wider cap diameter and longer stipe length mushrooms compared to strain S01 (Table 4.4). Additionally, all the substrate formulations grown with strain S02 had firm caps with regular edges, and showed more number of caps (Figure 4.4).

Based on the Table 4.4 (see Appendix D), there have been some improvements of utilisation CW, FWC and EFBC on growing *P. pulmonarius*. The total of the days required for mycelium running and from opened to first harvest have been shortened for strain S01 grown on 10 % (w/w) of CW and FWC supplementation (48 days, 47 days respectively) compared to control substrate (53 days). Besides, the days required for pinheads formation for strain S01 grown on 30 % (w/w) of EFBC supplementation (19.13±7.03 days) and strain S02 grown on 10 % (w/w) of CW (13.60±1.60 days) and 20 % (w/w) FWC (14.13±3.10 days) supplementation have reduced compared to its control substrate (19.93±7.33 days, 14.33±2.23 days respectively).

Table 4.4: Evaluation of growth performance of two strains of *P. pulmonarius* grown on selected substrate formulations.

Strains	Formulations	Days required for mycelium running	Days required for pinheads formation	Days required from opened to first harvest	Cap diameter (centimetre)	Stipe length (centimetre)
S01	Control	31.20±2.78^{e}	19.93±7.33^{cd}	21.80±7.79^{abc}	7.62±2.89^{bc}	4.58±1.66^{bc}
	10 % CW	21.27±1.53^{c}	25.07±8.41^{d}	27.20±8.99^{c}	6.19±2.25^{ab}	3.80±1.36^{abc}
	10 % FWC	22.13±3.11^{c}	22.93±13.00^{cd}	24.73±13.66^{bc}	5.43±2.82^{a}	3.53±1.69^{ab}
	30 % EFBC	30.47±3.00^{e}	19.13±7.03^{bc}	21.00±7.43^{ab}	6.23±2.39^{ab}	3.15±1.49^{a}
S02	Control	14.64±4.67^{a}	14.33±2.23^{ab}	16.20±2.31^{a}	7.21±1.80^{abc}	4.76±1.11^{c}
	10 % CW	18.20±1.52^{b}	13.60±1.60^{a}	16.40±1.40^{a}	7.24±2.30^{abc}	4.39±0.91^{bc}
	20 % FWC	24.87±3.10^{d}	14.13±3.10^{ab}	17.13±3.10^{a}	5.77±1.04^{ab}	4.54±1.26^{bc}
	30 % EFBC	20.87±2.61^{c}	25.13±3.94^{d}	27.13±3.94^{c}	8.44±2.23^{c}	4.17±0.93^{abc}

Values are means of 15 replicates ±; standard deviation. Values in a column followed by the same alphabet indicate no significant different ($p < 0.05$).

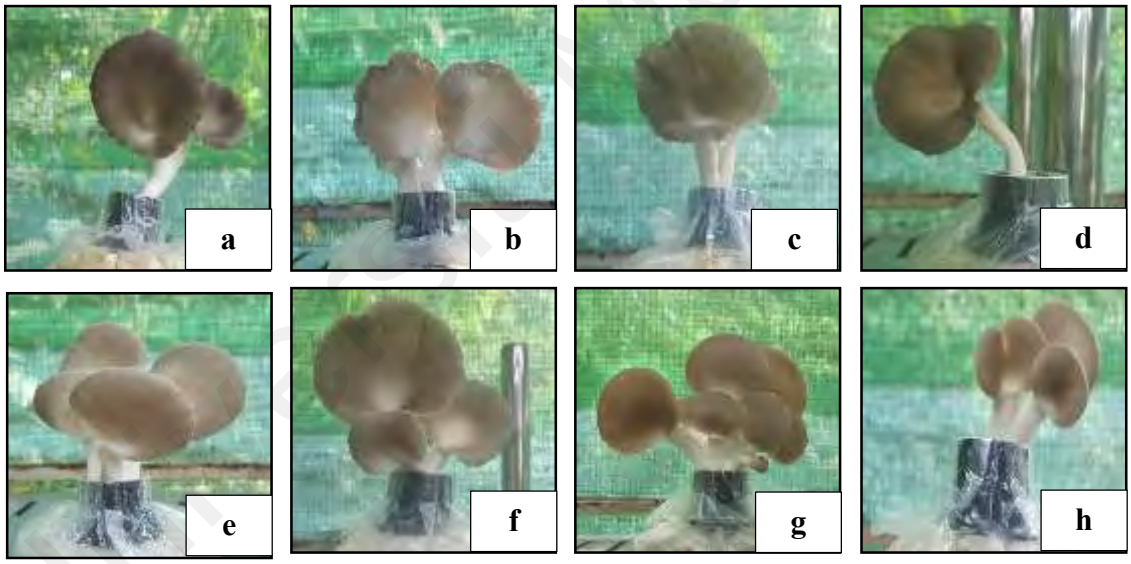

Figure 4.4: Fruiting bodies of *P. pulmonarius* grown on selected substrate formulations. Strain S01 grown on substrate formulations for **(a)** control **(b)** CW **(c)** FWC **(d)** EFBC whereas strain S02 grown on substrate formulations for **(e)** control **(f)** CW **(g)** FWC **(h)** EFBC.

4.2.2 Biological Yield and Biological Efficiency of *Pleurotus pulmonarius*

Based on the Table 4.5, the number of harvesting cycle, total biological yield and biological efficiency of two strains of *P. pulmonarius* grown on selected substrate formulations were showed varying results. Strain S01 only have two harvesting cycle as

it stopped fruiting at the next harvest and attacked by bugs. This eventually caused the occurrence of contamination among substrate formulations of strain S01.

Meanwhile, strain S02 were able to be cultivated and harvested until fourth harvest. Moreover, the highest biological yield were obtained from first harvest, followed by the second harvest. Then the trend gradually declined at the next two harvest. In the end of harvesting cycle, the weight of bags were found to be lighter than previously.

The biological yield and BE of strain S01 and S02 were significantly difference ($p<0.05$) on different substrate formulations (Table 4.5) (see Appendix E). It was found that strain S02 grown on control substrate produced the highest biological yield (78.77±19.93 g) followed by its 20 % (w/w) of FWC supplementation (60.84±17.95 g); while strain S01 grown on 30 % (w/w) of FWC supplementation produced the lowest biological yield (22.07±14.80 g). In general, substrate gave the higher yield and higher value of BE. Strain S02 grown on control substrate shown the maximum BE (131.28±33.22 %) followed by its 20 % (w/w) of FWC supplementation (101.40±29.92 %). Strain S01 grown on 10 % (w/w) of FWC supplementation of (36.78±24.67 %) shown the lowest BE.

Based on the Table 4.5, strain S02 grown on control substrate and 20 % (w/w) of FWC supplementation shown low percentage of contaminated bags. In addition, strain S02 grown on control substrate had the highest percentage of successfully fruiting bags (86.67 %) whereas strain S01 grown on 30 % (w/w) of FWC supplementation had the lowest percentage (26.67 %).

Strain S02 has proved to have higher production yields with strong pathogenicity than strain S01. Strain S01 showed lower performance in the majority of the measurements and low productivity, therefore this strain was not selected for further study. This study showed that strain S02 displaying markedly higher growth performances and productivity. Hence, it showed to be preferable for the production and was selected for nutrient analysis.

Table 4.5: Evaluation of number of harvesting cycle, total biological yield and biological efficiency of two strains of *P. pulmonarius* grown on selected substrate formulations.

Strains	Formulations	First harvest[1] (grams)	Second harvest[1] (grams)	Third harvest[1] (grams)	Fourth harvest[1] (grams)	Total biological yield[1] (grams)	Total BE[1] (%)	Contaminated bags[2]	Successful fruiting bags[3] (%)
S01	Control	20.93±11.24^{ab}	16.14±16.14^{bc}	-	-	37.67±22.18^{b}	62.78±36.97^{b}	High	38.33
	10 % CW	20.07±13.13^{a}	21.47±18.34^{bc}	-	-	41.53±23.23^{b}	67.11±37.47^{b}	High	40.00
	10 % FWC	17.47±10.25^{a}	4.60±10.13^{a}	-	-	22.07±14.80^{a}	36.78±24.67^{a}	High	26.67
	30 % EFBC	17.40±10.87^{a}	4.93±8.86^{a}	-	-	23.33±13.40^{a}	37.22±22.32^{a}	High	30.00
S02	Control	29.97±14.34^{c}	23.60±11.51^{c}	16.60±10.44^{c}	8.60±11.44^{b}	78.77±19.93^{d}	131.28±33.22^{d}	Low	86.67
	10 % CW	22.13±7.74^{abc}	19.87±10.97^{bc}	5.40±8.49^{a}	2.07±8.00^{a}	49.47±18.80^{bc}	82.45±31.34^{bc}	Moderate	56.67
	20 % FWC	29.14±7.51^{bc}	16.57±7.69^{bc}	13.07±10.67^{c}	2.07±4.99^{a}	60.84±17.95^{c}	101.40±29.92^{c}	Low	73.33
	30 % EFBC	24.23±6.40^{abc}	12.73±10.44^{ab}	4.53±5.86^{bc}	1.07±2.84^{a}	42.57±16.40^{b}	70.94±27.34^{b}	Moderate	55.00

[1]Values are means of 15 replicates ±; standard deviation. Values in a column followed by the same alphabet indicate no significant different ($p < 0.05$)) (see Appendix E).
[2]The percentage of contaminated bags (low: less than 30 %, moderate: 30-50 %, high: more than 50 %).
[3]The percentage of bags that had successful fruiting (Total number of harvesting cycle per bag / (4 cycles x 15 bags) x100)

4.3 Nutrient Assays of Cultivated *Pleurotus pulmonarius* Strain S02

Sample of fresh cultivated *P. pulmonarius* strain S02 were subjected to freeze dry and proximate nutrient analysis. In Table 4.6, the nutritional value (total sugar content, total protein content, total phenolic content, and β-glucan content) from the fruiting bodies of strain S02 was presented. Although strain S02 grown on control substrate also gave good growth performances and yield, but the nutritional attributes of mushrooms grown on supplemented substrates CW, FWC and EFBC surpassed those grown on control substrate.

The values of sugar content ranged from 32.32-72.32 mg/g. The fruit bodies from strain S02 grown on 30 % (w/w) of EFBC supplementation had the highest sugar content (72.32 ± 0.01 mg/g) and showed a significant different ($p < 0.05$) compared other to control substrate and supplemented substrates (Table 4.6) (see Appendix G).

High protein content in the fruiting bodies of *P. pulmonarius* strain S02, ranging from 93.33-165.43 mg/g. The fruit bodies from strain S02 grown on 30 % (w/w) of EFBC supplementation had highest protein content (165.43± 0.01 mg/g) followed by the 20 % (w/w) of FWC supplementation (163.21± 0.01mg/g). It was found that protein content was significantly higher ($p < 0.05$) in all substrate supplemented with wastes compared to control substrate (Table 4.6) (see Appendix G). There has been an improvement in protein contents for the fruiting bodies of *P. pulmonarius* grown on supplemented substrates.

Meanwhile, there were low content of phenolic substances in the fruiting bodies of *P. pulmonarius*, ranging from 17.05-20.83 mg/g. The fruit bodies from strain S02 grown on

20 % (w/w) of FWC supplementation (17.05±0.02 mg/g) had lowest content of phenolic compared to the control substrate (17.50±0.04 mg/g).

As shown in Table 4.6, β-glucan content in the fruiting bodies of *P. pulmonarius* strain S02, was detected in this study. The percentage of β-glucan content ranged from 28.84 to 34.09 %. The fruit bodies from strain S02 grown on control substrate had the highest β-glucan content (34.09±0.01 %) followed by 20 % (w/w) of FWC supplementation (29.58± 0.01 %).

Table 4.6: Nutritional content of *P. pulmonarius* strain S02.

Formulations	Nutritional value			
	Total sugar content[1] (mg/g of mushroom)	Total protein content[1] (mg/g of mushroom)	Total phenolic content[1] (mg/g of mushroom)	β-glucan content[12] (%)
Control	53.17 ± 0.02[b]	93.33 ± 0.01[a]	17.50 ± 0.04[a]	34.09 ± 0.01[a]
10 % CW	32.32 ± 0.02[a]	146.67 ± 0.04[b]	19.58 ± 0.03[b]	28.95 ± 0.01[a]
20 % FWC	51.46 ± 0.03[b]	163.21 ± 0.01[b]	17.05 ± 0.02[a]	29.58 ± 0.01[a]
30 % EFBC	72.32 ± 0.01[c]	165.43 ± 0.01[b]	20.83 ± 0.02[c]	28.84 ± 0.01[b]
RDA[3]	50g[4]	60g[5]	1g[6]	3g[7]

[1]All values are expressed as means ± standard deviation of triplicate measurements. Values in a column followed by the same alphabet indicate no significant different ($p < 0.05$).
[2]Estimation by using kit protocol.
[3]Recommended Daily Allowance.
[4] World Health Organization, 2015 (no more than 10% of total calories and a further reduction to below 5% or roughly 25 grams per day would provide additional health benefits).
[5] National Research Council, 1989 (based on 0.8 g/kg of body weight, 56 grams per day for the average of sedentary male and 46 grams per day for the average of sedentary female).
[6] PerézGimenéz *et al.,* 2008; Saura-Calixto, 2008.
[7]Malaysian Dietary Guideline, 2010 (minimum amount required 0.75g)

CHAPTER 5: DISCUSSIONS

5.1 Effects of Agricultural and Food Wastes on Linear Growth Study

For this study, the CW, FWC and EFBC were used to explore as components of the growth medium for mushroom cultivation. In order to optimise the standard substrate supplemented with agricultural and food wastes, two strains of *P. pulmonarius* were tested with varying concentrations of wastes based on the linear growth study.

5.1.1 Coffee Wastes on Linear Growth

In the current study, CW can be used to support mycelium growth of *P. pulmonarius* in the race tubes. However, there are certain drawbacks associated with the used of CW. Coffee spent-ground contains caffeine, tannins and polyphenols which have low toxicity towards fruiting bodies development in *P. pulmonarius* and prolong the vegetative growth phase (Leifa *et al.*, 2001; Velázquez-Cedeño *et al.*, 2002). Furthermore, the increased amount of caffeine and tannic acid would lower the mycelia growth rate when used as substrate for mushroom cultivation (Martínez Carrera *et al.*, 1988; Fan *et al.*, 2003). Ballesteros *et al.* (2014) reported that spent coffee waste consisted a variety of minerals including zinc (Zn), manganese (Mn), cadmium (Cd), cobalt (Co) and lead (Pb) (Table 2.1). A small quantity of essential heavy metals such as Co, Mn, and Zn are required for mycelia growth, but these metals might impede mycelium growth in term of morphological and physiological changes (Baldrian, 2003).

In an analysis of the used Starbucks coffee grounds, Soil and Plant Laboratory Incorporation has found that there was 2.28% nitrogen and the estimated C/N ratio in the CW was about 24:1. It has been shown that there is more than adequate nitrogen present in the coffee grounds for the soil microorganisms in the degradation for

organic fraction (Sunset, 2017). Other studies have considered the relationship of C/N ratio between mycelium growth and laccase production in basidiomycetes. The higher C/N ratio increased mycelium growth and decreased laccase production in solid substrate cultivation of *P. ostreatus*. (D'Agostini *et al.*, 2011). On the contrary, mycelium growth rate decreases under lower C/N ratio. Results from this study is in agreement with that the standard substrate supplemented with increasing concentration of CW, mycelium growth of *P. pulmonarius* decreases (Table 4.1) with decreasing of C/N ratio (Table 3.2). Besides that, CW consisted high nitrogen content could inhibit the mycelia growth which was result in hyphal plasmolysis (Ballesteros *et al.*, 2014; Hachiicha *et al.*, 2012; Baysal *et al.*, 2003). Hence, high concentration (40 % (w/w) of CW supplementation delays the mycelium growth rate and develop a sparse mycelium.

5.1.2 Food Waste Compost on Linear Growth

The UM ZWC compost used in this study have been produced by standard method and commercialised for plantation due to rich of biodegradable organic matters and free of pathogen. In addition, it contained important macronutrients (N: 2.39 %; P: 2.82 %; K: 0.21 %; Mg: 0.36 %) for plants which have been transcribed in the test report of UM ZWC compost (UM Zero Waste Campaign, 2018). It has been found that FWC improved the gaseous exchange between soil and as a good fertiliser to feed any plants. Zervakis *et al.* (2001) discussed that fast mycelium growth rate is often indicated of hyphal progression on an unfavourable and nutritionally poor medium but a slower and denser growth can be interpreted of fully exploitation of the nutrient resources of the medium by the fungus. Hence, the substrates supplemented with higher concentration (40 % (w/w)) of FWC are not favourable to mycelium growth as the mycelium are not fully utilised the nutrient resources.

Food waste compost contained a good supply nutrients for mushroom production and minerals such as calcium, copper, iron, magnesium, and molybdenum for fungi growth (Jennings, 1995; Royse *et al.*, 2013). Carbon is the most abundant mineral element in FWC, followed by calcium and copper (Table 2.1). Calcium contained in the substrate and inside the fungal cells affect the fungi growth, differentiation and sporulation (Royse *et al.*, 2013). Besides, the standard substrate incorporated with FWC provides a good gaseous environment which promote mycelium growth. Aeration among the substrate and FWC affect the oxygen and carbon dioxide required for aerobic growth and metabolism; moisture alteration, temperature adjustment, water vapour and some volatile metabolite elimination (Bellettini *et al.*, 2019). Low degree of mycelium compactness might due to the aeration system which limits the oxygen and carbon dioxide exchange that required for growth.

5.1.3 Empty Fruit Bunch Compost on Linear Growth

Carbon and nitrogen are essential for mycelium growth which obtained from cellulose, hemicellulose and lignin materials (Tabi *et al.*, 2008). Empty fruit bunch compost is one of the lignocellulose material residues also as a by-product of the industrial palm oil process after removal of the fruits (Parshetti *et al.*, 2013). According to Baharuddin *et al.* (2010), the final matured compost of empty fruit bunch comprised considerable amounts of nutrients, low heavy metals and was proved that to be safe as soil fertiliser and amendment. In this study, the increased of amount EFBC in the substrate was likely to be the consequences of gradually increasing the growth rate and mycelium compactness due to sufficient nutrient resources (Table 4.3). However, it was a decline of the mycelium growth rate at higher supplementation of EFBC when compared to the control substrate. This growth pattern was most probably due to over excessive nutrient in the substrate for the mycelium growth.

Muthangya *et al.* (2013) reported that the structure and the porosity levels of substrate are significant factors to be considered for growth and penetration of the mycelium into substrates. In this regard, the mycelium grown on higher supplementation of EFBC was most probably due to the particle size of EFBC which provide enough oxygen availability to promote lignin degradation. Tabi *et al.* (2008) reported that *P. ostreatus* was unable to grow on empty fruit bunch due to the complex structure lignin which was relatively resistant to degradation. However, Rizki and Tamai (2011) had successfully cultivated that *P. ostreatus* using empty fruit bunch. These contradictory results might be due to the different composting stage of the empty fruit bunch which result in different particle size of texture. The C/N ratios of empty fruit bunch used by Rizki and Tamai (2011) was 20:1, while the C/N ratio of EFBC used in this study was 12:1. From these C/N ratios, the used of EFBC by Rizki and Tamai (2011) were partially decomposed in coarse textured while in this study utilises fully decomposed empty fruit bunch in fine textured like SD.

5.2 Determination of Best Strain for Nutrient Analysis

The results obtained from this study showed that two strains of *P. pulmonarius* were successfully cultivated on different concentrations of wastes respectively. This part was aimed to select a comparatively more suitable strain to be used in nutrient analysis for *P. pulmonarius* by investigating the growth performance, yield and BE with its related studies.

5.2.1 Evaluation Study on Growth Performance of *Pleurotus pulmonarius*

According to Shah *et al.* (2004), days required for mycelium running in mushroom bags ranged from 16 to 25 days for cultivation *Pleurotus* spp. on wheat straw, leaves and SD. In this study, the days required for completing mycelium running was varied from 14 to 30 days. Akinmusire *et al.* (2011) who studied the cultivation performance of *P.*

pulmonarius using rice straw substrate showed similar results with this study. The total days required for completing mycelium running took about 21-31 day. However, it was found that strain S01 showed sparse or moderate growth of mycelium compared to strain S02 to complete mycelium running in selected substrates. This might be attributed to strain S01 has low adaptability and sustainability compared to strain S02, which slow down the rate of mycelium growth.

In *Pleurotus* spp., the pinheads formation was generally on the 24th-30th days (Naraian *et al.*, 2009). Results from this study showed the pinheads formation observed took longer period. This might be related to the transferring of mushroom bags from a dark warm place to a new environmental condition. They were adopted with the dark room in laboratory and were not able to make their appropriate development in mushroom house in the beginning. Thus, time required for pinning was longer so the pinheads have been appeared lately. However, the spawn run in strain S02 grown on control substrate, 10 % (w/w) of CW and 20 % (w/w) of FWC supplementations that reached to fruiting level was in agreement with the findings reported by Stamets (2000), where spawn run and fructification for *P. pulmonarius* grown on ligninocellulose wastes took about 8-14 days. Hence, the cultivation performance in the fruiting bodies vary with the strains.

From the results obtained in this study, the number of days required from opening to first harvest ranged 16 to 27 days. The total days include the days required for pinheads formation and mature at harvest, not meaning that it took longer times for pinheads grow into fully mature mushroom. The actual days required from pinheads to mature mushrooms were actually took an average of 2-3 days for *P. pulmonarius* observed under this study. Similarly, Mkhize *et al.* (2017) reported the time it took for *P. pulmonarius* to

initiate pinheads until matured was also 2 to 3 days only. In fact, the mushrooms were easily to pick out once they were going to be ready for harvest.

The results of this study showed that cap size and stipe length of *P. pulmonarius* varied markedly in different substrates formulations. These findings are in agreement with previous study by Yang *et al.* (2013) which reported *P. ostreatus* exhibited variation in stipe length and mushroom cap diameter in the nine substrate formulations used. The substrate included rice straw, wheat straw, cotton seed hull, and wheat straw or rice straw supplemented with different proportions (15 %, 30 %, and 45 % in rice straw substrate, 20 %, 30 %, and 40 % in wheat straw substrate) of cotton seed hull. Mane *et al.* (2007) reported that different substrates affect the yields and mushroom fruiting bodies characters such as pileus size and stipe length in *P. sajor-caju.* In this study, the cap diameter of strain S02 grown on substrate supplemented with EFBC was particularly wider among others. This may be attributed to the addition of supplement that changed the physical properties and C/N ratio of substrate formulas. If long stipe and underdeveloped caps were observed in fruiting body, which may result from the inadequate light and excessive of carbon dioxide in the mushroom house (Sánchez, 2010). In addition to that, environmental conditions as well as supplementation of substrates with various additives including nitrogen sources have been found to affect the quantity and quality of mushroom (Royes, 2002; Onyango *et al.*, 2011)

The variation of different growth performance and parameter of cultivated mushrooms are influenced by several factors such as type of substrates and supplements used, the species or strain employed, spawning method as well as mushroom growing conditions (Mshandete, 2008 ; Tripathy *et al.*, 2010). In addition, extrinsic factors such as temperature of culture house, humidity, light intensity, air composition and size of

polyethylene bags were also affecting the survival and multiplication of mushroom (Bellettini *et al.*, 2016).

5.2.2 Evaluation Study on Biological Yield and Biological Efficiency of *Pleurotus pulmonarius*

Obviously, there were high production and BE grown among substrate formulation for strain S02. In this regard, a recent study by Myronycheva *et al.* (2017) assess the fruiting of 19 oyster mushroom (13 strains of *P. ostreatus* and 6 strains of *P. pulmonarius*) strains grown on mixture substrate of wheat straw and sunflower husks. The authors reported that BE of five strains of *P. pulmonarius* strains were low (9.8-35.0 %), with the exception of one strain produced higher BE result (62 %). Furthermore, results from this study is in agreement with the study conducted in South Africa examining *P. pulmonarius* grown on maize stalk supplemented with varying levels of wheat bran and maize flour (Mkhize *et al.*, 2017). The BE ranging from 32 % to 132 % whereby 14 % of maize flour supplementation exhibited higher BE among others. It can be concluded that amount and type of nutrients as well as their interaction with mushroom strains had a significant impact on mushroom productivity.

On other hands, the failure of strain S01 to produce fruit body for subsequent harvesting cycle might be due to poor recovery of mycelium during resting period between harvest times. As a result, mycelium is easily contaminated by competing air-borne microorganisms such as the green mould *Trichoderm*a spp. in mushroom house. The contaminated substrate may also attract pests (mites and fly) which bring other contaminants. According to Sánchez (2010), mushroom produced only in first flush and fail to produce subsequent flushes was caused by inadequate substrate nutrition and competitor moulds. Thus, the ability of *P. pulmonarius* strain to grow successfully on

CW, FWC and EFBC may be associated with the nutrients contained in it. Wabali and Wocha (2013) revealed that occurrence of contamination can caused by an increased nutrient concentration of the substrate due to fungal contaminants compete with the cultivated mushroom. Ambi *et al*. (2011) also mentioned that variations observed in the production may be linked with differential nutritional status of the substrates and to some extent the physical nature of substrates as well as nature of mushroom.

Cultivation of grey oyster mushroom is very dependent on natural occurrence as well as environmental conditions, especially favourable in humid climate. Climate of Malaysia is usually hot and sunny with temperature reaches 28-35 °C and humidity around 60 % to 70 % all over the year (Malaysian Meteorological Department, 2017). Small fluctuations in temperature affect the mushroom yield and could encourage contamination in the mushroom house. This result revealed strain S01 had low resistance to strain S02 under the studied cultivation conditions. This may be attributed to the influence of temperature changes, which is not preferred fruiting temperature and adequate moisture for fruiting body growth condition (Sánchez, 2010). Additionally, prolonged rainy weather could cause the bags exposure to excessive high humidity and eventually lead to soggy substrate, mould contamination and standing water. In this study, strain S02 stopped fruiting when no more available substrate or substrate nutrients may be too limited which resulted in mushroom abortion and contaminated by available contaminated substrate. Hence, there were a numbers of factors which may act individually or have interaction effects with mycelium growth and development of mushrooms.

Generally, the purpose of supplementing a substrate is to improve growth of mushroom mycelium. The recorded results of *P. pulmonarius* mushroom indicate that

high concentration of EFBC supplementation result in lower mushroom yield compared to low concentration of FWC supplementation. In this regard, Assan and Mpofu (2014) reported that lower yield of *P. pulmonarius* grown on higher concentration of supplementation was most probably due to heat generated and excess nitrogen within substrate. Besides, Stamets (1993) asserts that when a substrate supplementing with nitrogen source, extra care is required to avoid influence on yield and growth of mushroom. The use of agricultural and food wastes as growth medium faced challenges in the survival and spread of certain pathogens might infect the host after spawning. This is because supplementing a substrate alter the number and the type of organisms existed in the substrate. Hence, prolong sterilisation of the supplemented substrate is highly recommended.

The result of this study showed that strain S02 grown on standard substrate supplemented with 20 % (w/w) of FWC recorded the highest yield and BE among all the agricultural and food wastes. This study particularly showed that strain S02 grow well on this supplemented substrate not only because of the susceptibility of strain S02 towards environmental and also due to FWC contained various nutrients to support the mushroom growth. Furthermore, FWC is mainly made up of carbon due to the optimum composting process required a C/N ratio between 25 and 30 parts of carbon (Kumar *et al.*, 2010), Naraian *et al.* (2009) and Bellettini *et al.* (2019) reported that 28 to 30 % of carbon and 1% of nitrogen of C/N ratio is an important condition for mushroom production. Likewise, Jo *et al.* (2013) reported that 15 % of FWC gave the highest yield of *Ganoderma lucidum*.

In the overall evaluations, strain S02 proved to have fast colonisation, short pinheads development, higher percentages of successfully fruiting bags, better yield and BE. These

superior properties could satisfy market requirements. Hence, this strain with all the substrate formulations was selected and proceed to nutrient assays.

5.3 Nutrient Profile of Dried *Pleurotus pulmonarius* Strain S02

In general, the fruiting bodies of mushrooms contain 56.8% carbohydrate (Hung & Nhi, 2012). Carbohydrate content comprises fibre, for example the structural polysaccharides, β-glucans, chitin, hemicelluloses and pectic substances (Rathore *et al.*, 2017). The high carbohydrate content is due to the higher level of non-fiber carbohydrates such as sugars (Reis *et al.*, 2012). The sugars present in mushrooms are mostly glucose, sucrose, fructose, mannitol and trehalose (Birch, 1973; Valverde *et al.*, 2015). In this study, total sugar content was vary in the range of 32.32-72.32 mg/g that grown on different substrate formulations. However, the values were slightly low compared to the total sugar content conducted by Obodai *et al.* (2012) on dried mushroom of *P. sajor-caju* (8.92 g/100 g) where the total sugar content included fructose, mannitol and trehalose.

The efficiency of fungi in transforming substrate to protein is far supreme to that of numerous plants and even animals (Kües & Liu, 2000). Alam *et al.* (2007) reported that the dried mushroom of *P. sajor-caju* containing high amount of protein (24.63 g/100 g). The high amount of protein in the mushroom revealed that it can be a protein source in the diet for humans. In this study, the total protein content was significantly higher ($p < 0.05$) in all substrate supplemented with wastes compared to the control substrate. This indicated that agricultural and food wastes that contained high protein content can contribute as a protein source by the mycelium (Silva *et al.*, 2002).

Besides sugar and protein, phenolic considered to be major contributors to the antioxidant capacity of the mushroom extract. They have been reported as natural

antioxidant which commonly found in mushroom with redox properties because they act as free radical scavengers, reducing agents, singlet oxygen quenchers, or metal ion chelators (Ferreira *et al.*, 2009; Khatun *et al.*, 2015; Valverde *et al.*, 2015). In this study, different results were recorded for the total phenolic content (17.05-20.83 mg/g) of *P. pulmonarius* grown on different substrate formulations. These results were relatively low compared to the reported values by Gogavekar *et al.* (2014), whereby total phenols was recorded at 52.20 mg/g in *P. sajor-caju*. However, lower phenol contents was reported by Yanga *et al.* (2002) for *P. cystidiosus* and *P. ostreatus* which contained 10.24mg/g and 15.7 mg/g of total phenols, respectively.

The β-glucans are essential polysaccharides and found in the cell wall of mushrooms (Valverde *et al.*, 2015). Beta-glucans are responsible for strengthening the immune system by inducing both the adaptive and innate immune response (Brown & Gordon, 2005; Sari *et al.*, 2017). Hence, mushrooms are an ideal source for making nutraceutical dietary supplement. Based on the results from this study, β-glucan content obtained by difference between total and α-glucans varied from 28.84 to 34.09 % in *P. pulmonarius* grown on different substrate formulations. Avni *et al.* (2017) studied the effects of relative concentration of olive mill solid waste (OMSW) and yield of glucan in the caps and stalks of *P. eryngii.* In the study, there were four types of treatment applied on *P. eryngii*, which included control substrate (100 % SD), 20 %, 60 % and 80 % of OMSW. It was found that the caps of *P. eryngii* grown on 20 % of OMSW had high β-glucan content (39.10 % w/w) whereas the stalks of *P. eryngii* grown on 80 % of OMSW had the highest β-glucan content (49.70 % w/w). It can be concluded that the contents of polysaccharides in the fruiting bodies also vary with the concentrations of wastes supplemented.

The nutritional values may be attributed to the species and strain of mushroom, environmental factors, cultivation times, substrates and supplement chemical compositions (Manzi *et al.*, 2001; Nunes *et al.*, 2012). Therefore, the efficiency of mycelium in transforming the chemical compositions of substrate and different wastes into fruiting bodies itself can cause the variations among the nutritional attributes of *P. pulmonarius*. It is evident from various studies that oyster mushrooms have a great nutritional value due to rich in carbohydrates, protein, fiber and low in fat levels (Agarwal *et al.*, 2017). Thus, oyster mushroom could be a protein source in our foods and able to promote health benefits in stimulating synergistic effects with all the bioactive compounds present (Elmastas *et al.*, 2007; Ferreira *et al.*, 2009). Stamet (1993) mentioned that populations which consumed mushroom meet the recommended daily allowance (RDA) and daily recommended intake (DRI) for calcium (Ca), cobalt (Co), iron (Fe), magnesium (Mg), phosphorus (P), zinc (Zn), folate, niacin, riboflavin, thiamin, vitamin A, B6, B12, C, E, energy, carbohydrate, fiber and protein compared to those who do not consume mushroom. Therefore, mushroom lover have a balanced diet than those who do not eat mushrooms.

In this study, the cultivation of grey oyster mushroom can be very time-consuming and costly. It was not only about growing mushroom in farm, but required laboratory experiences (microbiological sterile techniques) for the production of spawn as it is often regarded as non-practical for the common mushroom grower**.** Furthermore, the cost efficiency of mushroom cultivation was including the required time for mushroom growing, the costs of operating the mushroom house and the nutritional attributes for fruiting bodies of *P. pulmonarius*. However, the main concerns were the supplemented substrate used and the required times for mushroom growing along with analysing the nutrient analysis in this project. Hence, it was found that the time taken for mycelium

running was a bit longer when grown on supplemented substrates, but the times for pinhead formations was shortened as compared to its control substrate. It might due to supplemented substrates allowed mycelium to grow by providing sufficient nutrition and the mycelium was uniquely transform the nutrients. Ultimately, it caused the variations among the growth performance, biological yield and nutritional attributes of *P. pulmonarius* as compared to its control substrate. It also can been seen that the total protein and total phenolic contents grown on supplemented substrates CW, FWC and EFBC were surpassed grown on control substrate. Therefore, present results indicated that the effectiveness of supplemental CW, FWC and EFBC can help to improve and alter the nutritional attributes for fruiting bodies of *P. pulmonarius* strain S02.

CHAPTER 6: CONCLUSIONS

6.1 Conclusions

This study provided an important opportunity to advance the knowledge of the potential used of CW, FWC and EFBC as a substrate component of a growth medium in linear growth study. It was observed that the supplementation of various wastes at their lower concentrations (10 %, 20 % and 30 % (w/w)) of CW, FWC and EFBC) had enhanced the mycelium growth rate of *P. pulmonarius* (strain S01 and S02). It was revealed that only fast mycelium growth rate with dense mycelium may reflect the utilisation of nutrient resources is optimum in linear growth study. In the evaluation and comparison study, *P. pulmonarius* grew and fruiting bodies were produced in the formulated substrates in bag cultures. Strain S01 grown on control substrate was recorded the slowest (31.20±2.78 days) in substrate colonisation while when grown on 10 % (w/w) of CW supplementation showed the highest number of days required from opened to first harvest (27.20±8.99 days). Strain S01 grown on 30 % (w/w) of FWC supplementation gave the lowest BE (36.78±24.67 %) and had the lowest percentage of successfully fruiting bags (26.67 %). On the contrary, strain S02 grown on control substrate was recorded the fastest (14.64±4.67 days) in substrate colonisation and showed the shortest number of days (16.20±2.31 days) required from opened to first harvest. Strain S02 grown on all substrate formulations were able to be harvested until fourth harvest whereas strain S01 only have two harvesting cycle. From the results, strain S02 considered to have the highest potential and growth performance grown on the substrate formulations tested and the fruit bodies were selected and analysed for nutritional attributes. It was found that the protein content in the fruiting bodies of *P. pulmonarius* grown on all substrate formulations was higher compared to its sugar, phenolic and β-glucan content. The protein content was significantly higher ($p < 0.05$) in all substrate supplemented with

wastes compared to the control substrate. Hence, the efficiency of mycelium in transforming the chemical compositions of substrate and different wastes into fruiting bodies itself can cause the variations among the nutritional attributes of *P. pulmonarius*. In the overall, strain S02 grown on standard substrate supplemented with 20 % (w/w) of FWC gave the highest BE (101.40±29.92 %) among all substrate supplemented with wastes. The fruiting bodies had the highest β-glucan content (29.58 ± 0.01 %) among the fruit bodies obtained from tested formulations containing wastes as supplement. The days required for mycelium running was 24.87±3.10 and the pinheads formation was 14.13±3.10. In conclusion, CW, FWC and EFBC can be utilised as supplement in the cultivation of grey oyster mushrooms and this use can be an alternative way for the solid waste management.

6.2 Future Study

Based on the outcome of the current work, the use of FWC as a substrate component of growth medium produced higher yield among others wastes. Further assessment and investigation is recommended for pilot-scale study on this waste to obtain a sustainable production of mushroom. Besides, further studies on the involvement of more possible agricultural and food wastes in mushroom cultivation will open up a pool of innovation for the mushroom industry.

REFERENCES

Abd Razak, D. L., Abdullah, N., Khir Johari, N. M., & Sabaratnam, V. (2013). Comparative study of mycelia growth and sporophore yield of *Auricularia polytricha* (Mont.) Sacc. On selected palm oil wastes as fruiting substrate. *Applied Microbiology and Biotechnology*, *97(7)*, 3207–3213.

Abdullah, N., & Sulaiman, F. (2013). The oil palm wastes in Malaysia. In *Biomass Now-Sustainable Growth and Use*, Miodrag Darko Matovic (Ed.), InTech. Retrieved on December 26, 2017 from https://www.intechopen.com/books/biomass-now-sustainable-growth-and-use/the-oil-palm-wastes-in-malaysia.

Adebayo, E. A., & Martinez-Carrera, D. (2015). Oyster mushrooms (*Pleurotus*) are useful for utilizing lignocellulosic biomass. *African Journal of Biotechnology*, *14*(1), 52-67.

Agarwal, S., Kushwaha, A., Verma, V., & Singh, M. P. (2017). Nutritional attributes of *Pleurotus* mushroom. SinghV. V. SinghA. K. (Eds.)*, Incredible World of Biotechnology, MP*, 13-24.

Akinmusire, O. O., Omomowo, I. O., & Oguntoye, S. I. K. (2011). Cultivation performance of *Pleurotus pulmonarius* in Maiduguri, North Eastern Nigeria, using wood chippings and rice straw waste. *Advances in Environmental Biology*, *8*, 2091-2095.

Alam, N., Khan, A., Hossain, M. S., Amin, S. R., & Khan, L. A. (2007). Nutritional analysis of dietary mushroom *Pleurotus florida* Eger and *Pleurotus sajor-caju* (Fr.) Singer. *Bangladesh Journal of Mushroom*, *1*(2), 1-7.

Aljuboori, A. H. R. (2013). Oil palm biomass residue in Malaysia: availability and sustainability. *International Journal of Biomass and Renewable*, *1*(2), 13-18.

Al-Momany, A., & Ananbeh, K. (2010). Conversion of agricultural wastes into value added product with high protein content by growing *Pleurotus ostreatus*. In H. Gökçekus, U. Türker and J. W. LaMoreaux (Ed.), *Survival and Sustainability* (pp. 1483-1490). Berlin, Heidelberg: Springer.

Ambi, A. A., Pateh, U. U., Sule, M. I., & Nuru, G. F. (2011). Studies on cultivation of some wild edible mush room on some lignocellulosic substrates in main campus, Ahmadu Bello University, Zaria. *International Journal of Pharmaceutical Research and Innovation*, *3*, 14-18.

Asghar, R., Tariq, M. U. H. A. M. M. A. D., & Rehman, T. A. H. I. R. (2007). Propagation of *Pleurotus sajor-caju* (oyster mushroom) through tissue culture. *Pakistan Journal of Botany*, *39*(4), 1383-1386.

Assan, N., & Mpofu, T. (2014). The influence of substrate on mushroom productivity. *Scientific Journal of Crop Science*, *3*(7), 86-91.

Avin, F. A., Bhassu, S., Rameeh, V., Tan, Y. S., & Vikineswary, S. (2016). Genetics and hybrid breeding of *Pleurotus pulmonarius*: heterosis, heritability and combining ability. *Euphytica*, *209*(1), 85-102.

Avni, S., Ezove, N., Hanani, H., Yadid, I., Karpovsky, M., Hayby, H., ... & Danay, O. (2017). Olive mill waste enhances α-Glucan content in the edible mushroom *Pleurotus eryngii*. *International Journal of Molecular Sciences*, *18*(7), 1564.

Baharuddin, A. S., Hock, L. S., Yusof, M. Z., Rahman, N. A. A., Shah, U. K., Hassan, M. A., ... & Shirai, Y. (2010). Effects of palm oil mill effluent (POME) anaerobic sludge from 500 m^3 of closed anaerobic methane digested tank on pressed-shredded empty fruit bunch (EFB) composting process. *African Journal of Biotechnology*, *9*(16), 2427-2436.

Baldrian, P. (2003). Interactions of heavy metals with white-rot fungi. *Enzyme and Microbial Technology*, *32*(1), 78-91.

Baldrian, P., & Šnajdr, J. (2006). Production of ligninolytic enzymes by litter-decomposing fungi and their ability to decolorize synthetic dyes. *Enzyme and Microbial Technology*, *39*(5), 1023-1029.

Ballesteros, L. F., Teixeira, J. A., & Mussatto, S. I. (2014). Chemical, functional, and structural properties of spent coffee grounds and coffee silverskin. *Food and Bioprocess Technology*, *7*(12), 3493-3503.

Baysal, E., Peker, H., Yalinkiliç, M. K., & Temiz, A. (2003). Cultivation of oyster mushroom on waste paper with some added supplementary materials. *Bioresource Technology*, *89*(1), 95-97.

Bellettini, M. B., Fiorda, F. A., Maieves, H. A., Teixeira, G. L., Ávila, S., Hornung, P. S., … & Ribani, R. H. (2019). Factors affecting mushroom *Pleurotus* spp. *Saudi Journal of Biological Sciences*, *26*(4), 633-646.

Birch, G. G. (1973). Mushroom sugar. *Plant Foods for Man*, *1*(1), 49-55.

Bonatti, M., Karnopp, P., Soares, H. M., & Furlan, S. A. (2004). Evaluation of *Pleurotus ostreatus* and *Pleurotus sajor-caju* nutritional characteristics when cultivated in different lignocellulosic wastes. *Food Chemistry*, *88*(3), 425-428.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254.

Brown, G. D., & Gordon, S. (2005). Immune recognition of fungal β-glucans. *Cellular Microbiology*, *7*(4), 471-479.

Chae, H. J., & Ahn, J. H. (2013). Optimization of rice bran and food waste compost contents in mushroom culture medium to maximize mycelial growth rate and fruit body yield of *Pleurotus ostreatus*. *International Biodeterioration & Biodegradation*, *80*, 66-70.

Chang, S. T. (1996). Mushroom research and development-equality and mutual benefit. *Mushroom Biology and Mushroom Products*, *2*, 1-10.

Chang, S. T., & Wasser, S. P. (2012). The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. *International Journal of Medicinal Mushrooms*, *14*(2), 95-134.

Cheung, L. M., Cheung, P. C., & Ooi, V. E. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry*, *81*(2), 249-255.

Curvetto, N. R., Figlas, D., Devalis, R., & Delmastro, S. (2002). Growth and productivity of different *Pleurotus ostreatus* strains on sunflower seed hulls supplemented with N–NH4+ and/or Mn (II). *Bioresource Technology*, *84*(2), 171-176.

D'Agostini, É. C., Mantovani, T. R. D. A., Valle, J. S. D., Paccola-Meirelles, L. D., Colauto, N. B., & Linde, G. A. (2011). Low carbon/nitrogen ratio increases laccase production from basidiomycetes in solid substrate cultivation. *Scientia Agricola*, *68*(3), 295-300.

Daim, S. P. & N. (2016, March 27). 3,000 tonnes a month: Why are Malaysians wasting so much food? *NST Online*. Retrieved on January 16, 2017 from http://www.nst.com.my/node/135395.

Das, N., & Mukherjee, M. (2007). Cultivation of *Pleurotus ostreatus* on weed plants. *Bioresource Technology*, *98*(14), 2723-2726.

Deraman, M. (1993). Carbon pellets prepared from fibers of oil palm empty fruit bunches: 1.A quantitative X-ray diffraction analysis. *PORIM Bull Palm Oil Rest Lust Malaysia*, *26*, 20-25.

Diamantopoulou, P., & Philippoussis, A. (2015). Cultivated Mushrooms: Preservation and Processing. In Y. H. Hui, and E. Özgül Evranuz (Eds.), *Handbook of Vegetable Preservation and Processing* (pp.495-525). Florida: CRC press.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350-356.

Dung, N. T. P., Tuyen, D. B., & Quang, P. H. (2012). Morphological and genetic characteristics of Oyster mushrooms and conditions effecting on its spawn growing. *International Food Research Journal*, *19*(1), 347.

Elmastas, M., Isildak, O., Turkekul, I., & Temur, N. (2007). Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *Journal of Food Composition and Analysis*, *20*(3-4), 337-345.

Fan, L., Soccol, A. T., Pandey, A., & Soccol, C. R. (2003). Cultivation of *Pleurotus* mushrooms on Brazilian coffee husk and effects of caffeine and tannic acid. *Micologia Aplicada International*, *15*(1), 15-25.

Fanadzo, M., Zireva, D. T., Dube, E., & Mashingaidze, A. B. (2010). Evaluation of various substrates and supplements for biological efficiency of *Pleurotus sajor-caju* and *Pleurotus ostreatus*. *African Journal of Biotechnology*, *9*(19), 2756-2761.

Ferreira, I. C., Barros, L., & Abreu, R. (2009). Antioxidants in wild mushrooms. *Current Medicinal Chemistry*, *16*(12), 1543-1560.

Forgacs, E., Cserhati, T., & Oros, G. (2004). Removal of synthetic dyes from wastewaters: a review. *Environment International*, *30*(7), 953-971.

Gogavekar, S. S., Rokade, S. A., Ranveer, R. C., Ghosh, J. S., Kalyani, D. C., & Sahoo, A. K. (2014). Important nutritional constituents, flavour components, antioxidant and antibacterial properties of *Pleurotus sajor-caju*. *Journal of Food Science and Technology*, *51*(8), 1483-1491.

Gregori, A., Švagelj, M., & Pohleven, J. (2007). Cultivation techniques and medicinal properties of *Pleurotus* spp. *Food Technology and Biotechnology*, *45*(3), 238-249.

Hachicha, R., Rekik, O., Hachicha, S., Ferchichi, M., Woodward, S., Moncef, N., & Mechichi, T. (2012). Co-composting of spent coffee ground with olive mill wastewater sludge and poultry manure and effect of Trametes versicolor inoculation on the compost maturity. *Chemosphere*, *88*(6), 677-682.

Hearst, R., Nelson, D., McCollum, G., Millar, B. C., Maeda, Y., Goldsmith, C. E., ... & Moore, J. E. (2009). An examination of antibacterial and antifungal properties of constituents of Shiitake (*Lentinula edodes*) and Oyster (*Pleurotus ostreatus*) mushrooms. *Complementary Therapies in Clinical Practice*, *15*(1), 5-7.

Hoa, H. T., Wang, C. L., & Wang, C. H. (2015). The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology*, *43*(4), 423-434.

Hung, P. V., & Nhi, N. N. Y. (2012). Nutritional composition and antioxidant capacity of several edible mushrooms grown in the Southern Vietnam. *International Food Research Journal*, *19*(2), 611-615.

Hussain, M., Khan, S. M., Khan, S. M., & Chohan, M. A. (2001). Effect of different sterilization methods on the production of oyster mushroom (*Pleurotus ostreatus*) on different substrates. In *Integrated plant disease management. Proceeding of 3rd National Conference of Plant Pathology, NARC, Islamabad 1-3 October 2001* (pp. 159-160). Pakistan: UAF.

Islam, M. T., Zakaria, Z., Hamidin, N., & Ishak, M. A. B. M. (2016). Characteristics of indoor mushroom cultivation of grey oyster (*Pleurotus pulmonarius*) by different stages of humidifying treatment. *World Applied Science Journal*, *34*(8), 1066-1075.

Iwade, I., & Mizuno, T. (1997). V. Cultivation of kawariharatake (*Agaricus blazei Murill*). *Food Reviews International*, *13*(3), 383-390.

Jeena, G. S., Punetha, H., Prakash, O., Chandra, M., & Kushwaha, K. P. S. (2014).Study on in vitro antioxidant potential of some cultivated *Pleurotus* species (Oyster mushroom). *Indian Journal of Natural Products and Research*, *5*(1), 56-61.

Jennings, D. H. (1995). *The Physiology of Fungal Nutrition*. Liverpool: Cambridge University Press.

Jo, E. Y., Cheon, J. L., & Ahn, J. H. (2013). Effect of food waste compost on the antler-type fruiting body yield of *Ganoderma lucidum*. *Mycobiology*, *41*(1), 42-46.

Kalmis, E., & Sargın, S. (2004). Cultivation of two *Pleurotus* species on wheat straw substrates containing olive mill waste water. *International Biodeterioration & Biodegradation*, *53*(1), 43-47.

Kalmis, E., Azbar, N., Yıldız, H., & Kalyoncu, F. (2008). Feasibility of using olive mill effluent (OME) as a wetting agent during the cultivation of oyster mushroom, *Pleurotus ostreatus*, on wheat straw. *Bioresource Technology*, *99*(1), 164-169.

Khatun, S., Islam, A., Cakilcioglu, U., Guler, P., & Chatterjee, N. C. (2015). Nutritional qualities and antioxidant activity of three edible oyster mushrooms (*Pleurotus* spp.). *NJAS-Wageningen Journal of Life Sciences*, *72*, 1-5.

Kim, M. K., Ryu, J. S., Lee, Y. H., & Kim, H. R. (2013). Breeding of a long shelf-life strain for commercial cultivation by mono–mono crossing in *Pleurotus eryngii*. *Scientia Horticulturae*, *162*, 265-270.

Kirbag, S., & Akyuz, M. (2008). Effect of various agro-residues on growing periods, yield and biological efficiency of *Pleurotus eryngii*. *Journal Food Agricultural Environment*, *6*, 402-405.

Knop, D., Yarden, O., & Hadar, Y. (2015). The ligninolytic peroxidases in the genus *Pleurotus*: divergence in activities, expression, and potential applications. *Applied Microbiology and Biotechnology*, *99*(3), 1025-1038.

Kües, U., & Liu, Y. (2000). Fruiting body production in basidiomycetes. *Applied Microbiology and Biotechnology*, *54*(2), 141-152.

Kuhad, R. C., Singh, A., & Eriksson, K. E. L. (1997). Microorganisms and enzymes involved in the degradation of plant fiber cell walls. In *Biotechnology in the Pulp and Paper Industry* (pp. 45-125). Berlin, Heidelberg: Springer.

Kumar, M., Ou, Y. L., & Lin, J. G. (2010). Co-composting of green waste and food waste at low C/N ratio. *Waste Management*, *30*(4), 602-609.

Kuo, M. (2005, February). *Pleurotus ostreatus:* The oyster mushroom. Retrieved on January 16, 2017 from http://www.mushroomexpert.com/pleurotus_ostreatus.

Lavi, I., Levinson, D., Peri, I., Tekoah, Y., Hadar, Y., & Schwartz, B. (2010). Chemical characterization, antiproliferative and antiadhesive properties of polysaccharides extracted from *Pleurotus pulmonarius* mycelium and fruiting bodies. *Applied Microbiology and Biotechnology*, *85*(6), 1977-1990.

Leifa, F., Pandey, A., & Soccol, C. R. (2001). Production of *Flammulina velutipes* on coffee husk and coffee spent-ground. *Brazilian Archives of Biology and Technology,* 44*(2*), 205-212.

Li, W., Li, X., Yang, Y., Zhou, F., Liu, Y., Zhou, S., & Yu, H. (2015). Effects of different carbon sources and C/N values on non-volatile taste components of *Pleurotus eryngii*. *International Journal of Food Science & Technology*, *50*(11), 2360-2366.

Machado, E. M. S., Rodriguez-Jasso, R. M., Teixeira, J. A., & Mussatto,S. I. (2012). Growth of fungal strains on coffee industry residues with removal of polyphenolic compounds. *Biochemical Engineering Journal*, *60,* 87-90.

Maftoun, P., Johari, H., Soltani, M., Malik, R., Othman, N. Z., & El Enshasy, H. A. (2015). The edible mushroom *Pleurotus* spp.: I. Biodiversity and nutritional values. *International Journal of Biotechnology for Wellness Industries*, *4*(2), 67-83.

Malaysian Dietary Guideline (2010). Guide to nutrition labelling and claims. *Food Safety and Quality Division Ministry of Health Malaysia* (10th ed.). Malaysia: Technical Working Group on Nutritional Guidelines.

Malaysian Meteorological Department. (2017). *Buletin Cuaca Bulanan*. Retrieved on April 4, 2018 from http://www.met.gov.my/iklim/laporanringkasan.

Mandeel, Q. A., Al-Laith, A. A., and Mohamed, S. A. (2005). Cultivation of oyster mushrooms (*Pleurotus* spp.) on various lignocellulosic wastes. *World Journal of Microbiology and Biotechnology*, *21*(4), 601-607.

Mane, V. P., Patil, S. S., Syed, A. A., & Baig, M. M. V. (2007). Bioconversion of low quality lignocellulosic agricultural waste into edible protein by *Pleurotus sajor-caju* (Fr.) Singer. *Journal of Zhejiang University Science B*, *8*(10), 745.

Manzi, P., Aguzzi, A., & Pizzoferrato, L. (2001). Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry*, *73*(3), 321-325.

Manzi, P., Gambelli, L., Marconi, S., Vivanti, V., & Pizzoferrato, L. (1999). Nutrients in edible mushrooms: an inter-species comparative study. *Food Chemistry*, *65*(4), 477-482.

Marino, R. H., Eira, A. F. D., Kuramae, E. E., & Queiroz, E. C. (2003). Morphomolecular characterization of *Pleurotus ostreatus* (Jacq. Fr.) kummer strains in relation to luminosity and temperature of frutification. *Scientia Agricola*, *60*(3), 531-535.

Martínez Carrera, D., Sobal, M., & Morales, P. (1988). El efecto de la cafeína sobre el crecimiento e intracruzamiento de *Pleurotus ostreatus* en el laboratorio. *Revista Mexicana de Micologia*, *4*, 131-135.

Mattila, P., Salo-Väänänen, P., Könkö, K., Aro, H., & Jalava, T. (2002). Basic composition and amino acid contents of mushrooms cultivated in Finland. *Journal of Agricultural and Food Chemistry*, *50*(22), 6419-6422.

McCleary, B.V. & Draga, A. (2016). Measurement of β-Glucan in Mushrooms and Mycelial Products. *Journal of AOAC International*, *99*(2), 364-373.

Mkhize, S. S., Zharare, G. E., Basson, A. K., Mthembu, M. S., & Cloete, J.(2017). Performance of *Pleurotus pulmonarius* mushroom grown on maize stalk residues supplemented with various levels of maize flour and wheat bran. *Food Science and* Technology, *37*(4), 570-577.

Meier, E., 2013. The wood database. Retrieved on January 11, 2018 from http://www.wood-database.com/wood-articles/wood-allergies-and-toxicity/.

Miles, P. G., & Chang, S. (1997). *Mushroom Biology: Concise Basics and Current Developments*. Singapore: World Scientific.

Miles, P. G., & Chang, S. T. (2004). *Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact*. Florida: CRC press.

Ministry of Agriculture Malaysia. (2011). *National Agro-Food Policy*. Malaysia: Kemubu Agriculture Development Authority. Retrieved on March 7, 2017 from http: www/moa.gov.my.

Mohamed Imran, M., Mohamed Mahroop Raja, M., Abdul Basith, J., & Asarudeen, A. (2011). Determination of total phenol, flavonoid and antioxidant activity of edible mushrooms *Pleurotus florida* and *Pleurotus eous*. *International Food Research Journal*, *18*(2), 70-81.

Morais, M. H., Ramos, A. C., Matos, N., & Oliveira, E. J. S. (2000). Production of shiitake mushroom (*Lentinus edodes*) on lignocellulosic residues. Cultivo del hongo shiitake (*Lentinus edodes*) en residuos lignocelulósicos. *Food Science and Technology International*, *6*(2), 123-128.

Mshandete, A. M., & Cuff, J. (2008). Cultivation of three types of indigenous wild edible mushrooms: *Coprinus cinereus*, *Pleurotus flabellatus* and *Volvariella volvocea* on composted sisal decortications residue in Tanzania. *African Journal of Biotechnology*, *7*(24), 4551-4562.

Mukhopadhyay, R., Chatterjee, B. P., & Guha, A. K. (2002). Biochemical changes during fermentation of edible mushroom *Pleurotus sajor-caju* in whey. *Process Biochemistry*, *38*(5), 723-725.

Murthy, P. S., & Naidu, M. M. (2012). Sustainable management of coffee industry by-products and value addition—A review. *Resources, Conservation and Recycling*, *66*, 45-58.

Mussatto, S. I., Ballesteros, L. F., Martins, S., Maltos, D. A. F., Aguilar,C. N., & Teixeira, J. A. (2013). Maximization of fructooligosaccharides and β-fructofuranosidase production by *Aspergillus japonicusunder* solid-state fermentation conditions. *Food and Bioprocess Technology*, *6(8)*, 2128–2134.

Muthangya, M., Mshandete, A. M., Hashim, S. O., Amana, M. J., & Kivaisi, A. K. (2013). Evaluation of enzymatic activity during vegetative growth and fruiting of *Pleurotus* HK 37 on Agave sisalana saline solid waste. *Journal of Chemical, Biological and Physical Sciences (JCBPS)*, *4*(1), 247-258.

Myronycheva, O., Bandura, I., Bisko, N., Gryganskyi, A. P., & Karlsson, O. (2017). Assessment of the growth and fruiting of 19 oyster mushroom strains for indoor cultivation on lignocellulosic wastes. *BioResources*, *12*(3), 4606-4626.

Naraian, R., Sahu, R. K., Kumar, S., Garg, S. K., Singh, C. S., & Kanaujia, R. S. (2009). Influence of different nitrogen rich supplements during cultivation of *Pleurotus florida* on corn cob substrate. *The Environmentalist*, *29*(1), 1-7.

Narayanasamy, P., Suganthavel, P., Sabari, P., Divya, J., Vanchinathan, M., & Kumar, M. (2008). Cultivation of mushroom (*Pleurotus florida*) by using two different agricultural wastes in laboratory condition. *The Internet Journal of Microbiology*, *7*(2), 1457-1462.

National Research Council. (1989). Dietary Reference Intakes for Macronutrients. *Food and Nutrition Board Recommended Dietary Allowances* (pp. 52-77). Washington: National Academy Press.

Ng, S. H., Mohd Zain, M. S., Zakaria, F., Wan Ishak, W. R., & Wan Ahmad, W. A. N. (2015). Hypoglycemic and antidiabetic effect of *Pleurotus sajor-caju* aqueous extract in normal and streptozotocin-induced diabetic rats. *BioMed Research International*, *2015*, 1-8. http://dx.doi.org/10.1155/2015/214918.

Nunes, M. D., Maria, J., Paes, S. A., Júlio, J., & Ribeiro, O. (2012). Nitrogen supplementation on the productivity and the chemical composition of oyster mushroom. *Journal of Food Research*, *1*(2), 113–119.

Obodai, M., Ferreira, I. C., Fernandes, Â., Barros, L., Mensah, D. L. N., Dzomeku, M., & Takli, R. K. (2014). Evaluation of the chemical and antioxidant properties of wild and cultivated mushrooms of Ghana. *Molecules*, *19*(12), 19532-19548.

Oei, P. (2003). *Mushroom Cultivation: Appropriate Technology for Mushroom Growers* (Ed. 3). Netherlands: Backhuys Publishers.

Okhuoya, J. A., Akpaja, E. O., & Oghenekaro, A. (2005). Cultivation of *Lentinus squarrosulus* (Mont.) Singer on sawdust of selected tropical tree species. *International Journal of Medicinal Mushrooms*, *7*(3), 440-441.

Onuoha, C. I., Uchechi, U., & Onuoha, B. C. (2009). Cultivation of *Pleurotus pulmonarius* (mushroom) using some agrowaste materials. *Agricultural Journal*, *4*(2), 109-112.

Owaid, M. N., Abed, A. M., & Nassar, B. M. (2015). Recycling cardboard wastes to produce blue oyster mushroom *Pleurotus ostreatus* in Iraq. *Emirates Journal of Food and Agriculture*, *27*(7), 537-541.

Onyango, B.O., Palapala, V.A., Arama, P.F.Wagai., & Gichumu B.M., (2011). Suitability of selected supplemented substrates for cultivation of Kenyan native wood ear mushrooms (*Auricularia auricular-judae*). *American Journal of Food Technology*, *6*(5), 395-403.

Oyetayo, V. O., & Ariyo, O. O. (2013). Micro and macronutrient properties of *Pleurotus ostreatus* (Jacq: Fries) cultivated on different wood substrates. *Jordan Journal of Biological Sciences*, *6*(3), 223-226.

Pandey, A. (2001). *Solid-state Fermentation in Biotechnology: Fundamentals and Applications*. New Delhi: Asiatech Publishers Inc.

Pant, D., Reddy, U. G., & Adholeya, A. (2006). Cultivation of oyster mushrooms on wheat straw and bagasse substrate amended with distillery effluent. *World Journal of Microbiology and Biotechnology*, *22*(3), 267-275.

Parshetti, G. K., Hoekman, S. K., & Balasubramanian, R. (2013). Chemical, structural and combustion characteristics of carbonaceous products obtained by hydrothermal carbonization of palm empty fruit bunches. *Bioresource Technology*, *135*, 683-689.

Pathmashini, L., Arulnandhy, V., & Wijeratnam, R. S. (2009). Cultivation of oyster mushroom (*Pleurotus ostreatus*) on sawdust. *Ceylon Journal of Science (Biological Sciences)*, *37*(2). 177-182.

Paul, J. S., Tiwari, K. L., & Jadhav, S. K. (2015). Long term preservation of commercial important fungi in glycerol at 4 C. *International Journal of Biological Chemistry*, *9*(2), 79-85.

Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz-Rubio, M. E., Serrano, J., Goñi, I., & Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Research International*, *41*(3), 274-285.

Pérez-Martínez, A. S., Acevedo-Padilla, S. A., Bibbins-Martínez, M., Galván-Alonso, J., & Rosales-Mendoza, S. (2015). A perspective on the use of *Pleurotus* for the development of convenient fungi-made oral subunit vaccines. *Vaccine*, *33*(1), 25-33.

Philippoussis, A. N. (2009). Production of mushrooms using agro-industrial residues as substrates. In *Biotechnology for Agro-industrial Residues Utilisation* (pp. 163-196). Netherlands: Springer.

Philippoussis, A., Zervakis, G., & Diamantopoulou, P. (2001). Bioconversion of agricultural lignocellulosic wastes through the cultivation of the edible mushrooms *Agrocybe aegerita*, *Volvariella volvacea* and *Pleurotus* spp. *World Journal of Microbiology and Biotechnology*, *17*(2), 191-200.

Pujol, D., Liu, C., Gominho, J., Olivella, M. À., Fiol, N., Villaescusa, I., & Pereira, H. (2013). The chemical composition of exhausted coffee waste. *Industrial Crops and Products*, *50*, 423-429.

Rathore, H., Prasad, S., & Sharma, S. (2017). Mushroom nutraceuticals for improved nutrition and better human health: a review. *PharmaNutrition*, *5*(2), 35-46.

Rizki, M., & Tamai, Y. (2011). Effects of different nitrogen rich substrates and their combination to the yield performance of oyster mushroom (*Pleurotus ostreatus*). *World Journal of Microbiology and Biotechnology*, *27*(7), 1695-1702.

Roshita, I., & Goh, S. Y. (2018). Effect of exposure to different colors light emitting diode on the yield and physical properties of grey and white oyster mushrooms. *AIP Conference Proceedings, 2030*(1), 020110.

Rosmiza, M. Z., Davies, W. P., Jabil, M. J., & Mazdi, M. (2016). Prospects for increasing commercial mushroom production in Malaysia: challenges and opportunities. *Mediterranean Journal of Social Sciences*, *7*(1), 406-415.

Royse, D. (2002). Influence of spawn rate and commercial delayed release nutrient levels on *Pleurotus cornucopiae* (oyster mushroom) yield, size, and time to production. *Applied Microbiology and Biotechnology*, *58*(4), 527-531.

Royse, D. J. (1997). Speciality mushrooms and their cultivation. *Horticultural Reviews, Volume 19*, 59-97.

Royse, D. J. (2010). Effects of fragmentation, supplementation and the addition of phase II compost to 2nd break compost on mushroom (*Agaricus bisporus*) yield. *Bioresource Technology*, *101*(1), 188-192.

Royse, D. J., & Sanchez-Vazquez, J. E. (2003). Influence of precipitated calcium carbonate ($CaCO_3$) on shiitake (*Lentinula edodes*) yield and mushroom size. *Bioresource Technology*, *90*(2), 225-228.

Saavedra, M., Benitez, E., Cifuentes, C., & Nogales, R. (2006). Enzyme activities and chemical changes in wet olive cake after treatment with *Pleurotus ostreatus* or Eisenia fetida. *Biodegradation*, *17*(1), 93-102.

Sánchez, C. (2004). Modern aspects of mushroom culture technology. *Applied Microbiology and Biotechnology*, *64*(6), 756-762.

Sánchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied Microbiology and Biotechnology*, *85*(5), 1321-1337.

Sari, M., Prange, A., Lelley, J. I., & Hambitzer, R. (2017). Screening of beta-glucan contents in commercially cultivated and wild growing mushrooms. *Food Chemistry*, *216*, 45-51.

Saura-Calixto, F., & Goñi, I. (2006). Antioxidant capacity of the Spanish Mediterranean diet. *Food Chemistry*, *94*(3), 442-447.

Shah, Z. A., Ashraf, M., & Ishtiaq, M. (2004). Comparative study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates (wheat straw, leaves, saw dust). *Pakistan Journal of Nutrition*, *3*(3), 158-160.

Sharma, S., Yadav, R. K. P., & Pokhrel, C. P. (2013). Growth and yield of oyster mushroom (*Pleurotus ostreatus*) on different substrates. *Journal on New Biological Reports*, *2*(1), 03-08.

Sher, H., Al-Yemeni, M., Bahkali, A. H., & Sher, H. (2010). Effect of environmental factors on the yield of selected mushroom species growing in two different agro ecological zones of Pakistan. *Saudi Journal of Biological Sciences*, *17*(4), 321-326.

Silva, E. G., Dias, E. S., Siqueira, F. G., & Schwan, R. F. (2007). Chemical analysis of fructification bodies of *Pleurotus sajor-caju* cultivated in several nitrogen concentrations. *Food Science and Technology*, *27*(1), 72-75.

Silva, S. O., Costa, S. M. G. D., & Clemente, E. (2002). Chemical composition of *Pleurotus pulmonarius* (Fr.) Quél., substrates and residue after cultivation. *Brazilian Archives of Biology and Technology*, *45*(4), 531-535.

Singh, M., Vijay, B., Kamal, S., & Wakchaure, G. C. (2011). *Mushrooms: Cultivation, Marketing and Consumption*. New Delhi, India: India Council of Agricultural Research (ICAR).

Smiderle, F. R., Olsen, L. M., Carbonero, E. R., Baggio, C. H., Freitas, C. S., Marcon, R., ... & Iacomini, M. (2008). Anti-inflammatory and analgesic properties in a rodent model of a (1→ 3),(1→ 6)-linked β-glucan isolated from *Pleurotus pulmonarius*. *European Journal of Pharmacology*, *597*(1-3), 86-91.

Smith, J. E., Rowan, N. J., & Sullivan, R. (2002). Medicinal mushrooms: a rapidly developing area of biotechnology for cancer therapy and other bioactivities. *Biotechnology Letters*, *24*(22), 1839-1845.

Stamets, P. (1993). *Growing gourmet and medicinal fungi*. Berkeley, California: Ten Speed Press.

Stamets, P. (2000). *Growing gourmet and medicinal fungi*. Berkeley, California: Ten Speed Press.

Stoknes, K., Høiland, K., Norgaard, E., & Hammer, J. P. (2008). From food to waste to food: a high yield of mushrooms from food waste compost. In *Science and Cultivation of Edible and Medicinal Fungi: Mushroom Science XVII. Proceedings of the 17th Congress of the International Society for Mushroom Science 20-24 May 2008* (pp. 20-24). South Africa: Penguin Random House.

Subbu Lakshmi, S., & Sornaraj, R. (2014). Utilization of seafood processing wastes for cultivation of the edible mushroom *Pleurotus flabellatus*. *African Journal of Biotechnology*, *13*(17), 1779-1785.

Sunset (2017).The Starbucks coffee compost test. Retrieved on December 10, 2017 from https://www.sunset.com/garden/earth-friendly/starbucks-coffee-compost-test.

Tabi, A. N. M., Zakil, F. A., Fauzai, M. F., Ali, N., & Hassan, O. (2008). The usage of empty fruit bunch (EFB) and palm pressed fibre (PPF) as substrates for the cultivation of *Pleurotus ostreatus*. *Jurnal Teknologi*, *49*(F), 189-196.

Thambirajah, J. J., Zulkali, M. D., & Hashim, M. A. (1995). Microbiological and biochemical changes during the composting of oil palm empty-fruit-bunches. Effect of nitrogen supplementation on the substrate. *Bioresource Technology*, *52*(2), 133-144.

The Star Online (2015). University Malaya aims for zero waste. Retrieved on March 10, 2017 from www.thestar.com.my/lifestyle/features/2015/03/02/um-aims-for-zero-waste/.

The Star Online (2016). Research shows Malaysians waste enough to feed millions daily. Retrieved on April 8, 2017 from https://www.thestar.com.my/news/nation/2016 /05/31/ food- and- money- down- the- drain- research- shows- 69alaysians-waste-enough-to-feed-millions-daily/.

Thomas, L., Larroche, C., & Pandey, A. (2013). Current developments in solid-state fermentation. *Biochemical Engineering Journal*, *81*, 146-161.

Thomsen, M.H. (2005). Complex media from processing of agricultural crops for microbial fermentation. *Applied Microbiology and Biotechnology*, 68, 598–606.

Tripathy, A., Patel, A. K., & Sahoo, T. K. (2009). Effect of various substrates on linear mycelial growth and fructification of *Volvariella dysplasia*. *Asian Journal of Plant Sciences*, *8*(8), 566.

Tripathy, A., Sahoo, T. K., & Begera, S. R. (2010). Yield evaluation of paddy straw mushrooms (*Volvariella* spp.) on various lignocellulosic wastes. *International Journal of Applied Agricultural Research*, *5*(3), 317-326.

UM Zero Waste Campaign (2018). UM ZWC annual report 2017, 46-50. Retrieved on August 8, 2018 from https://www.um.edu.my/about-um/umique/um-living-lab/zero-waste.

Valverde, M. E., Hernández-Pérez, T., & Paredes-López, O. (2015). Edible mushrooms: improving human health and promoting quality life. *International Journal of Microbiology*, *2015*, 14.

Velázquez-Cedeño, M. A., Mata, G., & Savoie, J. M. (2002). Waste-reducing cultivation of *Pleurotus ostreatus* and *Pleurotus pulmonarius* on coffee pulp: changes in the production of some lignocellulolytic enzymes. *World Journal of Microbiology and Biotechnology*, *18*(3), 201-207.

Wabali, C. V., & Wocha, I. (2013). The effect of nutrient concentration on the yield of mushroom (*Pleurotus ostreatus*). *Greener Journal of Agricultural Sciences*, *3*(6), 437-444.

Wang, H.H. (1999). Development and/or reclamation of bioresources with solid state fermentation. *Proceeding of Natural Science Council*, *23(2)*, 45–61.

Wang, L. & Yang, S.T. (2007). Solid state fermentation and its application. In Yang, S.T. (Ed.). *Bioprocessing for Value-added Products from Renewable Resources.* Elsevier B.V., 465-488.

World Health Organization (2015). WHO calls on countries to reduce sugars intake among adults and children. Retrieved on November 16, 2018 from https://www.who.int/mediacentre/news/releases/2015/sugar-guideline/en/.

Wolfe, R. R., Cifelli, A. M., Kostas, G., & Kim, I. Y. (2017). Optimizing Protein Intake in Adults: Interpretation and Application of the Recommended Dietary Allowance Compared with the Acceptable Macronutrient Distribution Range–. *Advances in Nutrition*, *8*(2), 266-275.

Yang, F. C., Hsieh, C., & Chen, H. M. (2003). Use of stillage grain from a rice-spirit distillery in the solid state fermentation of *Ganoderma lucidum*. *Process Biochemistry*, *39*(1), 21-26.

Yanga, J. H., Lin, H. C., & Mau, J. L. (2002). Antioxidant properties of several commercial mushrooms. *Food Chemistry*, *77*(2), 229-235.

Zadrazil, F., Compare, G., & Maziero, R. (2004). Biology, cultivation and utilization of Pleurotus species [Abstract]. *Science and Cultivation of Edible and Medicinal Fungi, 16*(49), 687-696.

Zervakis, G.I., Philippoussis, A., Ioannidou, S., & Diamantopoulou, P. (2001). Mycelium growth kinetics and optimal temperature conditions for the cultivation of edible mushroom species on lignocellulosic substrates. *Folia Microbiologica*, *46*(3), 231-234.

Zhang, X., Wang, L., Ma, F., Yang, J., & Su, M. (2017). Effects of arbuscular mycorrhizal fungi inoculation on carbon and nitrogen distribution and grain yield and nutritional quality in rice (*Oryza sativa* L.). *Journal of the Science of Food and Agriculture*, *97*(9), 2919-2925.