COMPARISON OF USED GROUND COFFEE AND SAWDUST MIXTURE AS SUBSTRATES OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

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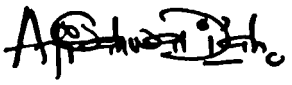

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ABSTRACT

A field experiment was conducted at the Faculty of Sustainable Agriculture in Universiti Malaysia Sabah, Sandakan, Sabah to investigate the comparison of used ground coffee (UGC) and sawdust mixture (SM) as substrates of oyster mushroom (*Pleurotus ostreatus*). The oyster mushroom, *P. ostreatus* was cultivated on used ground coffee and sawdust mixture with different percentage ratio in polyethene bags. The five treatments used are Treatment 1 (100% SM), Treatment 2 (75% SM, 25% UGC), Treatment 3 (50% SM, 50% UGC), Treatment 4 (25% SM, 75% UGC) and Treatment 5 (100% UGC). Each treatment was replicated five times. Completely Randomized Design (CRD) was used in this experiment. The spawning was done after the sterilization of the substrate bag then being left for incubation for 33 days with the temperature of 20-25°C and 75-90% relative humidity. Mycelium run rate, time taken for pinhead formation stage, biological efficiency, fresh weight of mushroom and total yield were measured and all data was analyzed using one way Variance Analysis Test (ANOVA) and Tukey analysis is used to analyze the difference of the average mean for each treatment at 5% level of significance. The experiment result indicates that there are significant difference of mycelium run rate, time taken for pinhead formation stage, biological efficiency, mushroom fresh weight and total yield. For Treatment 1 and Treatment 5 the mean for mycelium run rate, time taken for pinhead formation stage, biological efficiency, fresh weight of mushroom and total yield are 0.00 respectively. While for Treatment 2, Treatment 3 and Treatment 4, mean for mycelium run rate was 0.378 cm/day, 0.394 cm/day and 0.314 cm/day; mean for time taken for pinhead formation stage was 6.8 days, 9.8 days and 12.0 days; mean for biological efficiency was 0.354 %, 13.680 % and 5.984 %; mean for fresh weight of mushroom was 0.610 g, 25.262 g and 11.374 g and mean for total yield was 0.00354 g/g, 0.13678 g/g and 0.05984 g/g respectively. The result obtained from the experiment in this study indicates that there is potential of using used ground coffee (UGC) as an alternative substrate of oyster mushroom other than using sawdust mixture (SM) substrate.

PERBANDINGAN HAMPAS KOPI DENGAN CAMPURAN HABUK KAYU SEBAGAI SUBSTRAT CENDAWAN TIRAM (Pleurotus ostreatus)

ABSTRAK

Satu eksperimen lapangan telah dijalankan di Fakulti Pertanian Lestari, Universiti Malaysia Sabah, Sandakan, Sabah untuk menyiasat perbandingan antara hampas kopi dan campuran habuk kayu sebagai substrat cendawan tiram (Pleurotus ostreatus). Cendawan tiram P. ostreatus telah ditanam menggunakan hampas kopi dan campuran habuk kayu dengan perbezaan peratusan nisbah di dalam plastik polyethene. Lima rawatan yang digunakan dalam eksperimen ini adalah Rawtan 1 (100% campuran habuk kayu), Rawatan 2 (75% campuran habuk kayu, 25% hampas kopi), Rawatan 3 (50% campuran habuk kayu, 50% hampas kopi), Rawatan 4 (25% campuran habuk kayu, 75% hampas kopi) dan Rawatan 5 (100% hampas kopi). Setiap rawatan telah direplikasikan sebanyak lima kali. Completely Randomized Design (CRD) telah digunakan dalam eksperimen ini. Pembiakan dilakukan selepas pensterilan beg substrat kemudian ditinggalkan untuk pengeraman selama 33 hari dengan suhu 20-25°C dan kelembapan 75-90%. Kadar jangka miselium, masa yang diambil untuk peringkat pembentukan pinhead, kecekapan biologi, berat basah cendawan dan jumlah hasil telah diukur dan semua data telah dianalisis menggunakan Anova sehala dan analisis Tukey digunakan untuk menganalisis perbezaan purata min untuk setiap rawatan pada 5% tahap signifikan. Keputusan eksperimen menunjukkan bahawa terdapat perbezaan yang disignifikasikan daripada kadar jangka miselium, masa yang diambil untuk peringkat pembentukan pinhead, kecekapan biologi, berat basah cendawan dan jumlah hasil. Untuk Rawatan 1 dan Rawatan 5 min untuk kadar jangka miselium, masa yang diambil untuk peringkat pembentukan pinhead, kecekapan biologi, berat basah cendawan dan jumlah hasil ialah 0.00. Manakala bagi Rawatan 2, Rawatan 3 dan Rawatan 4, min untuk kadar jangka miselium ialah 0.378 cm/hari, 0.394 cm/hari dan 0.314 cm/hari; min untuk masa yang diambil untuk peringkat pertumbuhan pinhead ialah 6.8 hari, 9.8 hari dan 12.0 hari; min untuk kecekapan biologi ialah 0.354 %, 13.680 % dan 5.984 %; min untuk berat basah cendawan ialah 0.610 g, 25.262 g dan 11.374 g dan min untuk jumlah hasil ialah 0.00354 g/g, 0.13678 g/g dan 0.05984 g/g. Keputusan yang diperolehi daripada eksperimen kajian ini menunjukkan terdapat potensi untuk menggunakan hampas kopi sebagai substrat alternatif cendawan tiram selain menggunakan substrat campuran habuk kayu.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percentage
+	Plus
=	Equal
BE	Biological Efficiency
C	Carbon
cm/day	Centimetre per day
g	Gram
g/g	Gram per gram
MRR	Mycelial Run Rate
N	Nitrogen
°C	Degree celsius
PF	Pinhead Formation
PSI	Pressure
SM	Sawdust Mixture
UGC	Used Ground Coffee

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Mushrooms are fleshy, spore-bearing reproductive structures of fungi grown on organic substrates and for a long time, have played an important role as human food due to its nutritional and medicinal properties (Etich *et al.,* 2013). The cultivation of edible mushrooms is a worldwide important commercial activity (Chang, 2000). Several aspects have contributed to the development of this activity: (a) the raw materials used are waste from agribusiness that have little commercial value and are easy to acquire (Rajarathnam and Bano, 1991), (b) some species can be grown with relatively simple technology and low investment.

Worldwide commercial mushroom production has progressively improved during the last decade, but only a few genera of Basidiomycetes (*Agaricus, Lentinula, Pleurotus, Auricularia, Volvariella, Flammulina, Thermella* and few others) are industrially cultivated (Stamets, 1993). The edible mushroom *Pleurotus ostreatus* is considered a good alternative for protein rich food production in tropical countries (Chang, 1989; Martinez and Quirarte, 1984). Oyster mushroom can be grown on various substrates including paddy straw, maize stalk/cobs, vegetable plant residues, bagasse (Hassan *et al.*, 2011). The substrates most utilised are agricultural residues, such as corncobs, cottonseed husk, sugarcane bagasse, besides sawdust (Chang, 1989; Yang, 1986; Fan and Ding, 1990; Wang, 1995; Royse, 1995).

Many countries have abundance of agro-industrial wastes that can be used as substrates to produce mushrooms. Large quantities of agro-industrial wastes that are produced worldwide often cause environmental and health problems (Garg and Gupta, 2009). In addition, the ever growing need of cheap nutritious food, and the lack of protein in developing countries led to the development of the mushroom cultivation industry (Sivaprakasam and Kandasawmy, 1981; Levanon *et al.,* 1993; Yildiz *et al.,* 1997; Croan, 2000; Zervakis *et al.,* 2001). *Pleurotus sp.* shows good ability in producing fruiting body and simultaneously reducing or degrading toxic substances present in the substrate (Chang, 1989; Hadar, 1993; Thiclke, 1989).

Coffee husk and coffee is one of the most important beverages of the world, and its yearly production is about one million tons in more than 50 countries (Pandey *et al.,* 2000). At different stages from harvesting to the processing and consumption, coffee husk and spent-ground are generated in more than two million tons quantity yearly (Tango, 1971; Soccol, 1995; Pandey and Soccol, 2000). Coffee contains compounds such as caffeine, tannins, and polyphenols (Fan *et al.,* 1999a, 1999b). Due to the presence of the compounds (caffeine, tannins and polyphenols), these organic solid residues show toxic nature and thus have not been utilized potentially. This also led the problem of the environmental pollution.

1.2 Justification of Study

The production of new species of edible mushroom is an innovative way to recycle agro-industrial wastes into food production. The genus *Pleurotus* is a genus of gilled mushrooms which includes one of the most widely eaten mushrooms *Pleurotus ostreatus*. Species of *Pleurotus* may be called oyster, abalone, or tree mushrooms and are some of the most commonly cultivated edible mushrooms in the world. The substrate that was used in this experiment was coffee residue. Using ground coffee helps to reduce the amount of wasted materials as well as emissions of greenhouse gases and other air pollutants. Different percentage of used ground coffee mixed with sawdust mixture was used as substrate to test *P. ostreatus* cultivation.

1.3 Objective

i. To compare the growth and yield of *P. ostreatus* on sawdust mixture and used ground coffee as substrates at different percentage ratio.

1.4 Hypothesis

H_o: There is no significant difference in the growth and yield of *P.* ostreatus on the used ground coffee and sawdust mixture as substrates.

H_a: There is significant difference in the growth and yield of *P.* ostreatus on the used ground coffee and sawdust mixture as substrates.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction of Mushroom

A mushroom is defined as a macrofungus with a distinctive fruiting body which can be either epigeous or hypogeous. The macrofungi has fruiting bodies that are large enough to be seen using the naked eye and to be picked up by hand (Chang and Miles, 1988). In a narrow sense, the word mushroom also refers only to the fruit body.

Mushroom used to be classified into the Kingdom Plantae, but now, they belong to the Kingdom Fungi due to the unique fungal characteristics which draw a clear line from animals and plants. Unlike green plants, mushroom are heterotrophs. Not having chlorophyll, they cannot generate nutrients by photosynthesis, but take nutrients from outer sources. Most mushroom species are under Basidiomycota and Ascomycota, the two phyla under the Kingdom Fungi (Table 2.1).

Table 2.1 Kingdom Fungi

Ascomycota	Sac fungi (Yeast to large cup fungi)
Basidiomycota	Higher fungi (Toadstool, Puffball, Bracket Fungi)
Zygomycota	Molds, Mychorrhiza Fungi and soil decomposers
Chytridiomycota	Primitive fungi, chytrids
Deutromycota	Asexually reproducing fungi

Source: Song, 2004

Mushrooms breed by spores (seeds for plants). Under the proper conditions, spores germinate into hyphae (Collectively, mycelia). Mycelia are filamentous and generally unseen with the naked eye. Germinated hyphae form primary mycelia, and then secondary mycelia through plasmogamy (hyphal fusion). They accumulate nutrients from the substrate (soil for plants) and colonize substrate. When stimulated

by temperature, humidity and others, mycelial colony forms pins under certain conditions to grow into fruiting bodies (fruits for plants). Young fruiting bodies are called pins (buds for plants). Pins differentiate into a cap and stem forming fruiting bodies (Song, 2004).

2.2 Nutritional Value of Mushroom

Mushroom has been studied for nutritional and medicinal purposes and various potential antitumor and immune modulator substances, mainly polysaccharides have been identified (Zhang *et al.,* 2007) for medicinal purposes. Other than as the source of extra ordinary power and virility and are used in the preparation of many continental dishes and have medicinal properties like anticancerous, anticholestral and antitumorous (Shah *et al.,* 2004).

Mushrooms are useful against diabetes, ulcer and lung diseases (Quimio, 1976) and also been consumed to prevent cancer and cardiac diseases, to improve blood circulation and to reduce cholesterol (Wasser and Weis, 1991). They are used for physical and emotional stress, asteoporosis, gastric ulcers and chronic hepatitis; for the improvement for the quality of life of patients with diabetes and especially for the stimulation of immunity (Menoli *et al.,* 2004, Guterrez *et al.,* 2004, Angeli *et al.,* 2006, Choi *et al.,* 2006, Grind *et al.,* 2006). Moreover, mushrooms have been evaluated for their nutritional status mainly on the basis of their chemical composition.

Mushrooms are the good source of protein, vitamins and minerals (Khan *et al.,* 1981). Mushroom contain about 85-95% water, 3% protein, 4% carbohydrates, 0.1% fats, 1% minerals and vitamins (Tewari, 1986). It contains 19-35% protein on dry weight basis as compared to 7.3% in rice, 13.2% in wheat and 25.2% in milk (Chang and Miles, 1988). It is rich in essential minerals and trace elements (Chandha and Sharma, 1995). Mushrooms contain appreciable amount of potassium, phosphorus, copper and iron but low level of calcium (Anderson and Feller, 1942). It has now been well established that cultivated mushrooms contain reasonable amounts of proteins, carbohydrates, minerals and vitamins, and nutritionally they rank between low grade vegetables and high grade meats. Mushroom protein is intermediate between that of animals and vegetables (Kurtzman, 1976) and is for superior quality because of

presence of all the essential amino acids (Purkayastha and Nayak, 1981). *Pleurotus* species contains high potassium to sodium ratio, which makes mushrooms an ideal food for patients suffering from hypertension and heart diseases (Patil *et al.*, 2010). Mushroom also contain appreciable amount of Niancin, panthothenic acid and biotin (Subramanian, 1986).

A lectin isolated from the fruiting bodies of *P. ostreatus* demonstrated antitumor activity in mice bearing sarcoma and hepatoma (Wang *et al.,* 2000). In animal studies, oyster mushrooms significantly enhanced plasma cholesterol turnover by 50% with a corresponding 25% decrease in liver cholesterol levels as compared to controls (Bobek *et al.,* 1995). Other animal studies have shown significant reductions in serum and liver cholesterol levels when dried and powdered mushrooms were included in the animal diets, even with high-fat diets and in animals with hereditary high cholesterol levels (Bobek *et al.,* 1991).

Nutritional analyses of several mushroom species of different origins have been carried out in many laboratories in the world. But nutritional value of locally cultivated mushrooms remains speculative (Alam *et al*, 2008). Moreover, nutritional composition is affected by many factors; these include differences among strains, the composition of growth substrate, the method of cultivation, stage of harvesting, specific portion of the fruiting bodies used for analysis (Benjamin, 1995).

2.3 Mushroom Cultivation

Mushroom cultivation is a potential biotechnological process where waste plant materials or negative value crop residues can be converted into valuable food (Alemu, 2013). Mushroom can be grown on agricultural and industrial waste. More than the total produce from the land remain unused as waste in the form of straws, leaves, stems, roots and others (Zadrazil, 1978). These wastes can be recycled into food and environment may be less endangered by pollution (Hayes, 1978). Cultivation of mushroom is eco-friendly and profitable agribusiness but labour intensive (Chandha and Sharma, 1995). Mushrooms have been recognized as a high potential converter of cheap celluloses into valuable protein (Poppe, 2000). *Pleurotus* species also known as oyster mushrooms can be cultivated on different cellulosic waste (Oyetayo and Ariyo,

2013). The rapid growth and the ability to utilize various lignocellulosic substances, make *Pleurotus* species cultivation possible in different part of the world (Manso *et al.,* 2011).

Oyster mushrooms are popular and widely cultivated throughout the world mostly in Asia and Europe owing to the simple and low cost production technology and higher biological efficiency (Mane *et al.,* 2007). It belongs to class *Basidiomycetes,* subclass *Hollobasidiomycetidae,* order *Agaricales* (Ibekwe *et al.,* 2008). *Pleurotus* species are efficient lignin degraders which can grow on wide variety of agricultural wastes with broad adaptability to varied agro-climatic conditions (Jandaik and Goyal, 1995). The practice of mushroom cultivation not only produces food but also improves the straw quality. This takes place by reducing lignin, cellulose, hemicellulose, tannin and crude fiber content of straw making it ideal for animal feed (Ortega *et al.,* 1992). The spent straw contains large quantity of nitrogen, phosphorus, potassium and can be used as manure (Maher, 1991).

Mushroom cultivation represents the only current economically viable biotechnology process for the conversion of waste plant residues from forests and agriculture (Wood and Smith, 1987). At present mushroom production is approximately 1.5 million tons in the world (Shah *et al.,* 2004). *P. ostreatus* produce in large quantities in a short time and provides more protein per unit area than any other crop (Gupta, 1986). These mushrooms can be used industrially for mycoremediation purposes. Hence, its cultivation can play an important role in managing organic wastes whose disposal has become a problem (Das and Mukherjee, 2007).

2.4 Mushroom Substrate

Mushrooms can be classified into three categories by their tropic patterns; saprophytes, parasites or mychorrhizae. The most common grown mushrooms are saprophytes, decomposers in an ecosystem growing on organic matters like wood, leaves and straw in nature (Song, 2004). Mushrooms depend on substrates for nutrition and the substrate is normally source of lignocellulose material which support growth, development and fruiting of mushroom (Chang and Miles, 2004). The different substrates used in cultivating mushrooms do have effect on the functional, organoleptic and chemical properties on the mushrooms (Oyetayo and Ariyo, 2013).

Oyster mushroom is one example of edible mushrooms that can utilize lignocellulosic materials as a substrate. The capability of oyster mushroom is due to the presence of its lignocellulolytic enzymes, which help it convert cellulose and lignin into useful carbohydrates such as glucose that can be used as an energy source for the fungi (Custodio, 2004). Thus, most organic matters containing cellulose, hemicellulose or lignin can be used as mushroom substrate (Song, 2004).

Compost wheat and paddy straw, banana leaves, sugarcane bagasses and leaves, wheat barn, rice husk, sawdust and other can be used as substrate for growing mushroom (Gupta, 1986). Organic supplements are usually added to substrates to provide organic sources of nitrogen (Upadhyay *et al.,* 2003). Some frequently used organic supplements are rice straw and rice bran. Rice bran is commonly used to provide nitrogen, especially during the formation of fruiting body (Custodio, 2004). Rice straw has been recommended as the growth substrate for the economic production of *Pleurotus* species, a 17% increase in yield has been observed when composted sawdust of *Thermella scleroxylon* was mixed with the rice straw. Rice straw and rice husk have been identified as rich in cellulose (Datta and Chakravarty, 2001; Obodai *et al.*, 2003).

Substrates may also be obtained from various plant remnants without enrichments by expensive additives. In almost all cases the efficiency of these waste constituting substrates is considerably enhanced when supplemented with protein-rich materials such as rice bran and wheat (Manso *et al.,* 2011). Although the nutrients of the substrate always affect mushroom production, there are three substrate formulae for growing oyster mushroom. The substrate formula number one is not recommended for any poorly equipped mushroom farm because of its high risk of contamination and formula number three is used only for fresh sawdust (Truong, 2004). *Pleurotus* species have grown on different kinds of sawdust, straw and many other agricultural and industrial wastes (Hadder *et al.,* 1993).

Table 2.2 Substrate Formulae for Growing Oyster Mushroom

Formula 1 (Do, 1999)		**Formula 2** (Chau *et al.*, 2003)		**Formula 3** (Thang, 1993)	
Sawdust	: 75%	Sawdust	: 85%	Sawdust	: 99%
Rice bran	: 10%	Rice bran	: 10%	Lime	: 1%
Corn bran	: 5%	Lime	: 1%	(only used	
Lime	: 2%	Ammonium sulphate	: 0.5%	fresh	
Peanut waste	: 5%	Sugar	: 1%	sawdust)	
Super phosphate	: 1%	gypsum	: 2%		
Ammonium sulphate	: 0.5%				
Magnesium sulphate	: 0.05%				

Source: Truong (2004)

The different substrates used in cultivating these mushrooms obviously have significant effect on the nutritional and functional properties of oyster mushroom (Oyetayo and Ariyo, 2013).

2.5 Coffee Residues as an Alternative Substrate

There are several different coffee residues including coffee husks, coffee pulp and spent grounds. Coffee husk is produced in the dry process of the separation of the coffee berries while coffee pulp is obtained by the wet process of extracting coffee from the berries. Spent coffee grounds are produced during the processing of raw coffee powder to prepare instant coffee (Fan *et al.*, 2005). The nutrition and harmful substances of main coffee residues are shown in the table below.

Table 2.3 Nutrition and Harmful Substances in Coffee Residues (%)

Component	Coffee husk	Coffee pulp	Coffee spent ground
Protein	9.2-11.3	8.5-12.1	10.3-12.2
Lipids	2.0-2.3	1.5-2.0	15.2-17.9
Cellulose	13.2-27.6	15.1-20.3	13.2-18.4
Ash	3.3-4.1	5.5-6.8	4.5-6.3
Extract not-nitrogen	57.8-66.1	45.5-54.3	41.0-49.8
Tannins	4.5-5.4	1.8-2.4	1.2-1.5
Caffeine	0.8-1.1	0.5-0.7	0.02-0.08

Source: Fan and Soccol (2005)

Unfortunately, coffee husk and pulp have large amounts of caffeine and tannins, and the harmful substances, especially caffeine, have a negative effect on mushroom growth and inhibit the growth of mushroom mycelium. While coffee residues are highly nutritious, most do require treatment before used as substrate and the costs of pre-treating coffee husk and pulp can hinder their wide usage. Spent coffee grounds are the exception and do not require a caffeine removal processing stage (Thiclke, 1989).

CHAPTER 3

METHODOLOGY

3.1 Experiment Method

The experiment was conducted at Faculty of Sustainable Agriculture, Universiti Malaysia Sabah. The type of experiment conducted was a field experiment. The substrates used in this experiment were sawdust mixture (SM) as the control treatment, used ground coffee (UGC) and different percentage mixture of both SM and UGC. Substrate compositions used shown in Table 3.1.

Table 3.1 Mushroom Substrate Composition for Treatments

Treatments	Substrate Composition
Treatment 1, T (Control)	100% SM consist of 85% sawdust, 10% rice bran, 2% gypsum, 1% lime, 1% sugar, 0.5% Ammonium sulphate (Chau *et al.*, 2003)
Treatment 2, T_2	75% SM, 25% UGC
Treatment 3, T_3	50% SM, 50% UGC
Treatment 4, T_4	25% SM, 75% UGC
Treatment 5, T_5	100% UGC

3.2 Substrate Preparation

UGC were obtained from Rock, Paper, Scissors Café and Urban Café. After obtaining the used ground coffee, it is left in the sun to dry off the moisture (Fan *et al.*, 1999) to avoid molding of the used ground coffee. After the coffee grounds are dried, it was sieved using a sieve before being stored in an airtight container to avoid any wet clumped coffee ground mixed with the dried ones.

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