MUSHROOM CULTIVATION BY USING AGRICULTURAL WASTES

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"To my beloved mother, Salmiah binti Abdullah,
father, Mohd Hanafi bin Ab. Rahman,
and my siblings,
Mohd Shakir bin Mohd Hanafi and Shazlin binti Mohd Hanafi
for their everlasting love, support, pray and concern."

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ABSTRACT

Agricultural waste also known as lignocellulosic residue is a renewable resource that can be used for mushroom cultivation. Empty fruit bunch (EFB) and paddy straw (PS) are categorized as agricultural waste and abundant in every Malaysian palm oil plantation and paddy field. Mushroom cultivation using agricultural waste promises nutritious mushroom and spent mushroom substrate (SMS) that can be used for producing beneficial products such as ruminant feedstock and fertilisers in post-harvest cultivation. SMS is defined as residual biomass generated by commercial mushroom industry after harvesting period. This study aims to reuse agricultural waste generated from the two largest agricultural sectors i.e. palm oil plantation and paddy field, in mushroom cultivation and application of SMS in the post-harvest cultivation which have potential to be used as supplement for ruminant feedstock. The techniques used in this study were named as Process A, B, C and D which consist of drying, grinding, substrates mixing, sterilization, spawning, incubation and harvesting process. Then, the optimum process was used in subsequent cultivation to determine the optimum ratio between ratio A, B, C and D. At the end of harvesting period, SMS was tested for nutrient composition, feeding analysis, hazardous metal composition and bacteriological properties. The results showed novelty in Process D, which obtained optimum yield for three substrates i.e. sawdust, EFB and PS substrates as 232.5±50.3 g, 134.8 ± 82.4 g, and 127.7 ± 25.6 g, respectively. In addition, ratio B comprises 76.0% EFB, 20.0% rice bran and 4.0% agriculture hydrated lime obtained the highest percentage of recovering EFB at the end of mushroom cultivation of 63.2%. Another finding indicated that EFB based-SMS contains adequate nutrients to be applied as supplement for ruminant feedstock when compared to animal feed pellets. Furthermore, EFB-based SMS contains desirable feeding analysis and acceptable amount of *Escherichia coli* which is suitable to be used as ruminant feeding. In summary, this study shows that both, EFB and PS are suitable agricultural waste to be reused in mushroom cultivation, henceforth, reduced the generation of waste in oil palm plantations and paddy fields. EFB was the most applicable substrate to be used for commercialization purposes compared to PS due to high production of mushroom. Furthermore, EFB substrate can be applied further as supplement to ruminant feedstock in the post-harvest cultivation of SMS. Hence, this cycle promotes zero waste discharge. This study can be extended using other abundant agricultural waste in Malaysia such as cocoa or pineapple waste in order to minimise agricultural waste generation.

ABSTRAK

Sisa pertanian juga dikenali sebagai sisa lignoselulosa adalah sumber yang boleh diperbaharui yang mana boleh digunakan untuk penanaman cendawan. Tandan buah kosong (EFB) dan jerami padi (PS) dikategorikan sebagai sisa pertanian dan dihasilkan secara meluas di setiap ladang kelapa sawit dan sawah padi Malaysia. Penanaman cendawan menggunakan sisa pertanian menjanjikan cendawan yang berkhasiat dan sisa substrat cendawan (SMS) yang boleh digunakan untuk menghasilkan produk yang bermanfaat seperti bahan makanan haiwan dan baja dalam pasca penanaman. SMS ditakrifkan sebagai sisa biomas yang dihasilkan oleh industri komersil cendawan selepas tempoh penuaian cendawan. Kajian ini bertujuan untuk menggunakan semula sisa pertanian yang dihasilkan dari dua sektor pertanian terbesar iaitu ladang kelapa sawit dan sawah padi, dalam penanaman cendawan dan aplikasi SMS pada pasca penanaman yang berpotensi digunakan sebagai makanan tambahan untuk bahan makanan haiwan. Teknik yang digunakan dalam kajian ini dinamakan sebagai Proses A, B, C dan D yang terdiri daripada proses pengeringan, pengisaran, pencampuran substrat, pensterilan, pemijahan, pengeraman dan penuaian. Kemudian, proses yang optimum digunakan dalam penanaman berikutnya untuk menentukan nisbah optimum di antara nisbah A, B, C dan D. Pada akhir tempoh penuaian, SMS telah diuji untuk komposisi nutrien, analisis makanan, komposisi logam berbahaya dan sifat bakteriologi. Keputusan mendapati hasil pembaharuan dalam Proses D, dimana hasil optimum dari ketiga-tiga substrat; habuk kayu, EFB dan PS, direkodkan sebagai 232.5 ± 50.3 g, 134.8 ± 82.4 g, dan 127.7 ± 25.6 g. Di samping itu, nisbah B yang terdiri daripada 76.0% EFB, 20.0% dedak beras dan 4.0% kapur pertanian, memperolehi peratusan tertinggi pemulihan EFB pada penghujung penanaman cendawan iaitu 63.2%. Keputusan lain menunjukkan bahawa SMS berasaskan EFB mengandungi nutrien yang mencukupi untuk digunakan sebagai bahan tambah makanan haiwan apabila dibandingkan dengan pelet makanan haiwan. Tambahan pula, SMS berasaskan EFB mengandungi analisis makanan yang dikehendaki dan jumlah *Escherichia coli* yang boleh diterima dalam makanan haiwan. Secara ringkasnya, kajian ini menunjukkan bahawa kedua-dua sisa, EFB dan PS adalah sisa pertanian yang sesuai untuk digunakan semula dalam penanaman cendawan, dan seterusnya, mengurangkan penjanaan sisa di ladang kelapa sawit dan sawah padi. EFB merupakan substrat yang paling sesuai digunakan untuk tujuan pengkomersilan berbanding PS disebabkan penghasilan cendawan yang tinggi. Tambahan pula, substrat EFB boleh digunakan semula sebagai bahan tambah makanan haiwan dalam pasca penanaman SMS. Oleh itu, kitaran ini menggalakkan pelepasan buangan sifar. Kajian ini boleh diperkembangkan dengan menggunakan sisa pertanian lain yang banyak di Malaysia seperti sisa koko atau nenas untuk meminimumkan penjanaan sisa pertanian.

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LIST OF ABBREVIATIONS

EFB	Empty fruit bunch
PS	Paddy straw
SMS	Spent mushroom substrate
SD	Sawdust
N	Nitrogen
K	Potassium
P	Phosphorus
CO_2	Carbon dioxide
Ca	Calcium
Na	Sodium
NDF	Neutral detergent fiber
ADF	Acidic detergent fiber
C	Carbon
Mg	Magnesium
Zn	Zinc
MC	Moisture content
CP	Crude protein
CFT	Crude fat
CF	Crude fiber
$CaCO_2$	Lime hydrated agriculture
EE	Ether extract
WRF	White-rot fungi
N/A	Not available

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Agricultural waste is an abundant raw material of crops dry matter that is easy to obtain. They are also called as lignocellulosic biomass which brings definition of wastes generated in agricultural activities prior process and vice versa. The term "lignocellulosic" is due to the existence of cellulose and hemicellulose in their structure of plant (Mahesh and Mohini, 2013). Interestingly, the lignocellulosic materials not just known as renewable resource, but at the meantime, they are low cost and abundantly available (Sarkar et al, 2012). Typically, agricultural wastes that often generated in the fields were obtained from straws, wheat, paddy, oats, corncob, meanwhile, remnants of plantation activities were most of the time come from oil palm wastes; empty fruit bunch (EFB), trunks, palm kernel shells, and pineapple (Adebayo, 2015; Nicolcioiu et al. 2016). These wastes are known possess high nutrient levels of nitrogen (N), potassium (K), and phosphorus (P) which able to increase crop yields (Elly, 2011).

Embrandiri (2013) in his review stated that, palm oil and paddy plantation are among of important agricultural products which spurred the growth of economic condition in most ASEAN countries including Malaysia, Thailand, and Indonesia. The oil palm industry has become a significant icon to Malaysia and Indonesia as both countries become the major contributors in world's palm oil production (Sudirman et al., 2011). Furthermore, Malaysia as a tropical climate country experiencing mild humid weather throughout the year, has encouraged the growth and development of the oil palm plantation (Pei et al., 2012). These agricultural activities can play important role in stabilizing the climate change and act as food security for the countries (Schaffnit-chatterjee et al., 2011). Every mill of oil palm generated solid waste such as EFB, palm oil trunks, palm fiber and palm kernel shell. Oil palm plantations produced 1.27 tons of EFB per hectare per year of the surplus mill (Elbersen et al., 2013).

Other sectors that also become the larger contributor for agricultural waste in Malaysia is rice paddy plantation-Malaysia recorded 730.0 thousand hectares of rice crop by 2015 (DOSM, 2016) and this expected to keep expanding along with other contributors in the agricultural sector throughout the year. Typically, in Malaysia, paddy field are double-cropping throughout the year, during off season and main season. From this cultivation process, about 80% of rice straw is produced by improper disposal management, which results in a series of pollution (Shafie et al., 2014a). The paddy straw disposal is usually done through open burning known as the cheapest and easiest way to prepare the field for the next cultivation season (Rosmiza et al., 2014; Shafie et al., 2014b).

Waste disposal was introduced into the plantation and enforced under the Environmental Quality (Amendment) Act 2012 to control the open burning and open waste dumping that contributed to the release of carbon dioxide (CO_2) and methane (CH_4) into the atmosphere (MPOB, 2015). Conventionally, from previous practice, EFB will either incinerated or applied on top of soil as mulch, a direct composting system for plantations (Ali et al., 2013). Wan and Surya (2012) in their study stated, the mulching was able to improve soil conditions by maintaining soil moisture and has also been applied to cover crops for oil palm plantation.

An alternative method and application to recycle these wastes is seen as so important in order to recover this waste resource through its value proposition toward zero waste discharge. Reuse of agricultural wastes is more worthwhile as this not only provides another new application but also minimizes the waste generated in the field. Thus, this step can protect the environment from pollution. This waste recovery also contributes significantly other industries as it introduces low-cost primary source and potentially producing profitable products in future. Therefore, a number of researches on reuse agricultural wastes have been successfully carried out such as fertilisers, animal feedstock, and coal tailing (Elly, 2011; Mahesh and Mohini, 2013; Pattanayak, 2015).

Agricultural wastes can also be converted into valuable food through mushroom cultivation (Lalithadevy and Many, 2014). Since two decades ago, paddy straw was introduced and widely used as substrate media in mushroom cultivation of *P. sajor-caju, P. platypus and P. citrinopileatus* species (Ragunathan et al., 1996; Gurudevan, 2012). EFB, paddy straw, wheat straw, sugarcane bagasse, and maize cob were reported as great mushroom substrates media which produced higher yield compared to present practices substrates, sawdust (Chukwurah, 2012; Gurudevan, 2012; Kavitha et al., 2013). Out of listed media, paddy straw is the growth media that provided the shortest duration to complete mycelium colonization compared to other substrates (Lalithadevy and Many, 2014).

Additionally, the medium-scale mushroom industry is capable of producing approximately 13.6 million tonnes per year of spent mushroom substrate (SMS) after the harvest cycle (Phan and Sabaratnam, 2012). In Korea, SMS production is estimated about 2 million tons yearly, with almost 1.2 million tons generated from *Pleurotus sajor-caju* (Lim et al., 2013). SMS defined as biomass generation from mushroom industry remaining after a crop of mushroom is harvested (Moon et al., 2012).

In fact, SMS contains enough digestible nutrition for ruminant feeding and reuse of SMS can protect the environment as well as increase growers' income, (Danny et al., 2004). As reported by Khattab et al., (2013), paddy straw-SMS can be forages for ruminants and possess the high possibility of replacing practices ruminant feedstock at high levels of up to 50% or 90% from diets. Besides, lignocellulosic characteristics in mushroom substrate suitable to be used as animal feed due to its upgraded properties and digestible carbohydrates (Georgios et. al., 2014; Kuijk et al., 2015). Both targeted waste residues; EFB and paddy straw, contain high protein and carbohydrates which is very suitable to be used as mushroom media for *Pleurotus* spp. production and supplement for ruminant feedstock (Silvana et al., 2006; Kowalski et al., 2014; Wyngaard et al., 2015).

Therefore, this study is purposely to reuse and recover agricultural wastes; EFB and paddy straw through mushroom media as to produce edible *Pleurotus* spp. and recover again the residues produced from cultivation of mushrooms called as SMS as supplement for ruminant animals.

1.2 Problem Statement

Oil palm and paddy plantation are the main important commodity product that become as transformation agent to the scenario of agricultural sector and economy in Malaysia (DOSM, 2016). The presence of wastes from oil palm plantation has created a major disposal problem such as open burning and *in situ* dumping. Currently, most of EFB are used as soil mulching as organic fertiliser to the plantation, otherwise, dumped in the same manner as palm oil mill effluent (POME) (Pei et al., 2012).

According to available literature, production of paddy straw is over 13 tons per hectares and reported mostly in North of Malaysia (Perlis, Kedah, Penang, and Perlis) and Central of Malaysia (Selangor, Negeri Sembilan, and Malacca) (Shafi et al., 2013c). Presently, paddy straw is disposed of by open burning which sparked lots of environmental issues, hence affecting weather and local communities (Rosmiza et al., 2014). An alternative application of agricultural wastes is needed in order to minimize the waste generation and protect the environment from being polluted.

Moreover, series of problems also arises in mushroom industries whereby generation and management of spent mushroom substrate (SMS) become a big challenge to the farmers. The application of SMS is still lacking as the current practices substrate which is sawdust that containing low nutrient composition is still become an option, hence limiting the potential its usage.

Various efforts have been spent to increase the potential of SMS application in the agricultural industry, given the use of present substrate; sawdust substrate is not suitable to be used as animal feedstock and fertilisers, and it end up dumped and burned *in situ* (Park et al., 2012). Hence, to minimize the problem, sawdust substrate should be substitute with lignocellulosic substrates such as paddy straw, palm wastes, or crop wastes, as to minimize the wastes from mushroom industry.

Another problem arises in the mushroom industries is the cultivation techniques of *Pleurotus* spp. which caused high contamination rate and put the farmers in the worrisome state. Mushroom commercial industries in Ulu Tiram, and Pontian, Johor, experienced approximately 20% and 14%, respectively, contamination rate in every 1000 beds per production. Contamination occurs probably because of the techniques and handling methods used are unhygienic. Besides, *Pleurotus* spp. is categorized as fungi and very prone to contamination. Contamination rate must to be reduced by improving the process in the mushroom cultivation.

Most of the studies conducted in Malaysia only emphasized on a single output, for instance, mushroom cultivation by using agricultural wastes (Ali, et. al., 2013). There are limitations in research especially on the use of agricultural wastes as mushroom substrate, the percentage of waste recovered from the process and utilization of agro wastes-SMS as ruminant feedstock. Besides, the study of minimizing contamination rate in mushroom cultivation process also still scarce.

1.3 Objectives of The Study

The main purpose of this research is to recycle and recover agricultural wastes generated from two (2) largest agricultural sectors; palm oil plantation and paddy field, through mushroom life-cycle. The following are the objectives to achieve the research aim:

i. To study the performance of reuse agricultural wastes; empty fruit bunch and paddy straw in *Pleurotus* spp. cultivation by comparing mushroom cultivation process,

ii. To determine the percentage of waste recovery by using optimum ratio of recycled agro-wastes substrates; empty fruit bunch and paddy straw,

iii. To compare the nutrient composition of mushroom fruiting bodies to the three substrates; sawdust, empty fruit bunch and paddy straw,

iv. To investigate the composition and feeding analysis of spent mushroom substrates (SMS) as a supplement for ruminant feedstock in order to promote zero waste discharge.

1.4 Scope of The Study

This study focused on reuse and recovery agricultural wastes mainly from two (2) largest agricultural sectors; palm oil plantation and paddy field through mushroom cultivation of well-known species; *Pleurotus* spp.,. The study was started from the observation of problem arises in plantation and mushroom industries. Then, continued to the laboratory set up to study the performance of *Pleurotus* spp. on the recycled agro-wastes substrates and its optimum ratio of agro-wastes. The parameter of mushroom harvesting interval, mushroom fruiting bodies and mushroom yield of three substrates; sawdust (SD), empty fruit bunch (EFB) and paddy straw (PS) were investigated and percentage of waste recovered was calculated.

Furthermore, this study compared the nutrient composition of *Pleurotus* spp. fruiting bodies of these three experimented substrates; SD, EFB and PS in order to determine the most nutritious mushroom. In the post-harvest cultivation, composition and feeding analysis of chosen substrate (EFB-SMS) were examined to analyze the compatibility as a supplement for ruminant feedstock.

Controllable parameters in the study were moisture content, substrates (SD, EFB, and PS) and additional nutrients used in cultivation (rice bran and lime) and *Pleurotus sajor-caju* spawn.

1.5 Significance of The Study

The significance of this study is to utilize abandoned waste from palm oil plantations and paddy field as mushroom substrate in order to reduce the generation of agricultural wastes from the prohibited activities; burning and open dumping. Besides, these wastes can be used as a bio-remediation solution in SMS production for ruminant feedstock (Foluke et al., 2014; Phan and Sabaratnam, 2012).

Recently, under the Economic Transformation Program (ETP), both the oil palm and biomass industries have been highlighted as the nation's premier niche National Key Economic Areas (NKEAs) (JPM, 2013). The utilization of palm biomass is increasing significantly over time, which creates a symbiotic situation where the "previous waste" serves as the input for other industries, leading the palm oil industry to a zero waste path (Pei et al., 2012). Hence, Malaysian Economic Transformation Programme (ETP) encourages the utilization of oil palm and biomass for another application in order to promote zero waste paths.

Mushroom cultivation is well-known as the easiest way to reduce generation of agricultural wastes, moreover, the result obtained in previous study indicated that high yield of mushroom can be produced by using agricultural wastes as mushroom substrate (Ali et al., 2013; Kavitha et al., 2013; Marlina et al., 2015). Furthermore, some techniques to improve local mushroom cultivation process are provided in this study in order to minimize contamination rate due to unhygienic handling and techniques. The result obtained in this study showed significant differences in terms of harvesting interval, flushes, production of fruiting bodies, fresh mushroom yield, and nutrients of mushroom compared to local practices cultivation. An optimum ratio and nutrient composition of SD, EFB and PS substrates is provided.

Moreover, this study provides an alternative to recycle and recover abundant of agricultural waste to another beneficial products. Reuse and recovering agricultural wastes in mushroom life-cycle can be concluded as promoting a zero waste discharge; hence, this is important to the farmers to practice in agriculture sector. In addition, this study hopes to help the country in controlling environmental pollution through the zero waste initiatives, thereby enabling Malaysia to take a step further towards sustaining sustainable growth.

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