

SUBSTRATE FORMULATION FOR CULTIVATION OF *PLEUROTUS*
SAJOR-CAJU FROM OIL PALM EMPTY FRUIT BUNCH AND
SUGARCANE BAGASSE

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF BACHELOR OF
AGRICULTURE SCIENCE WITH HONOURS

**PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH**

CROP PRODUCTION PROGRAMME
FACULTY OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH

2017



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JUDUL: FORMULASI SUBSTRAT DARIPADA SERAT TANDAN BUAH KOSONG
KELAPA SAWIT DAN HAMPAS TEBU UNTUK PENANAMAN CENDAWAN
RE PLEUROTUS SAJOR-CAJU

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ACKNOWLEDGEMENT

Above all, I would like to thank Allah s.w.t, for He provided me good health, strength, patience and made all things possible for me to complete my dissertation successfully. It is my pleasure to express my heartfelt appreciation and special thanks to my supervisor, Dr. Mohd Rakib bin Mohd Rashid for his advices, professional guidance, constructive comments and countless supports throughout the period of my final year project research from the first step of preparing thesis proposal, conducting the research work and writing of the dissertation.

I also wanted to express my gratitude to the lab assistants, Mrs. Nurul Syakina Marli and Mrs Ahjia Jekan for their willingness to prepare all the necessities for my project in the laboratory and opening the laboratory during the weekend during my laboratory research in Final Year Project Laboratory 2. My special thanks to Mr. Mattunjan Upin for giving me permission to use the mushroom house and for his kind help in facilitating my project in the field period.

Special words of thanks also extended to all my family members, my father, Mr. Donal bin Marizaman, my mother, Mrs. Suraya binti Ahmad; my brothers, Mohd Syaiknal bin Donal, Mohamad Syahaikal bin Donal and Mohammad Syamiel Ieman bin Donal for their financial help, affection, love, inspiration and patience during my study period in Universiti Malaysia Sabah.

Finally, I would like to thank all my friends who helped me a lot during this research project, Mohamad Ihsan Ismail, Marlina binti Aliamat, Faradila Fathin bin Eddie and all people that directly or indirectly involved in my research project.



ABSTRACT

Oyster mushroom, *Pleurotus sajor-caju*, was cultivated on empty fruit bunch of oil palm fibre and sugarcane bagasse. This study was carried out to determine the best formulations for the growth and yield of oyster mushroom and determines the composition of these substrates that could produce optimum growth and production of Oyster mushroom by using mixture response surface methodology (MRSM). By using the simplex centroid design, seven formulations were obtained. Four of the formulations repeated randomly, which make it became 11 formulations, for test of validation of data. Based on the results, the shortest spawn running period and primordia initiation was on 100% sawdust (Formulation 1 and 8) and 100% EFB (Formulation 4 and 5). For number of fruiting bodies which was 11, achieved by 100% SB (Formulation 7). Next, the largest size of mushroom was on 50% SD + 50% SB (Formulation 6), 6.26 cm. Substrate with 100% EFB fibre, Formulation 4 shows the most rapid mycelial extension every week which the growth of mycelia covered about 61.88% of the total height of the bags just in one week. The fresh weight on the 33% SD + 33% SB + 33% EFB fibre (Formulation 10) was 65.3 g. Formulation 2 showed the highest dry weight which was 11.25 g. The results indicated that the moisture contents of the mushroom ranged from 66.4%-92.3%. Dry matter content of mushroom cultivated on the combinations of 50% SB + 50% EFB fibre shows the highest which was 34.6%



FORMULASI SUBSTRAT UNTUK PENANAMAN *PLEUROTUS SAJOR-CAJU* DARI SERAT TANDAN BUAH KOSONG KELAPA SAWIT DAN HAMPAS TEBU

ABSTRAK

Cendawan tiram kelabu, *Pleurotus sajor-caju* ditanam menggunakan tandan buah kosong dan hampas tebu. Kajian ini bertujuan untuk mengenal pasti formulasi yang terbaik untuk pertumbuhan dan hasil cendawan tiram pada substrat-substrat tersebut dan menentukan komposisi substrat yang sesuai untuk mencapai pertumbuhan cendawan tiram yang optimum menggunakan kaedah campuran tindakbalas permukaan. Dengan menggunakan reka bentuk ssentroid simples, tujuh formulasi telah diperolehi. Empat daripada formulasi itu diulang menjadikan jumlah 11 formulasi, untuk tujuan pengesahan data. Berdasarkan keputusan yang diperolehi, tempoh mengkolonisasi keseluruhan beg dan tempoh permulaan pembentukan primordia adalah paling cepat pada substrat 100% habuk kayu (Formulasi 1 dan 8) serta 100% serat TBK (Formulasi 4 dan 5). Untuk pembentukan jasad buah yang efektif yang paling tinggi iaitu 11 jasad buah pada substrat 100% hampas tebu (Formulasi 7). Seterusnya, saiz cendawan paling besar diperolehi pada substrat 50% SD + 50% SB (Formulasi 6) iaitu 6.26 sm. Substrat daripada 100% serat TBK (Formulasi 4) menunjukkan kadar pertumbuhan miselia yang laju, ia berjaya mengkolonisasi 61.88% daripada beg cendawan pada minggu pertama selepas inokulasi. Berat segar pada substrat yang mengandungi ketiga-tiga bahan tersebut adalah yang paling tinggi iaitu 65.3 g manakala berat kering paling tinggi, 11.25 g dicapai oleh Formulasi 2. Keputusan menunjukkan kandungan kelembapan cendawan tiram dalam kajian ini adalah di antara 66.4% hingga 92.3%. Kandungan bahan kering cendawan paling tinggi adalah pada kombinasi 50% hampas tebu + 50% serat TBK iaitu 34.6%.

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LIST OF ABBREVIATIONS

LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

EFB	Empty fruit bunch
FAO	Food and Agriculture Organisation
POME	Palm Oil Mill Effluent
PKS	Palm kernel shell
PKC	Palm kernel cake
CPO	Crude Palm Oil
PK	Palm kernel
CPKO	Crude palm kernel oil
RSM	Response Surface Methodology
PFS	<i>Parasianthes falcataria</i>
BE	Biological efficiency
SB	Sugarcane bagasse
SD	Sawdust
PDA	Potato dextrose agar
TBK	Tandan buah kosong

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Mushroom are the reproductive structures produced by members of the division of fungi known as the Basidiomycota. The basic parts of mushroom technically known as a basidiocarp are the stipe (stem), the pileus (cap) and either lamellae (gills) or tubes which are covered with reproductive cells called basidia, from which spores are produced (Pathak, 1998).

Mushrooms have been recognized as a highly potential converter of cheap celluloses into valuable protein (Poppe, 2000). It is a reproductive structure of fleshy macro-fungi and rich with protein, vitamin and minerals. More than 2000 species of edible mushrooms are known, out of which only few species have been cultivated commercially by preparing beds (Nair, 1994). *Pleurotus* species are one of the most popular and widely cultivated throughout the world, particularly Asia and Europe (Mandeel *et al.*, 2005).

Pleurotus species was reported as an edible mushroom, could be cultivated on ligno-cellulosic materials, such as agricultural waste for human consumption, and that various species and strains of this fungus can utilize woody and non-woody materials efficiently by degrading their ligno-cellulosic ingredients. It is one of the most suitable mushroom for producing protein rich food from various agro-wastes without composting (Pathak, 1998).

The cultivation of edible mushroom offers one of the most feasible and economic method for bio-conversion of agro-lignocellulosic wastes (Bano *et al.* 1993). This

technology can also limit air pollution associated with burning agriculture wastes as well as to decrease environment pollution due to unutilized agricultural wastes. *Pleurotus spp.* are commercially important edible mushrooms commonly known as the oyster mushroom (Pokhrel *et al.*, 2013). Cultivation of oyster mushroom has recently increased tremendously throughout the world because of their abilities to grow at a wide range of agro-based residues. These white rot fungi are useful decomposers of various agricultural wastes (Kurt *et al.*, 2010).

Nowadays, researchers all over the world are focusing on ways of utilizing agricultural wastes as a source of raw materials for the industry (Muhamad *et al.*, 2008, Shah *et al.*, 2004). This waste utilization would not only be economical, but may also result to foreign exchange earning and environmental pollution control. Agriculture wastes are the major source of lignocellulosic materials which is suitable as substrate for solid state fermentation of edible fungi such as *P. sajor-caju*. These wastes are produced in big volumes during production of agricultural products every year causing lots of environmental problem in many countries (Belewu and Banjo, 2000).

According to Sudirman *et al.* (2011) empty fruit bunch (EFB) and palm oil mill effluent (POME) are still considered as unwanted wastes because of their storage, distribution and treatment costs. Concerning the environmental problem especially high air pollution, the EFB incineration is prohibited therefore palm oil mills have started to bring EFB back to the plantation and just dump them. Millions and tons of agriculture industrial wastes are discarded, burned and neglected. They mainly contain lignocellulose compounds. These by-products can be used to produce edible mushrooms such as oyster mushroom, straw mushroom and shiitake mushroom (Sudirman, 2011).

In 2016, there are total of 5.74 million hectares of oil palm planted in Malaysia. Sabah is the state with the largest area of oil palm cultivation with total area of approximately 1.6 million hectare, which is 27% from the total area of oil palm cultivation in Malaysia. As of March 2017, the fresh fruit bunch yield of oil palm was 1.39 tonne per hectare (MPOB, 2017).

The majority of the oil palm biomass is left on the fields or returned to the fields as soil amendment or organic fertilizer. This biomass plays an important role to ensure the sustainability of plantations and preserve soil fertility. However, there are also the

potential to utilise a share of this biomass for a variety of additional end uses including as substrate for cultivation of mushroom.

Sugarcane bagasse is the lignocellulosic solid residue from sugarcane after stripping the juice for sugar and ethanol production. It has been used for paper, paperboard and fertilizer production. It is also can be used as animal feed or burn directly to generate energy (Tsigie and Ju, 2012). However, all of the products mentioned above does not contribute significantly to the use of sugarcane bagasse. Thus, it is still generally considered as an agriculture waste and its disposal becomes an environmental problem.

Bagasse consists of approximately 50% cellulose and 25% of each of hemicellulose and lignin. Chemically, bagasse contains about 50% alpha-cellulose, 30% pentosans and 2.4% ash. Because of its low ash content, bagasse offers numerous advantages for usage in bioconversion processes using microbial cultures (Parameswaran, 2009). The production of sugarcane bagasse in Malaysia in 2014 shows that 9147 tonnes of sugarcane harvested in Malaysia on cultivation area of 237 hectares in 2014. Furthermore, the yield per hectare of the sugarcane is 385,949 tonnes (FAOSTAT, 2014).

In Malaysia, the conventional practice of cultivation of mushroom is by using the sawdust and rice husk as substrate for the cultivation of mushroom. Thus, there is need to find alternative and suitable substrate for mushroom cultivation (Saidu, 2011).

1.2 Justification

The cultivation of mushroom conventionally had been practiced commonly in Malaysia nowadays by using sawdust from rubber tree especially in the Peninsular Malaysia (Muhamad *et al.*, 2008). This is because they have plenty of this tree growing there. However, in East Malaysia, such as Sabah, we rely on sawdust from wood processing mill and it involved many processes to ensure the sawdust is free from any chemicals. Plus, using only the sawdust is not sufficient as it cannot supply enough nutrient for the growth of the mushroom. Sometimes, rice bran added as mixture and calcium carbonate to reduce the acidity of the substrate.

Moreover, the amount of agricultural waste had been increased from day to day. Some of the processing mill such as palm oil factory, they might burn the empty fruit bunch (EFB) as the fuel or they only dispose it to the plantation for mulching purpose. Empty fruit bunch of oil palm contain a lot of nutrients for mushroom to grow. Plus, the *Volvariella volvacea*, or known as straw mushroom always found growing on the empty fruit bunch of oil palm in the plantation.

Growing mushroom by using agricultural waste is one of method that can help reduce the cost of growing mushroom, and to reuse the waste is a good idea as it helps to manage the amount of agricultural waste being dispose daily from the processing mill. Recently, there are many research done to study the growth of mushroom in various agricultural waste such as corn husks and cobs, banana leaves and mesocarp fibre of the oil palm (Muhammad 2008, Belewu and Banjo, 2000).

Sugarcane leftover still have high sugar content after pressing. It contains mainly cellulose, hemi cellulose, pentosans, lignin, sugars, wax, and minerals (Zafar, 2015). Sugarcane bagasse is abundant in Malaysia and at present is mainly used as combustible material for energy supply in the sugar factory (Shahidan and Muhammed, 2011).

1.3 Objectives

The specific objectives of this study are to:

- i. To determine the effects of combination of different substrate formulation from EFB fibre, sugarcane bagasse and sawdust on the development and growth also yield of *Pleurotus sajor-caju*.
- ii. To determine the optimum combination of substrate formulation for production of *Pleurotus sajor-caju* using mixture response surface methodology (MRSM).

1.4 Hypothesis

H_0 = Different combinations of substrate formulation from EFB, sugarcane bagasse and sawdust give no significant differences on the development, growth and yield of *Pleurotus sajor-caju*.

H_a = Different combinations of substrate formulation from EFB, sugarcane bagasse and sawdust give significant differences on the development, growth and yield of *Pleurotus sajor-caju*.

H_0 = Combination of substrate formulation gives no significant difference for production of *Pleurotus sajor-caju*.

H_b = Combination of substrate formulation gives significant difference for production of *Pleurotus sajor-caju*.

CHAPTER 2

LITERATURE REVIEW

2.1 Cultivation of *Pleurotus sajor-caju*

2.1.1 History of Oyster mushroom

Pleurotus mushroom is generally referred to as 'Oyster Mushroom' and cultivated commercially in Malaysia (Arini and Tajul, 2016). The word "*pleurd*" originated from Greek word which means formed laterally on or in a side way position, referring to the lateral position of the stem (stipe) in relation to cap (pileus). It is one of particular basidiomycete mushroom genera that have received considerable attention for their nutritional value, medicinal properties and biodegradable activities (Pinkal and Ratna, 2013). The fungi of *Pleurotus* genus have about 40 species belong in it (Jose and Janardhanan, 2000).

This mushroom has gained importance only in the last decade. It is now being cultivated many countries in the subtropical and temperate zones. In China, it is known as abalone mushroom, *P. abalonus* or *P. cystidiosus*. Other species available for cultivation are *P. sajor-caju*, *P. florida*, *P. sapidus*, *P. eryngii*, *P. columbinus*, *P. cornucopiae*, *P. flabellatus*, *P. platypus*, *P. opuntiae*, *P. citrinopileatus* and *P. coticatus*. Jandaik (1974) first introduced the tropical species, *P. sajor-caju*. It can tolerate temperature up to 30 °C although it fruits faster and produce larger mushroom at 25 °C (Pathak, 2011).

The fruit bodies of this mushroom are distinctly shell, fan or spatula shaped with different shades of white, cream, grey, yellow or light brown depending upon the species. The colour of the sporophors is extremely variable character influenced by the temperature, light intensity and nutrient of the substrate. The oyster mushroom is one



of the most suitable fungal organism for producing protein rich food from various agro-wastes without composting (Vijay, n.d.).

First successful experimental cultivation of *Pleurotus ostreatus* was achieved in Germany by Falck in 1917. He inoculated three stumps and wooden logs with mycelium of *P. ostreatus* and could harvest fresh oyster mushroom. Etter (1929) produced fruiting bodies of *P. ostreatus* in culture. Block *et al.*, (1958) cultivated *P. ostreatus* for the first time under laboratory conditions on sawdust medium. They used a mixture of oat meal and sawdust for cultivation and found best results on Eucalyptus followed by pine sawdust.

Bisaria *et al.*, (1987) studied the growth of this mushroom on several different agricultural wastes, including paddy and wheat straws. Growing oyster mushrooms convert a high percentage of the lignocellulosic materials to fruiting bodies. Therefore cultivation of *P. sajor-caju* on various agricultural residues offers high value products with nutritional and medicinal properties.

In Ghana, *Pleurotus* species are cultivated on composted sawdust of African whitewood tree, *Triplochiton scleroxylon*, supplemented with rice bran and lime (Obodai, 2002). However, some wood dust possesses toxicity and allergic effect with long term hazardous effect on human health (Meier, 2013).

2.1.2 Advantages and importance of Oyster mushroom

P. sajor-caju is one important edible mushrooms from the genera of *Pleurotus* gaining popularity in recent years because of its high nutritional value and ability to grow on diverse agricultural wastes. It is characterized by its high protein content, 30% to 40% on dry weight basis which is twice of the vegetables (Sharma and Madan, 1993). Oyster mushroom cultivation can play an important role in managing organic wastes whose disposal has become a problem (Das and Mukherjee, 2007).

Mushrooms from *Pleurotus* genera have the ability to produce extracellular ligninolytic enzymes namely laccase and two peroxidases which modify and degrade lignin (Stajic *et al.*, 2006). Thus, it can grow well on various lignin-rich lignocellulosic materials (Rodriguez *et al.*, 2004) such as mixture of sawdust and rice bran, rice straw

and rice bran, sawdust and other combinations of tropical wastes, corn cobs, cotton waste, sugarcane bagasse and leaves, corn leaves, grasses, rice hulls and water hyacinth leaves (Pathak, 2011). It also shows rapid growth on agro-wastes such as olive cake, tomato tuff, pine needles, wheat straw, banana leaves (Ananbeh and Almomany, 2005), leaf of hazelnut (Yildiz *et al.*, 2002) and many other wastes.

Oyster mushroom is very well-known in Thailand. It is becoming increasingly popular for consumption, because it is clean and white, has high nutritional value, has a delicious flavour and the flesh is not chewy just like the other types of mushroom. It also have high protein level, second only to beans, and has many other constituents, such as Vitamin B1 and B2, plus low in calories. This makes the mushroom popular for consumption among people who are dieting (Bano and Rajarathnam, 1988). Some of the advantages of growing oyster mushroom are simple cultivation technologies, highest productivity, choice of species, variety of substrates and longer shelf life. *Pleurotus* mushroom can help in solving the problem of malnutrition and disease. Poppe (2000) reported that there are about 200 kinds of waste in which edible mushrooms can be produced. Various agricultural wastes rich in cellulose are being used as substrates for cultivation of *Pleurotus* mushrooms (Thomas *et al.*, 1998).

Mushrooms have long been used as for medicinal and food purposes. *Pleurotus* species contain high potassium to sodium ratio, which make mushrooms ideal food for people suffering from hypertension and heart diseases. They are also rich source of proteins, minerals and vitamins (Caglarirmark, 2007). In general, edible mushrooms are low in fat and calories, rich in vitamin B and C, contain more protein than any other food of plant origin and are also a good source of mineral nutrients (Bahl, 1998). Consequently, now oyster mushrooms are the second largest produced mushrooms in the world (Kuforiji and Fasidi, 2009).

2.2 Agricultural waste as substrate for cultivation of mushroom

2.2.1 Importance of recycling agricultural wastes

Recycling agricultural waste can help reducing environmental pollution. One of profitable way to recycle organic solid waste is by bioconversion of vast quantities of these wastes into mushroom. Organic waste are a kind of biomass, which are generated annually

through the activities of the agricultural, forest and food processing industries (Imtiaj and Rahman, 2008). They consist of mainly three components namely cellulose, hemicellulose and lignin or its general term, lignocellulose. One cannot carelessly disposed of these wastes by dumping or burning because it may lead to environmental pollution and consequently health hazards. It should be recognized that the wastes are resources out of place and their proper management and utilization would lead to further economic growth as well (Wasser, 2002).

Agriculture residues are the major source of lignocellulosic materials which is suitable as substrate for solid state fermentation of edible fungi such as *P. sajor caju*. These wastes are produced in big volumes during production of agricultural products every year causing lots of environmental problems in many countries (Belewu and Banjo, 2000). Chiu *et al.* (2000) stated that one of successful example of agro-waste recycling is production of edible or medicinal mushroom. These agro-wastes with or without fertilizers or other nutrient supplements have been converted into many edible and medicinal mushrooms creating a total value of more than USD 0.24 billion per year contributing to prosperous economy of rural areas and the development of the mushroom industry in Taiwan.

Mushrooms are grown on a wide variety of substrates. The choice of substrates depends on availability and cost. The rapid growth and ability to utilize various lignocellulosic substances, makes *Pleurotus* species cultivation possible in different parts of the world. *P. ostreatus* is an easily cultivable mushroom that colonizes various crop residues as substrates. It is able to degrade and covert lignocellulosic compounds into protein-rich biomass (Mamiro and Mamiro, 2011). In this way, the residues can be converted into human food in the biological recycling process (Skrobiszewski *et al.*, 2013).

Rice husk are used for cultivation of *Pleurotus ostreatus* (Mahmoud, 1989) and these are available in large quantities. Although, 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 *T. scleroxylon* was mixed with rice straw (Narh and Obodai, unpublished data).

Vetayasuporn *et al.* (2006) in their studies to determine bagasse as a possible substrate for *Pleurotus ostreatus* cultivation for the local mushrooms farm in Thailand found out that 75% of bagasse combined with 25% sawdust produced the highest biological efficiency, 107.61, when compared to their control, 100% sawdust which the biological efficiency are 106.37. The high BE may be caused by bagasse, which is a reservoir of carbon that the *P. ostreatus* culture easily utilizes during the growth of spawn and colonization of substrates during the generative stage.

In Indonesia, *Paraserianthes falcataria* or commonly known as peacocksplume is the principal substrate for oyster mushroom cultivation, although adequate production can be achieved through use of sawdust with addition of supplements that substantially increase the yield per unit weight. In addition PFS contains cellulose 48.3%, lignin 27.8%, pentosan 16.2% (Nurhayati, 1988) but EFB of oil palm contains lower cellulose of 36%, lignin 27.3%, carbon 64.7%, N 1.1% and C/N ratio 57.8 (Sudirman, 2011).

2.2.2 Sugarcane bagasse as substrate for cultivation of Oyster mushroom

Sugarcane is a large, tropical grass that stores sucrose in its stem and serves as an important food and bioenergy crop. It has long been recognized as one of the world's most efficient crops in converting solar energy to chemical energy harvestable as sucrose and biomass. *Saccharum officinarum* L. was the first named sugarcane of domesticated species and is the primary species for production of sugar (Moore, 2012).

In sugarcane production, the whole plants are used. The average composition of sugarcane is 65-75% of water, 11-18% of sugar, 8-14% fibres and 12-23% soluble solids. The cane basically consists of juice and fibre. After extracting the juice, the material called bagasse is obtained (Noe Aguilar-Rivera, 2012). Table 2.1 shows the average composition of sugarcane bagasse on dry mass basis.

Table 2.1: Average composition of sugarcane bagasse on dry mass basis

Fraction/ component	% (w/w)
Hemicellulose	31.2
Xylose	25.2
Cellulose	42.4
Lignin	19.6
Ashes	1.6
Extractives	4.1

Source: Tsigie (2012)

A recent study by Aguilar *et al.*, (2012) on production of *Pleurotus ostreatus* grown on sugarcane biomass were done to see how their economic and nutritional potentials will increase the productivity of mushrooms on sugarcane substrates with better flavour, appearance, texture, nutritional qualities at low cost. The result shows that on sugarcane biomass, the biggest production reported is in trash as a substrate and mixed with bagasse in a ratio of 50:50 with biological efficiency of 106.64.

Other than potential as substrate for cultivation of oyster mushroom, sugarcane bagasse has been used as a reinforcement in composites on account of its low cost, low density, high specific strength, modulus and atoxic. The utilization of this biomass for processing of novel composites has attracted growing interest due to eco-friendly and renewable nature characteristics.

Dey *et al.* (2008) conducted an experiment on production of oyster mushroom on different substrates using cylindrical block system. They are using different substrates namely paddy straw, sugarcane bagasse and mustard straw using cylindrical block system to find out suitable substrate for cultivation of oyster mushroom. The results showed that each of the substrates significantly affected the number of primordia, fruiting bodies and the amount of fresh weight or yield of oyster mushroom in cylindrical block system. The highest number of primordia, fruiting bodies and the amount of fresh weight was obtained with sugarcane bagasse in all flushes whereas the lowest is with the mustard straw.

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