THE GROWTH AND YIELD QUALITY OF GREY OYSTER MUSHROOM, *Pleurotus sajor-caju* ON *Brachiaria mutica* AS SUBSTRATE

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ABSTRACT

Many places cultivate oyster mushroom (*Pleurotus spp.*) by using sawdust and other agricultural wastes. Oyster mushroom is capable of converting lignocellulosic residues from agricultural fields and forest into protein-rich biomass by degrading those. Like sabai grass as potential substrate, *Brachiaria mutica* belongs to the Poaceae family also contain lignocellulose that fulfils the characteristic as substrate for the cultivation of oyster mushroom. Since this grass is highly available in Sandakan areas, it is possible to reuse it as a substrate for cultivation of oyster mushroom. In the present study, *B. mutica* was used as substrates for the cultivation of grey oyster mushroom (*Pleurotus sajor-caju*). To study the possibility of using it, *B. mutica* was tested by using either as whole substrate or in combination with conventional substrate such as sawdust at different ratio in 3:1, 1:1 and 1:3 ratio. Each treatments had 5 replicates. Sawdust as control, unexpectedly, did not produce any fruiting bodies, resulting no yield attributes. It was most probably due to the contamination of green mould and flies. However, when *B. mutica* mixed with sawdust in any ratios or as whole substrate, it showed no significance different in yield by fresh weight of fruiting bodies and biological efficiency. Sawdust mixed with *B. mutica* at ratio 1:3 revealed as the best ratio recommended in term of primordial formation and biological efficiency. The results indicated that *B. mutica* can be utilized as a good substrate for the *P. sajor-caju* cultivation. This study also provided an alternative way to decrease the dependence on sawdust for the large scale of *P. sajor-caju* cultivation.

PERTUMBUHAN DAN HASIL KUALITI CENDAWAN TIRAM KELABU, *Pleurotus sajor-caju* PADA *Brachiaria mutica* SEBAGAI SUBSTRAT

ABSTRAK

Banyak tempat menanam cendawan tiram (*Pleurotus spp.*) dengan menggunakan habuk kayu dan sisa pertanian yang lain. Cendawan tiram adalah mampu menukarkan sisa lignoselulosa dari kawasan pertanian dan hutan kepada biomass yang kaya dengan protein dengan menguraikan sisa-sisa tersebut. Seperti rumput sabai yang merupakan potensi substrat, *Brachiaria mutica* tergolong dalam keluarga Poaceae juga mengandungi lignoselulosa yang memenuhi ciri-ciri sebagai substrat untuk penanaman cendawan tiram. Memandangkan rumput ini adalah sangat senang untuk didapati di kawasan Sandakan, ia mempunyai kemungkinan untuk menggunakannya semula sebagai substrat untuk penanaman cendawan tiram. Dalam kajian ini, *B. mutica* telah digunakan sebagai substrat untuk penanaman cendawan tiram kelabu (*Pleurotus sajor-caju*). Untuk mengkaji kemungkinan menggunakannya, *B. mutica* telah diuji dengan menggunakan sama ada secara keseluruhan substrat atau dalam gabungan dengan substrat konvensional seperti habuk kayu pada nisbah yang berbeza dalam 3:1, 1:1 dan 1:3 nisbah. Setiap rawatan mempunyai 5 replikasi. Habuk kayu sebagai kawalan, tanpa diduga, tidak menghasilkan apa-apa badan buah cendawan, menyebabkan tiada maklumat untuk hasil. Ia kemungkinan besar disebabkan oleh pencemaran kulat hijau dan lalat. Walau bagaimanapun, apabila *B. mutica* bercampur dengan habuk kayu dalam apa-apa nisbah atau secara keseluruhan substrat, ia tidak menunjukkan perbezaan yang ketara dalam hasil mengikut berat segar badan buah cendawan dan kecekapan biologi. Habuk kayu yang dicampur dengan *B. mutica* pada nisbah 1:3 didedahkan sebagai nisbah terbaik disyorkan dari segi pembentukan asal dan kecekapan biologi. Keputusan menunjukkan bahawa *B. mutica* boleh digunakan sebagai substrat yang baik untuk penanaman *P. sajor-caju*. Kajian ini juga memberi cara alternatif untuk mengurangkan kebergantungan kepada habuk kayu untuk skala besar penanaman *P. sajor-caju*.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percentage
$	US Dollars
°C	Degree Celcius
ANOVA	Analysis of Variance
BE	Biological Efficiency
cm	Centimetres
CO_2	Carbon Dioxide
FAO	Food and Agriculture Organization
FSA	Faculty of Sustainable Agriculture
g	Grams
kg	Kilograms
mm	Amount of Rain per Square Meter in One Hour
PDA	Potato Detoxes Agar
SPSS	Statistical Package for the Social Science
UMS	Universiti Malaysia Sabah
USDA	United States Department of Agriculture
wt	Weight

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Cultivation of Oyster Mushroom, *Pleurotus spp.*

Oyster mushroom can be said as one of the leading mushroom based on the term of customer preference and worldwide production technology (Mswaka and Mamiro, 1997; Baysal *et al.,* 2003). The simplicity of cultivation of oyster mushroom and low cost of the oyster mushroom cultivation have made them the better choice for mushroom growers. The substrate for the cultivation of oyster mushroom only requires pasteurization and does not require sterilization so it reduces the cost for the oyster mushroom cultivation. Cultivation of oyster mushroom (*Pleurotus spp.*) has showed increasing trend throughout the world in last decade (Chang, 1999; Royse, 2002). In 1997, 14.2% of the total world production of edible mushrooms produced are from this mushroom (Chang, 1999). Oyster mushroom is capable of converting lignocellulosic residues from agricultural fields and forest into protein-rich biomass by degrading those (Rowel *et al.,* 2000). Oyster mushroom normally grown on pasteurized paddy straw, sawdust and wheat straw. However, any type of organic matter that contain lignin and cellulose also can be used as substrate for the cultivation of oyster mushroom as they have the ability to degrade the organic matters with its enzymes. Therefore, cultivation of oyster mushroom can helps in solving the problem for the disposal of the organic waste by managing the organic wastes.

Oyster mushrooms can also produce fruiting bodies on goose grass (*Eleucine coracana* Gaertn.) and kikuyu grass (*Pennisetum typhoides)* straw; sorghum (*Sorghum vulgare* Pers.) and maize stem (*Zea mays* L.)(Bano *et al.*, 1987; Goswami *et al.,* 1987), and straw of some species of wild plants including: *Leonotis* sp., *Sida acuta* Burm, *Parthenium argentatum* Gray, *Ageratum conyzoides* L., *Cassia sophera* L., *Tephrosia purpurea* (Linn.) Pers., and *Lantana camara* L. (Das *et al.*, 2007).

1.2 Para grass, *Brachiaria mutica*

B. mutica is basically known as para grass. It is perennial stoloniferous grass that grow widely in the humid tropic and subtropic with rainfall of 1250mm per year. It belongs to the Poaceae family, just like other grasses and maize. Sometimes, people call it as *Urochloa mutica. B. mutica* can affects interest of farmers because of the potential of it to become aggressive weeds. Weeds are known as undesirable plants that grow in the place with interested plants. It is very common to appear as weed in sugarcane field, coconut plantation and paddy field. Basically, it is found in wet location such as drains but it will also grow in deep soil in non-swampy areas (Gamble and Fisher, 1915-1935).

1.3 Justification

B. mutica serve as a troublesome weed of drainage lines, coconut plantation, nurseries, paddy field and sugarcane field. *B. mutica* choke streams and wetlands, increase the sedimentation within the waterbody and impede the water flow. It results for the increased flooding as it changes such the hydrological and morphological of stream. It can also causes some of the environmental problem when farmers dispose these weeds through burning (Croan, 2000). It also increase the cost of farmers in the agricultural farming to remove those weeds.

The studies of Das *et al.* (2002) reported that sabai grass is a potential substrate for cultivation of grey oyster mushroom (*P. sajor-caju*). Like sabai grass, *B. mutica* belongs to the Poaceae family also contain lignocellulose that fulfil the characteristic as substrate for the cultivation of oyster mushroom. Since this grass is highly available in Sandakan area, it is possible to reuse it as a substrate for cultivation of oyster mushroom. It will not only minimize the problems such as environmental pollution and flooding but also increase the alternative choices for substrate to be chose for the cultivation of *P. sajor-caju.* Therefore, it is a need to evaluate the possibility of using *B. mutica* as substrate for the cultivation of *P. sajor-caju.*

1.4 Objective

To evaluate the performance of *B. mutica* as substitute substrate for sawdust in the growth and yield quality of *P. sajor-caju.*

1.5 Hypothesis

The hypothesis of the research as below:

H_o: There is no significant difference in growth and yield quality of *P. sajor-caju* in *B. mutica* as substrate

H_A: There is significant difference in growth and yield quality of *P. sajor-caju* in *B. mutica* as substrate

CHAPTER 2

LITERATURE REVIEW

2.1 Mushroom Industry

2.1.1 World Production

Worldwide mushroom production has shown an increase over 300%, reaching approximately 2,961,493 tons in 2002 (USDA, 2003). 40% of the world's supply for all edible mushrooms are came from the top-producing nation, China (USDA, 2003). The next largest producer of mushrooms is from US, contributing about 13%, while the Netherlands and France produce 9.5% and 5%, respectively (USDA, 2003). U.S. has been steadily rising the overall production in term of volume over the last decade. Operations are also diversifying as various specialty mushrooms are added in the production. The improvements in cultivation technologies and the expansion of market demand cause the expansion of industry in both output and diversity (Yamanaka, 1997).

For edible mushrooms, the cultivation methods are quite different around the world. Basically, the methods are depend on the mushroom types. For *Agaricus* species such as white button mushroom, portabello and crimini need to cultivate on composted substrate while white-rot fungi can be cultivated on uncomposted organic materials. Shiitake (*Lentinula edodes*), wood ears (*Auricularia spp.*), paddy straw mushrooms (*Volvariella volvacea*), oyster mushrooms (*Pleurotus spp.*) and many others are white rot fungi. Shiitake and wood ears are cultivated best on hardwoods, while paddy straws mushroom are cultivated best on straw just like what their names called. Oyster mushrooms are very popular cultivated mushroom because they capable to grow well on many types of substrates.

Many cultivated mushrooms can be grown using either indoor or outdoor methods based on their location of production. The majority of cultivation in China is done outdoors, while US has most of their cultivation done in indoors (Shen *et al.*, 2004). Normally, outdoor cultivation costs much lower operation fees and depends on nature to create the environmental conditions that are suited for the mushroom cultivation. Indoor cultivation is far more specific in the environmental conditions control and hygienic operation control. Indoor methods usually give higher yields but are very expensive to establish and operate if compare to outdoor methods.

2.1.2 Market Value

Among the edible mushrooms, the oyster mushroom and shiitake as cultivated mushrooms have a relatively high market value. In the United States, the value of the cultivated mushroom crop reached $37,676,000 in 2002-2003 (USDA, 2003). The oyster mushroom is a very common mushroom that used for cooking and eating and it has gain its reputation as it is easy to be cultivated (Stamets, 2000). In the US, the oyster mushroom has approximately $4.50 per kg for the current market value (USDA, 2003). Shiitake mushrooms have around $6.60 per kg in 2002 to 2003 and it has higher selling value (USDA, 2003). According to the historical and more recent trends, it shows that the cultivated mushroom industry will continue to flourish (Royse, 1996).

2.2 Substrate

For more than two thousand years, the highly priced delicacy such as mushroom even now are still consumed by many people. Mushroom cultivation is agribusiness that can earn a considerable profit. For the cultivation of *Pleurotus* species, many agricultural and industrial wastes can be used as substrates (Zadrazil and Brunnert, 1981).

The studies from Tan (1981) showed that the best substrate for the cultivation of *P. ostreatus* was cotton waste. Addition of cereal bran which is rich in protein into the substrate in cultivation of *P. ostreatus* help in the stimulation of mycelia growth and increase the yield of mushroom (Kinugawa *et al.*, 1994)

For growing of oyster mushroom, sawdust and sugarcane bagasse were the best substrates among the other agro-based substrates (Ahmed, 1998).

Obodai *et al.* (2002) reported that sawdust substrate for production of mushroom should composting for a period of time to breakdown the cellulose and lignin components of the wood so that the essential materials can be released for the establishment of mushroom mycelium. The lignocellulosic materials in sawdust usually contains low protein content and thus it is not enough for the mushrooms cultivation, and therefore it needs additional nitrogen, phosphate and potassium.

Baysal (2003) investigated rice husk, chicken manure and peat as supplement to paper waste for *P. ostreatus* cultivation. Substrate that contain 20% rice husk showed the highest yield for fresh weight which is 350.2 g.

The availability of large quantities of several agro–industrial wastes in a developing economy like Nigeria which can serve as substrates for the mushrooms cultivation have its value of commercial mushroom cultivation. (Banjo *et al.*, 2004) has been reported that mushrooms can grow on chopped cocoa pods, cotton waste, dried chopped maize straw, oil palm (fibre and bunch) wastes, tobacco straw, used tea leaves, rice straw, sugarcane bagasse, newsprint, old rags and sawdust.

Silva *et al.* (2005) reported that there are relationship of mycelium extension with bio availability of nitrogen when they discovered that cereal bran as supplement to eucalyptus residue helped fast growth. However, the lignocellulosic substrate of wood components with low nitrogen availability is often considered as a limitation to be utilized as mushroom substrate.

Das *et al.* (2006) studied on weed plants for the cultivation of *P. ostreatus*. It showed that best substrates in fruit body production of *P. ostreatus* was *Leonotis sp.* when it was mixed with rice straw in ratio of 1:1. In the investigation, *Leonotis sp.* had lesser fruiting time for *P. ostreatus* if compare to any other weed substrates tested. It also reported that the least suited weed for oyster mushroom cultivation among the weed substrates was *Tephrosia purperea*.

The simple and low cost production technology and higher biological efficiency of *Pleurotus* species have turned them well known and widely cultivated throughout the world especially in Asia and Europe (Mane *et al.*, 2007).

Rajapakse *et al.* (2007) investigated the effect of six substrates on the growth and yield of American oyster mushrooms. Although banana leaf substrate had highest mycelial growth rate followed by paddy straw and bagasse mixture, only mixture of paddy straw and bagasses obtained higher mushroom yield and they showed no significant difference to the control, sawdust media. This investigation shows that paddy straws and sugar cane bagasses can considered as good substrates same like sawdust media for cultivation of oyster mushrooms.

Olfati *et al.* (2008) studies on lawn clippings for the oyster mushroom cultivation. Lawn clippings alone and rice + lawn clippings can considered as the best substrates for fruit body production of *P. ostreatus*. The lawn clippings also had the shorter fruiting time. It is also suitable for lawn clippings as substrate for production of oyster mushroom. This study shows that it needs further research in the effects of the volume of components of the lawn clippings to the suitability of the substrate.

Moonmoon *et al.* (2010) studied king oyster mushroom *Pleurotus eryngii* on sawdust and rice straw in Bangladesh and found that sawdust showed the highest biological efficiency (73.5%) than other strains. He has also reported sawdust had better yield and efficiency than those cultivated on rice straw. However, he also reported that the mushroom fruiting bodies were larger in size when cultivated on straw. This study shows the prospects of cultivation of *P. eryngii* in Bangladesh and further study in controlled environment for higher yield and production is recommended.

Stanley *et al.* (2011) has evaluated the effect of rice bran as supplement to corn cob substrate on yield of *Pleurotus pulmonarius* (Fr) Quel. Corn cob without supplement (0% supplementation) gave the best yield in terms of the mean diameter of pileus 5.50 cm, mean fresh weight of fruiting bodies 53.2 g, mean height of stipe 3.64 cm and number of healthy fruiting bodies as 12. 30% supplementation showed the least yield as follows: mean diameter 3.20 cm, mean fresh weight of fruiting bodies 30.0 g, mean height of stipe 1.65 cm and number of healthy fruiting bodies as 5. Substrate without supplement produced better edible mushrooms in terms of quantity and quality.

Shyamal *et al.* (2011) found that utilization of plenty of grasses that available in forest and wastelands of lateritic uplands of eastern India as substrate in commercial cultivation of *P. sajor-caju* can be success. He also revealed that the maximum yield of mushroom was reached by the paddy straw with 85.9% biological efficiency.

Nasir Ahmad Khan *et al.* (2012) have observed that the first flush of *P. ostreatus* gave the maximum yield followed by second and third flush. Kikar sawdust obtained the maximum yield as 282.2 g followed by Mango sawdust as 257.7 g, mixed sawdust as 233 g, Simbal sawdust as 216.5g and Kail as 200.5g. Control treatment of cotton waste in oyster mushroom showed relatively better yield as compared to other substrates. Kikar sawdust obtained maximum biological efficiency which was 70.56%. Kail sawdust also obtained the lowest biological efficiency which 50.12%. Among all substrates, it proved that the best substrate for the effective cultivation of oyster mushroom was Kikar sawdust.

The study of Fekadu Alemu (2013) revealed that both coffee and sholla leaves can be good substrates for the cultivation of mushroom. It proved that the cultivation of oyster mushroom on these substrates have promising effects for the sustainable development, food security and supply.

M. Manimuthu *et al.* (2015) reported that the seagrass with 70.65% biological efficiency can be a substitution for the paddy straw, wheat straw and other agricultural wastes in oyster mushroom cultivation.

Tesfaw *et al.* (2015) studied on optimization of oyster mushroom cultivation using locally available substrates and material. The study showed that higher yield can be obtained from waste paper and gabi wastes alone or in mixture with sawdust. It also reported that 25°C was the suitable temperature for the growth of oyster.

2.3 Oyster Mushroom

2.3.1 Historical Information

Oyster mushroom like other types of edible mushrooms have been collected in wild for many centuries. In the early 1900's, cultivation of these mushrooms only began. In early technique and methods for growing *Pleurotus*, tree stumps and logs are used as substrate to mimic their growth in nature (Ivors, 2003). In the 1950's, a historic milestone for mushroom cultivation is created by having successful attempts to grow oyster mushroom on sawdust in Germany. Start by the last 1960's, oyster mushrooms was produced in large amounts using a straw based substrate (Chang and Hayes, 1978). Oyster mushroom has been increasing their popularity and production ever since.

This edible basidiomycete has well developed commercial production techniques (Stamet, 2000; Oei, 1996; Zadrazil, 1978). Species of *Pleurotus* are relatively simple to cultivate if compare to other edible mushrooms (Zadrazil, 1978). In addition, *Pleurotus spp.* are known as the most adaptable genera of edible fungi as they can grow on a wide range of lignocellulosic materials (Stamets, 2000). Around the world, cultivation of oyster mushrooms are using many different organic materials as substrates. However, it mostly depends on the substrate availability in a particular region. Normally, *Pleurotus spp.* grow on the wood of broadleaf trees so wood and wood products are not rarely substrates for oyster mushroom cultivation (Zadrazil, 1978). Wheat straw is very common used as substrate for oyster mushroom cultivation in the continental US, while the high availability of rice straw in China is utilized as substrate (Chang and Hayes, 1978). There are few successful cultivation of oyster mushroom by using other substrates such as cotton waste, corn cobs, palm fronds, tea waste, and peanut shells (Cohen *et al.*, 2002, Thomas *et al.*, 1998, Kalita and Mazumder 2001, Philippoussis *et al.*, 2001).

2.3.2 Taxonomy and Morphology

Pleurotus spp. are belongs to the order Agaricales. They are wood-inhabiting ligninolytic white-rot Basisdiomycetes. There are over 30 species of *Pleurotus* mushrooms. Beside as saprophytic fungi, *Pleurotus spp.* also can grow parasitically on tress as well. The colour of fruit bodies are from grey to white to grey-brown. Fruit bodies' shape are mostly shell or spatula with a non-central stalk. *Pleurotus* mushrooms have thin, broad and dense gills that continuous to the upper part of the stipe and its gills colour can vary from white to light grey. Their spores have the colour from white to buff to grey-lilac. The spores mostly be produced in huge amounts and it is found that sometimes it can causes allergic or irritation reaction to the growers (Eger, 1978).

For *P. sajor-caju*, it has 5 to 14 cm pileus in diameter which often lobed and folded when it reaches maturity, white to grey or dull brown in colour intense towards the margin, smooth surface, irregular and curved margin (Subrata *et al.*, 2011). It has white gills when fresh and yellow gills when dry and 1 to 3 cm long stipe.

2.3.3 Physiology

Species of *Pleurotus* same as other white rot fungi secrete an arsenal of enzymes specific for the digestion of lignocellulose materials. Among different species of white-rot fungi and different growth conditions, the degradation of substrate, including types and quantities of enzymes produced are different (Freer and Detroy, 1982, Boyle *et al.*, 1992). Buswell *et al.* (1996) has studied and summarized the enzymatic profiles of three edible mushroom, *L. edodes*, *P. pulmonarius*, and *Vollvariella volvacea* (paddy straw). *L. edodes* grow naturally, and is cultivated, on wood logs and sawdust which contain high lignin. This fungus will produce both Mn peroxidase and laccase, two enzymes specific in the process of lignin degradation. The paddy straw mushroom like to grow on high cellulose substrates such as straw. It produces many cellulolytic enzymes but none of the lignin-degrading enzymes. *P. pulmonarius* produced higher levels of both cellulolytic and ligninolytic enzymes when enzyme production was quantified (Buswell *et al.*, 1996). *Pleurotus spp.* produce specific lignin-degrading enzymes include lignin peroxidase, Mn peroxidase and laccase (Orth *et al.*, 1993, Kaal *et al.*, 1995). Cellulolytic enzymes of *Pleurotus spp.* include endoglucanase, exoglucanase, β-glucosidase (Buswell *et al.*, 1996, Tan and Wahab, 1997) and cellobiohydrolase (Tan and Wahab, 1997, Velazquez-Cedeno *et al.*, 2002).

The growth and fruiting of *Pleurotus spp.* has also been shown to be influenced by lignin, cellulose, and mineral contents of substrates. Philippoussis *et al.* (2001) demonstrated that the cellulose:lignin ratio of the substrates was positively correlated to the mycelial growth rate and mushroom yield of both *P. ostreatus* and *P. pulmonarius*. Fasidi and Olorunmaiye (1994) verified that certain macroelements and trace elements are essential for *Pleurotus spp.* growth. In addition, medium supplemented with Cu, Fe, Mn and Zn produced greater fungal growth than basal medium.

2.4 Fruit Body Initiation and Production

Several factors are critical for successful fruiting of cultivation of *Pleurotus spp.* and all white-rot fungi. Moisture, temperature, gas exchange, and light are involved in mushroom initiation and development.

For optimal primordial formation, extremely high humidity is recommended (90 to 100%). Once primordial have formed, humidity should be lowered to 85% to 90%. Mushrooms can regularly receiving moisture but excess moisture can evaporate from fruit body surface by controlling the humidity levels (Stamets, 2000). Excessive moisture can promotes certain contamination and decrease the oxygen level in the substrate. Lack of moisture also can discourage the primordial formation and cause the fruit body become stunted.

Oyster mushrooms can grow and thrive in a wide range of temperature environment. Stamets (2000) suggests that temperature between 10°C and 21°C for oyster mushroom development. Pettipher (1987) achieved successful fruiting of *P. ostreatus* with daily temperature from 8°C to 33°C. For *P. sajor-caju*, the optimum temperature for fruiting is between 18°C and 25°C (Subrata Biswas *et al.*, 2011).

Since the decomposition of the substrate by fungus produces carbon dioxide, fresh air is needed to reduce the carbon dioxide build up and increase oxygen levels. Fungal mycelium can thrives at 20% CO_2 level as it is extremely tolerant of carbon dioxide. Formation of fruit bodies need oxygen. A significant decrease in ambient CO_2 level and increase in oxygen is critical for the initiation and primordial development. Thus it is vital to have sufficient air circulation within a mushroom fruiting site. However excessive influx of outside air greatly affects both temperature and humidity of the environment (Stamet, 2000).

Indirect natural light is considered ideal for the formation of *Pleurotus spp.* fruit bodies. Proper fruit body formation requires moderate light while the mycelium of the oyster mushroom does not require light. Too little or too much light can causes

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