**UNIVERSITI PUTRA MALAYSIA**

# *CULTIVATION OF GREY OYSTER MUSHROOM [Pleurotus pulmonarius (Fr.) Quél.] ON DIFFERENT ALKALINE MATERIALS AND ITS ANTIOXIDANT PROPERTIES*

**MUHAMAD FAKHRUL RADZI BIN MUHAMAD PIKRI**

**FP 2022 7**

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By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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**July 2022**

**Chair : Sumaiyah Binti Abdullah, PhD**
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The study aims to ascertain the effect of different alkaline materials' supplementation on mushroom spawn, yield and antioxidant properties of grey oyster mushroom. The mushroom spawn grown on wheat with the treatment of zeolite (0.5%, 1%, 2%) and gypsum (1%, 2%) showed the rapid growth in spawn-running time (10 days). The maize substrate coupled with all of the treatments and the wheat substrate with gypsum (1%, 2%) developed the thickest spawn mycelia. The antagonistic activity showed the wheat substrate with the treatment of zeolite (2%), and the maize substrate with zeolite (2%) exhibited a high growth inhibition rate against *Trichoderma koningii* with 91.67 ± 5.77 % and 90 ± 8.66 %, respectively. DNA analysis of mushroom pathogenic fungi proved the isolate of *Trichoderma* sp. was genetically similar with *Trichoderma koningii* at a bootstrap value of 97%. The cultivation of grey oyster mushrooms between different alkaline treatments was resolved to show the significant effect on mushroom production. The substrate in the presence of gypsum took minimum days for mycelial growth (30.7 ± 4.12 days) and the emergence of primordia (7.7 ± 4.55 days). The mushroom yield indicated that zeolite treatment showed the most significant yield (62.36 ± 9.67 g) as collected from the first until the fourth flush. The biological efficiency resembled by the treatment of zeolite stood out all the treatments by 26.05 ± 11.18 %. A maximum inhibitory effect against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, IC50, was demonstrated by zeolite treatment at the third flush (63.48 ± 28.804 µg/mL). The fruiting body produced from the zeolite treatment was highly containing phenolic compound (98.96 ± 10.07 µg GAE/mg), as noticed in the fourth flush. Therefore, supplementation of gypsum and zeolite in substrate formulation might be a great benefit to practice in promoting and increasing the quality of spawn and fruiting body of mushrooms.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENANAMAN CENDAWAN TIRAM KELABU [*Pleurotus pulmonarius* (Fr.) Quél.] PADA BAHAN BERALKALI YANG BERBEZA DAN SIFAT ANTIOKSIDAN CENDAWAN**

Oleh

**MUHAMAD FAKHRUL RADZI BIN MUHAMAD PIKRI**

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**Pengerusi : Sumaiyah Binti Abdullah, PhD**
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Kajian ini bertujuan untuk memastikan kesan penambahan bahan beralkali yang berbeza terhadap benih, hasil dan sifat antioksidan cendawan tiram kelabu. Anak benih cendawan yang ditanam pada gandum dengan rawatan zeolit (0.5%, 1%, 2%) dan gipsum (1%, 2%) menunjukkan pertumbuhan pesat dalam masa pembentukan benih (10 hari). Substrat jagung ditambah dengan semua rawatan, bersama-sama dengan substrat gandum dengan gipsum (1%, 2%) menghasilkan miselia paling tebal. Aktiviti antagonis menunjukkan substrat gandum dengan rawatan zeolit (2%), dan substrat jagung dengan zeolit (2%) menunjukkan kadar perencatan pertumbuhan yang tinggi terhadap *Trichoderma koningii* dengan masing-masing 91.67 ± 5.77 % dan 90 ± 8.66 %. Analisis DNA kulat patogen cendawan membuktikan pencilan *Trichoderma* sp. secara genetik serupa dengan *Trichoderma koningii* pada nilai bootstrap 97%. Penanaman cendawan tiram kelabu di antara rawatan beralkali yang berbeza telah diselesaikan untuk menunjukkan kesan yang ketara terhadap pengeluaran cendawan. Substrat dengan kehadiran gipsum mengambil hari minimum untuk pertumbuhan miselia (30.7 ± 4.12 hari) dan kemunculan primordia (7.7 ± 4.55 hari). Hasil cendawan menunjukkan bahawa rawatan zeolit menunjukkan dapatan terbesar (62.36 ± 9.67 g) seperti yang dipetik dari tuaian pertama hingga keempat. Koefisien biologi yang ditunjukkan oleh rawatan zeolit lebih menonjol daripada semua rawatan sebanyak 26.05 ± 11.18 %. Kesan perencatan maksimum terhadap radikal bebas 2,2-diphenyl-1-picrylhydrazyl (DPPH), IC50, ditunjukkan dengan rawatan zeolit pada tuaian ketiga (63.48 ± 28.804 µg/mL). Cendawan yang dihasilkan daripada rawatan zeolit mengandungi jumlah fenol yang tinggi (98.96 ± 10.07 µg GAE/mg) seperti yang diperhatikan dalam tuaian keempat. Oleh itu, penambahan gipsum dan zeolit dalam formulasi substrat mungkin memberi manfaat untuk digunakan dalam menggalakkan dan mengingkatkan kualiti benih dan badan buah cendawan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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I hereby confirm that:

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| | |
|---|---|
| $^{o}C$ | Degree celcius |
| % | Percentage |
| mL | Mililiter |
| µL | Microliter |
| mm | Milimeter |
| cm | Centimeter |
| g | Gram |
| mg | Miligram |
| µg | Microgram |
| s | Second |
| min | Minutes |
| hrs | Hours |
| PDA | Potato dextrose agar |
| EFB | Empty fruit bunch |
| C/N | Carbon-nitrogen ratio |
| pp | Polypropylene |
| OH- | Hydroxide ion |
| $CaCO_3$ | Lime |
| $CaSO_4$ | Gypsum |
| Ca | Calcium |
| DNA | Deoxyribonucleic acid |
| PCR | Polymerase chain reaction |
| ITS | Internal Transcribed Spacer |
| BLAST | Basic Local Alignment Search Tool |
| V | Voltage |

| | |
|---|---|
| Mm | Milimolar |
| DPPH | 1,1-diphenyl-2-picryl-hydrazyl |
| ROS | Reactive oxygen species |
| RSA | Radical scavenging activity |
| $IC_{50}$ | Half maximal inhibitory concentration |
| nm | Nanometer |
| TPC | Total phenolic content |
| GAE | Gallic acid equivalent |
| SAS | Statistical analysis system |
| ANOVA | Analysis of variance |
| CRD | Completely randomized design |
| SD | Standard deviation |
| Et al. | And others |
| FAO | Food and Agriculture Organization |
| SMI | Small and medium industry |
| MOA | Ministry of Agriculture and Agro-Based Industry |

# CHAPTER 1

## INTRODUCTION

Grey oyster mushroom, *Pleurotus pulmonarius* (Fr.) Quél (1827), also known as *P. sajor-caju* (Fr.) Singer (Li & Yao, 2005), is a species classified under the family Pleurotaceae, class Agaricomycetes, and in the order Agaricales. It is a popular edible mushroom worldwide over the demand of its consumption; as it is among of the important species under the genus of *Pleurotus,* which occupy third ranking of the edible mushroom production next to the top line of the genus *Agaricus* and *Lentinus* (Fernandes *et al*., 2015). To come with an overview of this high tradable industry showing the continuously growing market, China is the big country to produce mushrooms worldwide, followed by the other producer countries Europe and India. It has been estimated that China produced 3 million tons of mushrooms in 2016 (Zhang *et al*., 2019). Oyster mushroom is recognised as one of the high-valued commodities and listed as one of the seven commercial varieties in Malaysia (Amin *et al*., 2014). To date, oyster mushrooms kept thriving in snapping a tremendous demand across the globe due to their flavour, nutritional and medicinal properties.

Mushroom is not only a good additional source of high-quality vegetables, but it is likely the main course comprising the traditional diets among the local community in the secluded area in some countries. They promise a wide range of health benefits from their consumption on a daily basis in respect to the gastronomical delicacy varying in the culture from different places. It is renowned that it is a good source of dietary fibre over its protein and minerals such as phosphorus, calcium, iron, potassium, and sodium (Yamauchi *et al*., 2019). The extension of study on the mushroom potential to pose medicinal properties has been a big interest in pharmaceutical production and medical purposes. Oyster mushroom is well known for their medicinal value, such as antiviral, antibacterial, antifungal, antiparasitic, antitumor, antioxidant, antihypertensive, antidiabetic, and withstand hyperglycemia and insulin resistance and anti-inflammatory (Finimundy *et al*., 2018). This aside, this mushroom also contains many bioactive metabolites, including polysaccharides, polyphenols, flavonoids, terpenoids, ergosterols, and volatile organic compounds (Nguyen *et al*., 2016). The majority of folks back then, when mushroom consumptions were increasingly in awareness, exploited mushrooms as the traditional medicine they were described to possess with a high constituent of the bioactive compound, mainly phenolic compounds. The composition of these prerequisite chemical properties of the phenolic compound has been used to determine the antioxidant properties (Reid *et al*., 2017). The cultivated mushroom is, however, as has been reported, commonly has a higher chemical composition (Ashraf *et al*., 2013). Nevertheless, the different composition of substrates to grow mushroom chemical properties found in the fruiting body is yet to be thoroughly studied.

As opposed to other planted crops, mushrooms can be grown in various agricultural and plant residues due to their complex extracellular enzyme,

degrading and utilising the lignocellulosic waste. For instance, among the agricultural waste used, such as cotton waste, rice straw, and wheat straw (Yang *et al*., 2013; Sardar *et al*., 2017), sawdust (Obodai *et al*., 2003), banana leaves (Reddy *et al*., 2003), sugarcane bagasse (Hasan *et al*., 2015), elephant grass, coat cross and coffee husks (Corrêa *et al*., 2016). In Malaysia, rubber sawdust is the primary medium for cultivation as it is the most readily available because Malaysia plays the role of the exporter of rubber on such a colossal scale (Nur Aziera *et al*., 2015). As achieving a higher yield of mushroom production is the industrial player's main interest, maintaining the optimum uses of a suitable substrate in providing the nutrient requirement for mushroom growth is perhaps crucial to maximising the performance. The supplementation of materials consisting of wheat bran, rice bran, cottonseed hulls, and perilla stalks are important in improving mushroom's market value and yield (Yang *et al*., 2013; Li *et al*., 2017). Despite wide selective of mushroom substrates, the development of mycelia would have been undergoing an impediment as the implication of the slow growth rate of performance for the fungi to elongate the ramification on the nutritive medium is because of the unfavourable substrate exploitation such thus decrease the yield and nutritional content of mushroom (Khan *et al*., 2008). To best mention, the selection of suitable substrates with the type of mushroom cultivated is pivotal to elevate mushroom production.

A multitude of treatments is critically important during the preparation of the mushroom substrate to tackle the shortcomings potentially posed by the pest and disease attacks. The alkaline pre-treatment has offered an imperative method in the face of the increasingly high demand of mushroom commercially produced that is low cost in the establishment and familiar-opted of its application among those small businesses in the rural areas. Applying the alkaline treatment is akin to composing medium for growth with casing materials, an essential component covered on the surface had been vigorously used in hand on composting, which would be great to benefit upon their characteristics include exerting a range of neutral to alkaline pH, calcium source, a potent cationic exchange capacity, low magnesium content and low toxic trace compound (Pardo *et al*., 2010). The most common alkaline materials implemented in mushroom cultivation include zeolite, bentonite, sodium hydroxide, sodium carbonate, calcium hydroxide, and other alkaline bauxite (Awasthi *et al*., 2016; Kim *et al*., 2016). In general, the alkaline treatment was purposefully used to help in facilitating the pH value precedes in the substrate for complying under a suitable level. Likewise, the limestone application in mushroom substrates increased the pH values to 7.5, thus reducing contamination (Ritota & Manzi, 2019). The substrate treated with the insoluble calcium salts suppress the bacterial contamination (Choi *et al*., 2009). So the source of alkaline materials incorporated with this approach with non-toxic chemicals is an effective way to improve nutrient availability, taking into account alkaline materials chemical properties. Until then, the take up of the alkaline materials as an amendment to increase the nutrient absorption remains obscure.

For a while, as various materials have been adopted into the mushroom substrate, the alkaline material used as an additive in enhancing the mushroom production to achieve the acceptable outcome has been the most sought after

by many industrial players. Expect sustainable agriculture in wide of crop production, this alkaline materials were not only the best preference to utter in compensating the cost-effective technique at large scale of farming but more becoming mire to their labour intensive way without cost excessively on any operational works takes up along the process. Put another way, the efficacy of alkaline materials in assisting mushroom cultivation in inclining the yield of production and quality of mushroom produced is still unforeseen and remains at face value. However, a ground study pertaining to its beneficial uses will perhaps help navigate the local industry. Therefore, the study aimed to evaluate the different alkaline materials, including agricultural lime, zeolite and gypsum, in increasing the quality of spawn, yield and antioxidant status of grey oyster mushrooms.

## 1.1 Research Problems

Grey oyster mushroom is a variety under the genus *Pleurotus* revered as one of the economic crops in Malaysia that contribute to the national income. The mushroom industry is undoubtedly turning to hit rock bottom in the long run, and thus the cultivation technique is the most sought after. The most would have troubled mushroom cultivation, be it the mushroom contamination, from pre-harvest to post-harvest stage, had been affecting the industry in view of their net profit and financial loss. No less than that, the heavy metals pose threats against mushroom would be the other concern the locals might face. As the contamination took a count, mushroom cultivation frequently dealt with the downside in shelf life with a shorter life span.

Adding alkaline materials to mushrooms does not limit the positive effect of sterilizing mushroom substrates. However, it is a rigorous method nourishing adequate nutrients, increasing the productiveness and quality of mushrooms produced. The establishment of alkaline materials in cultivation is convenient because it is cheap, user-friendly, accessible, labour-efficient and polluted-free. This study will narrow down the application of three different alkaline materials - agricultural lime, zeolite and gypsum – as the additives *Pleurotus pulmonarius* supplemented with, and results are evaluated based on its performance.

## 1.2 General Objectives

This research aims to study the effect of alkaline materials application on mushroom spawn quality and cultivation of *Pleurotus pulmonarius* productiveness.

## 1.3 Specific Objectives

1. To determine the most suitable spawn substrate for the cultivation of grey oyster mushrooms.
2. To evaluate the performance of different alkaline materials on mushroom growth and yield.
3. To investigate the postharvest quality of grey oyster mushroom through the yield, biological efficiency and antioxidant activity at different flush cycles.

## 1.4 Scope of Study

This study is focussing on the effect of the application of alkaline materials on grey oyster mushroom reflected in mushroom spawn, growth, yield and antioxidant properties. As the study layout, it is designed into a completely randomized block design (CRD) with one factor: the treatment of alkaline materials. The study was conducted at a micro-scale, and all have been done at the Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. Following this, it could profoundly elucidate the important role of alkaline materials in mushroom cultivation and the effective substrate formulation.

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