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GROWTH RATE ENHANCEMENT OF TIGER MILK MUSHROOM USING ELECTRICAL STIMULATION TECHNIQUES

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By

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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

GROWTH RATE ENHANCEMENT OF TIGER MILK MUSHROOM USING ELECTRICAL STIMULATION TECHNIQUES

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Chair: Gorakanage Arosha Chandima Gomes, PhD
Faculty: Engineering

Slow growth rate of some varieties of mushrooms, including tiger milk mushroom, demands the application of such growth enhancement techniques including through electrical stimulation techniques. However, the only electrical technique that has been reported is through pulse electrical technique at high voltage, which is not cost effective and has many limitations in upscaling to suit commercial applications. Therefore, the objective of this study is to enhance the growth rate of tiger milk mushroom (*Lignosus rhinocerus*) by applying different electrical stimulation techniques, specifically at low electrical field strengths or high strength with feasible techniques. Mycelium of tiger milk mushroom at early developmental stage was exposed to injection of direct current, electric field and corona discharge respectively for five days, at various combinations of electric field strength, time duration and frequency of application. Results show that none of the treated mycelium exhibited growth rate acceleration upon discontinuous exposure to direct current at 0.03 A to 0.9 A, electric field at 350 V/cm from a Van de Graff generator and corona discharges at 5 kV electrical strength. Continuous exposure (five hours) to 1.1-1.3 A direct current has retarded the mycelium growth at the anode region. In contrast, discontinuous exposure to electric field for short period by a DC power supply at 30 V/cm has accelerated the mycelium growth rate up to 8%. The positive impact was declined with the reduction of electric field strength to 8.2 V/cm. The growth rate of the mycelium was successfully increased up to 10% by corona discharges through multiple needles at 5 kV for five hours continuously. Application of this technique on the mycelium at intermediate development stage for four weeks has resulted in 16% enhancement on the mycelium growth rate and up to 56% to the yield of tuber. Myco-chemical analysis on the tuber of the corona- treated group did not show a significant variation in the total flavonoid content and metabolite chromatogram pattern as compared to the control groups and the reference groups. It can be concluded that continuous application of this feasibly technique of corona discharges through multiple needles at 5 kV by using a small scale Van de Graff generator was the best electrical stimulation technique for growth rate and yield enhancement of tiger milk mushroom. This finding will help in

the development of a suitable design of the successful system to fit large scale mushroom cultivation without repeating the unsuccessful parameters.

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

PENINGKATAN KADAR PERTUMBUHAN CENDAWAN SUSU HARIMAU MENGGUNAKAN TEKNIK RANGSANGAN ELEKTRIK

Oleh

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Kadar pertumbuhan yang lambat bagi beberapa jenis cendawan, termasuk cendawan susu harimau, telah menimbulkan permintaan dalam pengaplikasian teknik-teknik yang dapat meningkatkan kadar pertumbuhan termasuk melalui teknik rangsangan elektrik. Walaubagaimanapun, satu-satunya teknik elektrik yang telah dilaporkan ialah melalui teknik elektrik pulsa pada voltan tinggi, di mana teknik ini agak mahal dan juga mempunyai pelbagai kekangan untuk diperluaskan ke tahap pengkomersilan. Maka, objektif kajian ini adalah untuk meningkatkan kadar pertumbuhan cendawan susu harimau (*Lignosus rhinocerus*) melalui aplikasi teknik ransangan elektrik yang berbeza, khususnya pada kekuatan medan elektik aras rendah, dan juga pada kekuatan medan elektik aras tinggi melalui teknik yang mudah untuk dilaksanakan. Miselium cendawan susu harimau pada peringkat pertumbuhan awal didedahkan kepada suntikan arus terus, medan elektrik dan pelepasan korona secara berasingan selama lima hari; melalui pelbagai kombinasi kekuatan medan elektrik, tempoh masa dan kekerapan pendedahan. Keputusan menunjukkan tiada miselium yang mempamerkan peningkatan kadar pertumbuhan selepas pendedahan secara berjeda kepada suntikan arus terus pada 0.03 A sehingga 0.9 A, medan elektrik pada 350 V/cm daripada penjana Van de Graff dan pelepasan korona pada kekuatan elektrik 5 kV. Pendedahan berterusan selama lima jam kepada suntikan arus terus pada amplitud 1.1-1.3 A telah merencatkan pertumbuhan miselium di kawasan anod elektrik. Pendedahan berjeda kepada medan elektrik sebanyak enam kali, selama satu minit setiap pendedahan, melalui mesin bekalan kuasa arus terus pada penetapan 30 V/cm telah mempercepatkan kadar pertumbuhan miselium sehingga 8%. Walaubagaimanapun, impak positif ini telah berkurangan dengan penurunan kekuatan medan elektrik kepada 8.2 V/cm. Kadar pertumbuhan miselium berjaya ditingkatkan sehingga 10% dengan aplikasi pelepasan korona melalui berbilang jarum pada kekuatan elektrik 5 kV selama lima jam berterusan, berbanding melalui jarum tunggal. Pengaplikasian teknik pelepasan korona ini ke atas miselium semasa peringkat perkembangan perantaraan selama empat minggu telah memberikan 16% peningkatkan kepada kadar pertumbuhan miselium dan hasil ubi cendawan. Analisis miko-kimia ke atas ubi cendawan tidak menunjukkan perbezaan ketara ke atas jumlah kandungan flavonoid dan corak kromatogram

metabolit antara kumpulan pendedahan korona, kumpulan kawalan dan juga kumpulan rujukan. Ini dapat disimpulkan bahawa pelepasan korona melalui jarum berbilang selama lima jam yang dihasilkan oleh penjana Van de Graff pada 5 kV merupakan teknik perangsangan elektrik yang terbaik bagi peningkatan kadar pertumbuhan dan hasil cendawan susu harimau. Penemuan ini akan membantu dalam pembangunan rekabentuk yang sesuai bagi sistem yang berkesan untuk digunakan di kawasan penanaman cendawan berskala besar tanpa mengulangi parameter-parameter yang tidak berjaya.

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I certify that a Thesis Examination Committee has met on 30 April 2018 to conduct the final examination of Nor Azreen binti Mohd Jamil on her thesis entitled "Growth Rate Enhancement of Tiger Milk Mushroom using Electrical Stimulation Techniques" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

Appendix **Page**

LIST OF ABBREVIATIONS

AC	alternating current
$AlCl_3$	aluminium chloride
$CaCO_3$	calcium carbonate
DC	direct current
ECC	extracted compound chromatogram
HPLC	high-performance liquid chromatography
IES	inductive energy storage
LCMS-QToF	liquid chromatography/mass spectrometry–quadrupole time of flight
$NaNO_2$	sodium nitrite
NaOH	sodium hydroxide
PDA	potato dextrose agar
QE	quercetin equivalent
RRLC	rapid resolution liquid chromatography
SPLG	small population lightning generator
TIC	total ion chromatogram
UV	ultra-violet

CHAPTER 1

INTRODUCTION

1.1 General introduction

Severe weather such as heat wave and heavy rain, and difficulty to identify the growth spot of some wild mushrooms has limited mushroom resources. Tiger milk mushroom (*Lignosus rhinocerus*) is one of well-known local mushroom that has very limited availability due the difficulty to identify it growth location as the sclerotium (tuber) remains dormant underground for years. This part of tiger milk mushroom has been proven to have various medicinal properties and have been practiced for hundreds years by medical practitioners. However, each step in mushrooms cultivation starting from development of pure mycelium culture, production of seeds, production of substrate block and until formation of fruiting body is also times consuming.

Electrical stimulation approach through pulse power technology has been mainly focused in many studies to improve the growth rate and yield of mushrooms. However, due to many limitation of this technology such as high investment cost and safety issue, application of other electrical stimulation technique should also been considered. Therefore, different electrical stimulation techniques especially at low electrical strength or at least feasible high electrical strength were conducted in this study to improve the growth rate of tiger milk mushroom, as it has been reported to cause improvement on plant growth and yield. Selection of treatment parameters by means of electrical strength, exposure time and frequency of application were based on the parameters that have been applied on various types of plants.

In summary, application of high voltage pulse power technology on mushroom growth has contributed a lot of constraints. Other feasible electrical stimulation methods at the parameters that have effectively improved plant growth were applied in this study with the focal aim to enhance the growth rate of tiger milk mushroom.

1.2 Problem statement

Slow growth rate is the main issue in the cultivation process of mushroom including tiger milk mushroom as the whole process of cultivation takes about seven months (Jamil *et al*., 2018). Electrical stimulation by pulsed power technique at very high voltage levels (50 kV to 150 kV) was reported to give positive results on the mushroom growth rate.

However, there are many limitations of using pulse power technology, especially for farmers' application. High investment cost is one of the concerns that limit the application of this technology. Cost of one unit of pulse power emitter is about 450,000 to 2,000,000 USD (Tsukamoto *et al*., 2003). A majority of the previous application of pulse power technology was specifically custom-made and are not available in the market; including inductive energy storage pulse power generator (Takaki *et al.,* 2014), small population lightning (SPLG) generator (Islam and Ohga, 2012) and automatic pulse power generator (Tsukamoto *et al*., 2005). This is another limitation of employing this technology. The SPLG was specially designed by a Toyota Company that consisted of a controller, connection cable, a high voltage generator and a wheel electrode (Takaki *et al.,* 2014). Furthermore, due to its complexity, the use of pulse power system is technically challenging. An experienced and skilled operator is required for handling Blumlein pulse power generator which has a complex switching element that requires impedance matching (Rebersek and Miclavcic, 2011).

Large space consumption and mobility issue due to huge size of pulse power generators such as Blumlein pulse power generator and Marx generator also contributes to the barriers in applying this technique in the field (Joler *et al*., 2008; Tsukamoto *et al*., 2005). Difficulty in up-scaling the system for industrial application for large scale production is another challenge of pulse power technology (Ricci *et al.,* 2017). Apart from that, safety issue is also the main concern of high voltage pulse power technology as high voltage may contributes high risk to users which then requires highly restricted area to keep the system and need special care to prevent energy leakage.

Therefore, investigation of other modes of electrical stimulation techniques especially at low voltage is an urging requirement at present. Up to date, there is no information available on the applications of other electrical stimulation techniques to enhance mushroom growth rate.

1.3 Aim and objective of study

The aim of this study is to improve the growth rate of tiger milk mushroom through electrical stimulation techniques with three specific objectives:

1) To determine a low voltage or feasible high voltage electrical stimulation technique with the best parameters for tiger milk mushroom.
2) To accelerate the growth rate of mycelium of tiger milk mushroom at different developmental stages.
3) To increase the yield of tuber of tiger milk mushroom without affecting its myco-chemical properties.

1.4 Scope of study

This study covers the process of determining the best electrical stimulation technique to enhance the growth rate of mushroom, focusing on tiger milk mushroom. Selection of techniques will be focused on the successful parameters that have been done on various types of plants, especially at low electrical strength, by using low voltage range of small scale equipment. The stimulation process will be applied only to the mycelium stage of mushroom development. The effects of electrical stimulation techniques on tiger milk mushroom will be determined through the mycelium growth diameter and migration length, weight of developed fruiting body (tuber), and also on the myco-chemical properties of the collected tuber.

1.5 Contribution of study

The finding of this study will provide a lot of information in developing a suitable design of the successful methodology to trigger mushroom growth rate and yield at large scale cultivation area without applying any unsuccessful parameters.

1.6 Research hypotheses

This study has three hypotheses, which are:

1. The growth rate of mycelium of tiger milk mushroom can be induced by low voltage or practically feasible high voltage electrical stimulation techniques.
2. Application of electrical stimulation technique at the mycelium stage of tiger milk mushroom can lead to the improvement in the tuber yield.
3. Enhancement of the mycelium growth rate by electrical stimulation technique does not cause variation in the myco-chemical properties of the tuber.

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