UNIVERSITI PUTRA MALAYSIA

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2008

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By

NOOR HAIDA BINTI SEBRAN

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

June 2008

This thesis is dedicated to my beloved family…

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

GROWTH REQUIREMENT, MASS PRODUCTION AND APPLICATION OF *Trichoderma harzianum* AS A GROWTH ENHANCER OF OIL PALM

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Chairman: **Professor Faridah Abdullah, PhD**
Faculty: **Science**

Several species of the genus *Trichoderma* were reported to be effective biological control agents of plant diseases with *Trichoderma harzianum* being one of the most documented biological control agents of various plant pathogens. Previous studies have shown that *T. harzianum* (isolate FA 1132) has good properties as a biological control agent against basal stem rot (BSR) of oil palms, caused by the bracket fungus *Ganoderma boninense*. This study found that FA 1132 grew well in both the solid (MFOP) and liquid media (PDB, POME) tested. The best carbon and nitrogen source were D-fructose and L-asparagine, respectively. The optimal growth temperature was at the ambient temperature of 28±2°C, while the optimum pH level was between pH 2.7 to pH 6.0. This study also found that 3% sucrose concentration in PDB and MFOP, and 9% molasses or jaggery in POME, gave maximum mycelial yield of FA 1132. Shake flask cultures at 12 hours/day gave higher mycelial yield of FA 1132

compared to static flask cultures, while shake flask cultures at 24 hours/day were not significantly different from 12 hours/day over 14 days of experiment. For its application in the field, *T. harzianum* has to be produced on a large scale in a suitable carrier. This isolate has to self-proliferate in a cheap and easily available organic media and within a relatively short period of time. Preliminary studies showed that wastes from the oil palm industry, namely mesocarp fibres of oil palm (MFOP) were good and practical substrates. A potent inoculant of FA 1132 was successfully prepared via submerged fermentation with an agitation speed of 1000 rpm and 50% dissolved oxygen tension level for 96 hours. The mean conidial count by this method was 7.73×10^9 conidia/ml. This conidial suspension was turned into a solid inoculum for solid substrate fermentation, at 5 kg per 50 tonnes palm press fibre waste, which were piled into windrows of 50 m dimension. Liquid palm oil mill effluent (POME) was given as a nitrogenous supplement at 16 tonnes within the first 8 weeks. The presence of *T. harzianum* was monitored every 3 weeks over a 27-week period. Results showed that the trend towards an increased *Trichoderma* population started at week 6 and reached its peak of 4.07×10^8 conidia/g at week 15, when the product was ready for packaging. Conidial counts of the product at 8 weeks after bagging in 25 kg bags was 5.10×10^8 conidia/cfu/g, indicating a slight increase in population during storage. Thus, *T. harzianum* (FA 1132) was found to sustain well during mass production and the FA 1132 inoculant was able to generate a pilot scale production of 22mt of *Trichoderma* end product per run. When the formulated FA 1132 was applied onto newly planted field palms, Treatment 2 (which is treatment applied directly into the planting hole) gave a significantly better growth performance than palms applied

with fertilizer and untreated control. Similarly, based on the summation of the growth response, T2 gave the best growth response to the treatment for every parameter. In conclusion, *T. harzianum* (FA 1132) produced good biological characteristics which were suitable to be used to upscale the propagule production of FA 1132 by submerged fermentation and mass produce it on a larger scale by solid substrate fermentation. Formulated FA 1132 was found to be a good growth enhancer of oil palms based on field trials.

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

KEPERLUAN PERTUMBUHAN, PENGHASILAN SKALA BESAR DAN APLIKASI *Trichoderma harzianum* SEBAGAI PENGGALAK PERTUMBUHAN POKOK KELAPA SAWIT

Oleh

NOOR HAIDA BINTI SEBRAN

Jun 2008

Pengerusi : Prof. Faridah Abdullah, PhD

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Beberapa spesies genus *Trichoderma* yang dilaporkan sebagai agen kawalan biologi yang efektif ke atas penyakit tumbuhan dengan *Trichoderma harzianum* dikenalpasti sebagai agen kawalan biologi untuk beberapa patogen tumbuhan. Kajian terdahulu menunjukkan *T. harzianum* (isolat FA 1132) mempunyai potensi yang baik sebagai agen kawalan biologi terhadap penyakit reput pangkal batang (RPB) pada pokok kelapa sawit yang disebabkan oleh kulat *Ganoderma boninense.* Kajian ini mendapati FA 1132 hidup dengan baik di dalam kedua-dua medium pepejal (MFOP) dan cecair (PDB, POME) yang dikaji. Sumber karbon dan nitrogen yang terbaik adalah D-fruktos dan L-asparagin. Suhu optimum pertumbuhan adalah pada suhu bilik (28±2°C) dan had pH optima adalah di antara pH 2.7 hingga pH 6.0. Kajian ini juga

mendapati 3% kepekatan sukros di dalam PDB dan MFOP, dan 9% molases atau gula merah di dalam POME, memberikan penghasilan miselia yang maksimum pada FA 1132. Kelalang kultur bergoncang selama 12 jam/hari memberikan penghasilan miselia FA 1132 yang tinggi berbanding dengan kelalang kultur statik manakala kelalang kultur bergoncang selama 24 jam/hari tidak memberikan perbezaan yang signifikan dengan 12 jam/hari selama 14 hari ujikaji. Untuk aplikasi di ladang, *T. harzianum* perlu dihasilkan dalam skala besar dengan pembawa yang sesuai. Isolat ini perlu berproliferasi dalam media organik yang murah dan dalam tempoh yang singkat. Kajian awal menunjukkan bahan buangan daripada industri kelapa sawit, serat mesokap kelapa sawit (MFOP) merupakan substrat yang baik dan praktikal. Inokulasi FA 1132 yang poten telah berjaya dihasilkan dengan kaedah fermentasi separa pepejal dengan kelajuan goncangan pada 1000 rpm dengan 50% kadar oksigen terlarut dalam masa 96 jam. Kiraan min konidia dengan menggunakan kaedah ini adalah 7.73×10^9 konidia/ml. Ampaian konidia ini berubah menjadi inokulum pepejal untuk fermentasi substrat pepejal pada 5 kg per 50 tan batas hampas kelapa sawit mampat sepanjang 50 m. Air kumbahan kelapa sawit (POME) disiramkan ke atas batas sebagai nitrogen tambahan sebanyak 16 tan selama 8 minggu yang pertama. Kehadiran *T. harzianum* dipantau setiap 3 minggu sehingga minggu ke-27. Keputusan menunjukkan peningkatan dalam populasi *Trichoderma* bermula pada minggu ke-6 dan mencapai bacaan konidia yang tertinggi iaitu 4.07×10^8 konidia/cfu/g pada minggu ke-15, di mana produk telah sedia untuk proses pembungkusan. Kiraan konidia produk pada minggu ke-8 selepas pembungkusan di dalam beg 25 kg adalah 5.10×10^8 konidia/cfu/g, menunjukkan peningkatan dalam populasi meskipun setelah

disimpan dan dibungkus. Oleh itu, semasa proses penghasilan skala besar, FA 1132 didapati stabil dan inokulum ini berjaya menghasilkan 22 mt produk akhir *Trichoderma* dalam satu sesi penghasilan produk. Apabila formulasi FA 1132 diaplikasi pada pokok yang baru di ladang, Rawatan 2 (aplikasi rawatan terus ke dalam lubang tanaman) memberikan pertumbuhan yang signifikan berbanding dengan pokok kelapa sawit dengan penggunaan baja dan yang tidak dirawat (kawalan). Rawatan 2 juga memberikan keputusan yang paling baik untuk setiap parameter di dalam respon pertumbuhan pokok kelapa sawit. Secara kesimpulannya, *T. harzianum* (FA 1132) menghasilkan ciri-ciri biologi yang baik dimana ia sesuai digunakan untuk meningkatkan penghasilan propagul FA 1132 dengan menggunakan kaedah fermentasi separa pepejal dan penghasilan skala besar dengan menggunakan kaedah fermentasi substrat pepejal. Formulasi FA 1132 didapati sebagai penggalak pertumbuhan kelapa sawit yang baik berdasarkan percubaan di ladang.

ACKNOWLEDGEMENTS

First of all, Alhamdullillah to Allah S.W.T for giving me this opportunity and strength to complete my study.

I wish to express my heartfelt thanks to Prof. Dr. Faridah Abdullah for her help and invaluable advice, encouragement and unfailing patience throughout the course of this study. Her guidance is truly appreciated and her constant guidance towards the completion of this thesis. A thank you note also goes to Prof. Dr. Arbakariya Ariff and Dr. Rosfarizan Mohamad for their advice and support.

I would like to thank my labmates, Husrita, Hefni, Zetty, Nazif and friends for their timely assistance in the laboratory. I also appreciate all the help from Mr. Christopher and Mr. Seth while at the sampling site in Sedenak, Johor.

My heartfelt thanks, gratitude and appreciation goes to my family especially my father and late mother for their endless effort in persuading me to complete this thesis and not forgetting my sister Noor Hazlin, Zurinanorhani and Noor Haryati for their support and encouragement throughout the finishing point of this thesis.

Finally, a special appreciation goes to my husband Md. Ayob Mustafa and my lovely son, Muhammad Aliff for their endless motivation, assistance, continuous encouragement and guidance during the process of completing this thesis. Thank you for everything.

I certify that an Examination Committee met on 11th June 2008 to conduct the final examination of Noor Haida binti Sebran on her Master of Science thesis entitled "Growth Requirement, Mass Production and Application of *Trichoderma harzianum* as a Growth Enhancer of Oil Palm' in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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Date:26 August 2008

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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Date : 11 September 2008

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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NOOR HAIDA BINTI SEBRAN

Date: 25 July 2008

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	:	Analysis of Variance
PDA	:	Potato Dextrose Agar
CDA	:	Czapek Dox Agar
PIRG	:	Percentage Inhibition of Radial Growth
ppf	:	palm press fibre
cfu	:	colony forming unit
g	:	gram
MFOP	:	Mesocarp Fibre of Oil Palm
POME	:	Palm Oil Mill Effluent
mL	:	millilitres
L	:	litre

cm	:	centimeter
kg	:	kilogram
C	:	celcius
rpm	:	rotation per minute

CHAPTER 1

INTRODUCTION

The last two decades saw a rapid expansion in land areas planted with oil palm in Malaysia. Oil palm planting area has increased by 2.7% from 4.05 millions hectares in 2005 to 4.16 in 2006 (Kppk-oil palm, 2007). Malaysia currently accounts for 51% of the world's palm oil production and 62% of world's exports, and therefore also for 8% and 22% of the world's total production and exports of oils and fats, respectively.

One of the constraints to maximum edible oil production is disease incidence; in Malaysia it is Basal Stem Rot (BSR) of oil palm, caused by the bracket fungus *Ganoderma boninense.* Chemical control has not been effective and sustainable, even though *in vitro* screening has identified several fungicides that were effective against *Ganoderma*, such as drazoxolone and cycloheximide (Ramasamy, 1972). Others include penconazole, tridemorph and tridemenol (Lim *et al*., 1990). One promising fungicide still in its initial stages of use is hexaconazole (Idris *et al*., 2004). Cultural practices do not guarantee a decreased disease incidence but merely delays the infection (Flood and Hassan, 2004). The demand for an alternative to chemical control of plant diseases has become stronger owing to concerns about the safety and the impact of chemicals on the environment.

The genus *Trichoderma* has many species which have been reported to be affective biological control agents of plant diseases with *T. harzianum* found to be

one of the largest biological control agents of several plant pathogens in the tropics. *In vitro* studies have shown that species of *Trichoderma* showed good antagonism against *G. boninense* (Lim and Teh, 1990). In a nursery trial, treatments using conidial suspension and a surface mulch were found to be the most successful in suppressing BSR, giving a Disease Severity Index (DSI) of 5 compared to the untreated controls which gave 75 on a scale of 0 to 100 (Abdullah *et al*., 2003). Nursery trials by Ilias (2000) found one strain of *T. harzianum* and one of *T. virens* to give equally good results in the disease suppression of basal stem rot.

Some isolates of *Trichoderma* are also good plant growth enhancers. Koppert (2001) reported that the application of *T. harzianum* to cosmos seedlings gave enhanced growth as well as conferred protection to the seedlings against certain fungal diseases. Inbar *et al*. (1994) showed that *T. harzianum* could act as a plant growth enhancer as well as control diseases of vegetable seedlings grown under commercial conditions. *T. harzianum* strains showed a positive effect on the growth of tomato transplants under field trials (Ozbay *et al*., 2004). Shivanna *et al*. (1996) isolated *Penicillium* and *Trichoderma* from the roots of zoysia grass and found many of them promoted growth of wheat and soybean under greenhouse conditions. An enhanced growth of marigold after treatment with *Trichoderma aureoviride* in combination with the mycorrhizal fungus *Glomus mosseae* was reported by Calvet *et al*. (1993).

The abundant positive results of *Trichoderma* on plant growth and disease control has made it necessary to test the locally-isolated fungus under field conditions in