



UNIVERSITI PUTRA MALAYSIA

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

NOOR HAIDA BINTI SEBRAN

FS 2008 28



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**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA
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

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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 $28\pm 2^{\circ}\text{C}$, 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**

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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 ($28\pm 2^{\circ}\text{C}$) 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, Alhamdulillah 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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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.

NOOR HAIDA BINTI SEBRAN

Date: 25 July 2008

TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xx
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	
2.1 Importance of the Oil Palm Industry	5
2.2 Taxonomy and Morphology of <i>T. harzianum</i>	7
2.3 Efficacy of <i>T. harzianum</i> as a Biological Control Agent (BCA)	8
2.4 <i>T. harzianum</i> as a Plant Growth Enhancer	9
2.5 Mechanism of Action of <i>T. harzianum</i>	10
2.6 Commercial Production of <i>T. harzianum</i>	13
2.7 Mass Production of <i>T. harzianum</i>	14
2.8 Submerged Fermentatation (SmF)	15
2.9 Solid Substrate Fermentation (SSF)	15
2.10 Application of <i>Trichoderma</i>	16
2.11 Fungal Dynamic in Compost	18
3 MORPHOLOGICAL CHARACTERISTICS AND GROWTH REQUIREMENTS OF <i>Trichoderma harzianum</i>	
3.1 Introduction	19
3.2 Materials and Methods	
3.2.1 Source of <i>Trichoderma harzianum</i> (FA 1132)	21
3.2.2 Morphological Characteristics of FA 1132	22
3.2.3 Conidial Production of FA 1132 on Agar Culture	22
3.2.4 Conidia Production using Rolling Bottle Culture	23
3.2.5 Effect of Inorganic Carbon and Nitrogen Compounds on Growth of FA 1132	24
3.2.6 Effect of Temperature and pH on Growth of FA 1132	25
3.2.7 Growth of FA 1132 in Liquid Media	26



3.2.8	Effect of Shake Flask <i>versus</i> Static Flask Cultures	27
3.2.9	Growth of FA 1132 in Solid Substrate Media	28
3.2.10	Statistical Analysis	28
3.3	Results	
3.3.1	Colony and Microscopic Characteristics of FA 1132	29
3.3.2	Conidia and Chlamyospore Counts	30
3.3.3	Effect of Carbon and Nitrogen on Growth of FA 1132	32
3.3.4	Effect of Temperature and pH	33
3.3.5	Growth of FA 1132 in Liquid Media	35
3.3.6	Shake Flask <i>versus</i> Static Flask Cultures	36
3.3.7	Growth of FA 1132 in Solid Substrate Media	37
3.4	Discussion	38
4	MASS PRODUCTION OF <i>Trichoderma harzianum</i>	
4.1	Introduction	43
4.2	Materials and Methods	
4.2.1	SmF : Preparation of FA 1132	46
4.2.2	Medium Composition	46
4.2.3	Fermenter	46
	Variations of Agitation Speed and DOT Level	48
4.2.4	Preparation of FA 1132 Inoculant	49
4.2.5	On-site Solid Substrate Fermentation	50
4.2.6	Statistical Analysis	53
4.2.7	Viability Test by Dual Culture Technique	53
4.2.8	Fungal Dynamics in Composting Windrow	55
	Dilution Plating Method	55
	Warcup's Alcohol Treatment Method	56
4.3	Results	
4.3.1	Submerged Fermentation: Effect of Agitation Speed	58
4.3.2	Submerged Fermentation : Effect of DOT Level	59
4.3.3	Conidia Production of FA 1132 in Palm Press Fibres	59
4.3.4	Large Scale Solid Substrate Fermentation	60
4.3.5	Viability Test by Dual Culture Technique	67
4.3.6	Fungal Dynamics in Composting Windrow	68
4.4	Discussion	90
4	A FIELD TRIAL ON THE APPLICATION OF <i>Trichoderma</i> FORMULATED PRODUCT AS A GROWTH ENHANCER OF OIL PALMS	
5.1	Introduction	94
5.2	Materials and Methods	
5.2.1	Experimental Design of Field Plot	97
5.2.2	Treatments	97



5.2.2	Growth Assessments	98
	Chlorophyll Content Analysis (ml/g) (Arnon, 1949)	99
5.2.3	NPK Content in the Formulated FA 1132 and Fertilizers	100
5.2.4	Overall Growth Response	100
5.2.5	Statistical Analysis	100
5.3	Results	
5.3.1	NPK Content in the Formulated FA 1132 and Fertilizers	101
5.3.2	Growth Assessments:	
	Girth Perimeter (cm)	102
	FronD Number Per Palm	102
	Longest Oil Palm FronD (cm)	102
	Number of Oil Palm Pinnae Per Longest FronD	102
	Chlorophyll Content Analysis (ml/g)	103
5.3.3	Overall Growth Response	106
5.4	Discussion	107
6	GENERAL DISCUSSION AND CONCLUSION	111
	REFERENCES	116
	APPENDICES	132
	BIODATA OF STUDENT	147
	LIST OF PUBLICATION	147



LIST OF TABLES

Table		Page
1	Production of conidia and chlamydo spores of <i>T. harzianum</i> on agar.	31
2	Production of conidia and chlamydo spores of <i>T. harzianum</i> (FA 1132) by rolling bottle technique.	32
3	Mycelial dry weight (g) of FA 1132 at different carbon sources.	33
4	Mycelial dry weight (g) of FA 1132 at different nitrogen sources.	33
5	Radial growth rate (mm/day) and conidia production of <i>T. harzianum</i> at different temperatures at 8 days growth.	34
6	Mycelial dry weight (g) of <i>T. harzianum</i> at different pH readings.	34
7	Mycelial dry weight (g) of <i>T. harzianum</i> at different sucrose concentrations.	35
8	Mycelial dry weight (g) of FA 1132 at different molasses and jaggery concentration.	36
9	Mycelial dry weight (g) of <i>T. harzianum</i> in PDB on static vs shake cultures at 25, 50, 75 and 100 rpm for 14 days.	36
10	Mycelial dry weight (g) of FA 1132 on static and shake cultures for 14 days.	37
11	Mycelial dry weight (g) of FA 1132 on static and shake cultures.	37
12	Conidia production of FA 1132 ($\times 10^{12}$ conidia/g) at different sucrose concentration.	38
13	Treatments in solid substrate fermentation.	52
14	Conidia production of FA 1132 in palm press fibres.	60
15	Mean reading of conidia count at 10^7 cfu/g of FA 1132 for each treatment over 27 weeks.	61



16	Mean reading of moisture content for each treatment over 27 weeks.	64
17	Mean reading of pH for each treatment over 27 weeks.	65
18	Mean reading of temperature for each treatment over 27 weeks.	66
19	Percentage of radial inhibition (PIRG) and colony overgrowth by FA 1132 by certain weeks.	67
20	Distribution of fungal species at 28±2°C and 35°C from different isolation methods in composting windrow for Treatment 1 (T1).	86
21	Distribution of fungal species at 28±2°C and 35°C from different isolation methods in composting windrow for Treatment 2 (T2).	87
22	Distribution of fungal species at 28±2°C and 35°C from different isolation methods in composting windrow for Control (C).	87
23	Number of fungal species on different weeks for Treatment 1 (T1).	88
24	Number of fungal species on different weeks for Treatment 2 (T2).	89
25	Number of fungal species on different weeks for Control (C).	89
26	Percentage of NPK content in the formulated FA 1132.	101
27	Percentage of NPK content in the fertilizers.	101
28	Summation of overall growth response.	106



LIST OF FIGURES

Figure		Page
1	Rolling bottle culture.	24
2	Surface (left) and undersurface (right) of colony culture of <i>T. harzianum</i> (FA 1132) at 8 days growth on PDA.	29
3	Microscopic characteristics of <i>T. harzianum</i> (FA 1132) showing (A) conidiophore, (B) phialide, (C) conidia and (D) hyphae.	30
4	Conidia and chlamydo spores production of <i>T. harzianum</i> on PDA at 7 to 10 days after culture.	31
5	(A) Conidia and (B) Chlamydo spore of FA 1132.	32
6	2L Bench-Top Stirred Tank Fermenter (B. Braun model 'Biostat ® B' 2L version 1.0) with tubes connecting to control unit and bottles.	47
7	Sampling conidia by using syringe and released to a universal bottle.	49
8	Fully colonized in palm press fibres at 4 weeks after inoculation with FA 1132.	50
9	One windrow of 50 m.t lignocellulosic wastes before treatment. MFOP windrow at 6 th weeks after treatment.	51
10	Application of FA 1132 inoculant mixed into 4 m.t POME and sprayed onto the windrow.	51
11	Propagule production of FA 1132 at different agitation speeds.	58
12	Propagule production of FA 1132 at different percentage of DOT levels.	59
13	Mean reading of cfu/g soil of FA 1132 between Week 0 to Week 27.	62
14	Dual culture technique.	68
15	<i>A. fumigatus</i>	69
16	<i>A. niger</i>	70



17	<i>A. flavus</i>	71
18	<i>A. glaucus</i>	72
19	<i>A. candidus</i>	73
20	<i>A. wentii</i>	74
21	<i>T. harzianum</i>	75
22	<i>P. expansum</i>	76
23	<i>P. islandicum</i>	77
24	<i>P. helicum</i>	78
25	<i>P. purpurogenum</i>	79
26	<i>P. luteum</i>	80
27	<i>P. rotundum</i>	81
28	<i>Allescheria sp.</i>	82
29	<i>Paecilomyces sp.</i>	83
30	<i>Mucor sp.</i>	84
31	Mean reading of girth perimeter in cm over 24 weeks.	103
32	Mean reading of frond number of the oil palm over 24 weeks.	104
33	Mean reading of the longest oil palm frond over 24 weeks.	104
34	Mean number of oil palm pinnae per longest frond over 24 weeks.	105
35	Mean reading of chlorophyll content over 24 weeks.	105
36	Summation of overall growth response based on 5 growth parameters.	107

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 tridemol (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