



**UNIVERSITI PUTRA MALAYSIA**

***INDUCING HOST RESISTANCE AGAINST FUSARIUM WILT OF  
BANANA THROUGH APPLICATION OF CHITOSAN***

**MOHAMMED YAHYA ABDULLAH AL HETAR**

**FP 2013 10**



**INDUCING HOST RESISTANCE AGAINST FUSARIUM WILT OF  
BANANA THROUGH APPLICATION OF CHITOSAN**

**By**

**MOHAMMED YAHYA ABDULLAH AL HETAR**

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

**May 2013**

## **COPYRIGHT**

All material contained within the thesis, including without limitation text, logos, icons, photographs, and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright© Universiti Putra Malaysia



بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ

﴿ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَىٰ وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ  
وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ ﴾

**"My Lord, enable me to be grateful for Your favor which You have bestowed upon me and upon my parents and to do righteousness of which You approve. And admit me by Your mercy into [the ranks of] Your righteous servants."**

صدق الله العظيم  
سورة النمل - الآية (19)

## DEDICATION

*Dedicated to my beloved parents*

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements of Doctor of Philosophy

**INDUCING HOST RESISTANCE AGAINST FUSARIUM WILT OF BANANA THROUGH APPLICATION OF CHITOSAN**

By

**MOHAMMED YAHYA ABDULLAH AL HETAR**

**May 2013**

**Chairman: Associate Professor Zainal Abidin Mior Ahmad, PhD**

**Faculty: Agriculture**

This study was undertaken with the aim to evaluate the effects of chitosan on the activation of defense related compounds to increase tolerance to *Fusarium oxysporum* f. sp. *cubense* Race 4 (FocR4) in susceptible banana seedlings var Berangan. In the *in vitro* studies, chitosan reduced the hyphal growth of FocR4 on Potato Dextrose Agar (PDA) medium and gave maximum inhibition of 76.36 % at 8 mg/ml. Inhibition of fungal growth was more efficient in Potato Dextrose Broth (PDB) where complete inhibition of mycelial growth of FocR4 was accomplished at all concentrations tested. Chitosan inhibited the sporulation of FocR4 by a maximum of 96.53 % at 8 mg/ml chitosan and 100% inhibition of spore germination was recorded at all concentrations tested. Chitosan was also found to induce morphological and cytological changes in FocR4 characterized by agglomeration of hyphae, abnormal shapes, and formation of vesicles or empty cells devoid of cytoplasm in the mycelia. Chitosan sprayed at concentrations of more than 0.3 mg/ml was found to cause damage to banana leaves. There was no significant difference in photosynthesis parameters including photosynthetic rates, stomatal

conductance and chlorophyll contents within four days after spraying with chitosan at 0.3 mg/ml. However, chitosan was found to increase significantly the activities of peroxidase (PO), phenylalanine ammonia lyases (PAL), lignothioglycolic acid (LTGA), chitinases and glucanase enzymes in banana roots. The occurrence of induced resistance enhanced tolerance of the seedlings to Fusarium wilt based on parameters such as delay in onset of foliar disease symptoms, lower in percentage of disease severity (DS), lower in percentage of disease incidence (DI), area under disease progress curve (AUDPC), and epidemic rate. Vegetative growth were measured based on increased in plant height, number of leaves, diameter of pseudostem, root and shoot dry mass showing significant improvement only between treatments challenged by inoculation with FocR4 (T1 and T2). The growth of banana plants was not influenced by chitosan itself as a growth stimulator but it enhanced the growth in those infected with FocR4. The increase in PO, PAL, LTGA, chitinase and  $\beta$ -1,3-glucanase activity in seedlings treated with chitosan had been shown to be associated with plant defense through the decrease percentage of disease severity, disease incidence and area under disease progress curve. This indicated effective role by chitosan in controlling banana seedlings against wilt disease caused by FocR4. It showed promise as an inducer in the activation of host defense systems and increasing tolerance to FocR4 infection in susceptible banana var Berangan. Further studies regarding application frequency and combination techniques are essential to more effective of control this disease.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KETAHANAN RANGSANGAN TERHADAP LAYU FUSARIUM PISANG  
MELALUI PERMOHANAN KITOSAN**

Oleh

**MOHAMMED YAHYA ABDULLAH AL HETAR**

**Mei 2013**

**Pengerusi: Profesor Madya Zainal Abidin Mior Ahmad, PhD**

**Fakulti: Pertanian**

Kajian ini telah dijalankan dengan tujuan untuk menilai kesan kitosan dalam pengaktifan sebatian berkaitan pertahanan untuk meningkatkan toleransi kepada *Fusarium oxysporum* f. sp. *cubense* Race 4 (FocR4) dalam benih pisang rentan var Berangan. Kitosan mengurangkan pertumbuhan hifa FocR4 atas medium PDA dan memberi perencatan maksimum 76.36% pada 8 mg/ml. Kitosan adalah lebih berkesan dalam PDB di mana ia menghalang sepenuhnya pertumbuhan miselium FocR4 pada semua kepekatan yang diuji. Kitosan menghalang pensporulan FocR4 di tahap maksimum 96.53% pada 8 mg/ml kitosan dan perencatan 100% untuk percambahan spora telah direkodkan pada semua kepekatan yang diuji. Kitosan juga telah didapati merangsang perubahan morfologi dan sitology pada FocR4 melalui ciri pengumpulan hifa, bentuk yang tidak normal, pembentukan vesikel atau sel-sel kosong tanpa sitoplasma dalam miselia. Kitosan disembur pada kepekatan melebihi lebih daripada 0.3 mg/ml menyebabkan kerosakan pada pisang. Tiada perbezaan yang bererti dalam parameter fotosintesis termasuk kadar fotosintesis, penyaluran stomata dan kandungan klorofil dalam masa empat hari selepas disembur dengan



kitosan pada 0.3 mg/ml. Walau bagaimanapun, kitosan telah didapati meningkatkan dengan bererti aktiviti peroksidase, fenilalanine ammonia liase, asid lignithioglicolic, kitinases dan glucanases dalam akar pisang. Pengeluaran kompaun teraruh meningkatkan ketahanan benih pisang terhadap layu Fusarium berdasarkan kepada parameter seperti kelewatan dalam kemunculan symptom, lebih rendah di peratusan keterukan penyakit (DS), lebih rendah dalam peratusan kejadian penyakit (DI), kawasan di bawah kemajuan penyakit keluk (AUDPC), dan kadar wabak. Tumbesaran tampang diukur berdasarkan ketinggian pokok daun, garispusat batang pseudo, jisim kering akar dan pucuk yang menunjukkan pembaikan hanya antara rawatan yang disuntik cabar dengan FocR4 (T1 dan T2). Tumbesaran tumbuhan pisang tidak dipengaruhi oleh kitosan sendiri sebagai perangsang pertumbuhan tetapi turut meningkatkan tumbesaran tumbuhan yang dijangkiti oleh FocR4. Peningkatan PO, PAL, LTGA, kitinase dan aktiviti  $\beta$ -1,3-glucanase dalam benih dirawat dengan kitosan telah ditunjukkan berkait dengan pertahanan tumbuhan melalui pengurangan peratusan keterukan penyakit, kejadian penyakit dan keluasan di bawah keluk kemajuan penyakit. Ini menunjukkan kesan efektif oleh kitosan dalam pengawalan anak benih pisang terhadap penyakit layu oleh FocR4. Ia menunjukkan kebaikannya sebagai perangsang dalam pengaktifan sistem pertahanan perumah dan peningkatan toleransi kepada jangkitan FocR4 pada pisang var Berangan yang rentan. Kajian lanjut mengenai kekerapan dan teknik gabungan adalah penting untuk lebih berkesan untuk mengawal penyakit ini.

## ACKNOWLEDGEMENTS

### **In the name of Allah, the Beneficent and the Merciful**

Alhamdulillah, all praises to Allah SWT for the strengths, His blessing and His permission to complete this thesis. Sincere thank and profound appreciation goes to the chairman of my supervisory committee, Assoc. Prof. Dr. Zainal Abidin Mior Ahmad, Department of Plant Protection, Faculty of Agriculture for his supervision and constant support. His invaluable help of constructive comments and suggestions throughout the experimental and thesis works have contributed to the success of this research. My sincere appreciation and grateful thanks also to Prof. Dr. Sariah Meon, my co-supervisor for her efforts, support, invaluable advice and intellectual guidance as well as for her patience in the conduct of my research and in the preparation of this thesis. Special thanks to Dr. Wong Mui Yun as a member of my supervisory committee for her help, critical comments and suggestions during the course of this project. My exceedingly grateful the staff members of Plant Diseases and Microbiology laboratories, Department of Plant Protection in particular Mr. Shamsudin Bujang, Mr. Nazri Abd. Rahman, Mr. Johari Sarikat, Mrs Asmalina Abu Bakar, Mr. Mohammed Yusof and Mrs. Junainah for their cooperation in carrying out this project.

Thanks are also extended to Prof. Dr. Abdelbasset EL Hadrami from University of Manitoba (Canada), Prof. Dr. Lee Hadwiger from Washington State University (USA), Dr. Silvia Bautista-Banos from National Polytechnic Institute (Mexico) and Leila Er rachiq from Bioersity International (France) for their help by sending of some of scientific papers and publications that's were not available on the network.

Sincere thanks to all my friends especially Dr. Monther Tahat, Dr. Abdul Raqeeb Al Eeryani, Low Ying Chiang , Bushra Subair, Elias Sadeq, Sadeq Al Sheraji, Fouad Hassan, Dr. Nabil Al Mekhlafi, Dr. Yasmeen Siddiqui, Michelle Chee Yea, Yuvarani Naidu, Elya Masya and others whom not listed along for their generous support and encouragement.

My deepest appreciation and gratitude goes to my beloved parents, I pray to Allah to forgive them, who guided and supported me to achieve the highest level of education. I also wish to express my sincere gratitude to my sisters, brothers and my wife for their endless love, encouragement, support and prayers.

I certify that a Thesis Examination Committee has met on 17 May 2013 to conduct the final examination of Mohammed Yahya Abdullah Al Hetar on his thesis entitled "Inducing Host Resistance Against Fusarium Wilt of Banana Through Application of Chitosan" 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 Doctor of Philosophy.

Members of the Examination Committee were as follows:

**Kamaruzaman Sijam, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Jugah Kadir, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

**Ganesan Vadamalai, PhD**

Senior Lecturer  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

**Richard Martin Cooper, PhD**

Professor  
University of Bath  
United Kingdom  
(External Examiner)



---

**NORITAH OMAR, PhD**  
Assoc. Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 2 August 2013

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

**Zainal Abidin Mior Ahmad, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Sariah Meon, PhD**

Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Wong Mui Yun, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

---

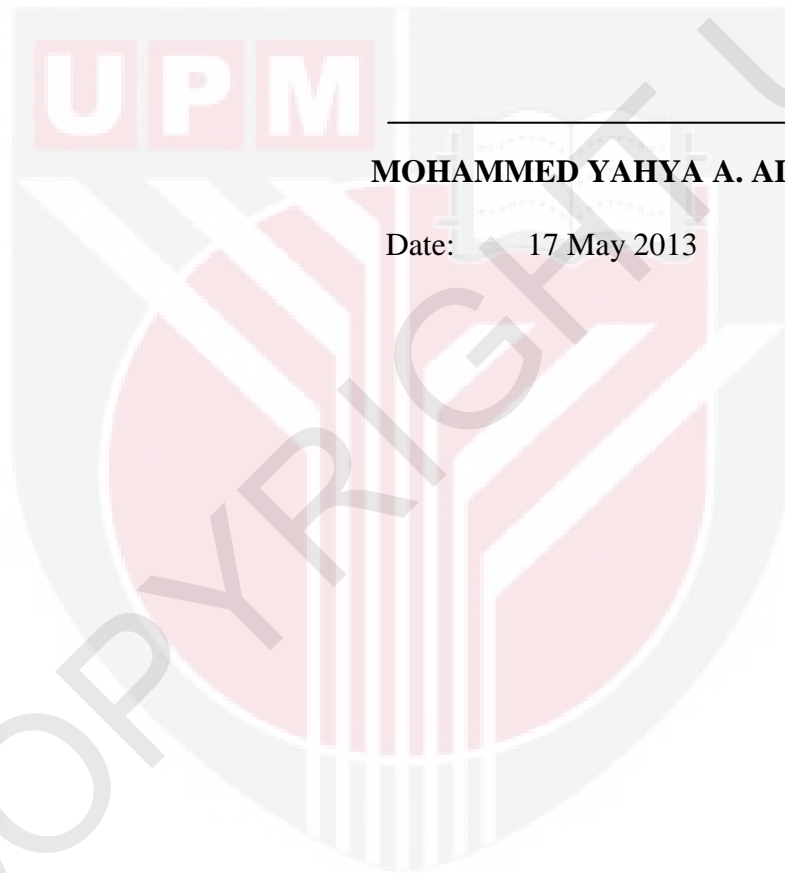
**BUJANG BIN KIM HUAT, PhD**

Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



---

**MOHAMMED YAHYA A. AL HETAR**

Date: 17 May 2013



## TABLE OF CONTENTS

	<b>Page</b>
<b>DEDICATION</b>	iii
<b>ABSTRACT</b>	iv
<b>ABSTRAK</b>	vi
<b>ACKNOWLEDGEMENTS</b>	viii
<b>APPROVAL</b>	x
<b>DECLARATION</b>	xii
<b>LIST OF TABLES</b>	xvii
<b>LIST OF FIGURES</b>	xviii
<b>LIST OF ABBREVIATIONS</b>	xx
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
<b>2 LITERATURE REVIEW</b>	<b>4</b>
2.1 Banana	4
2.2 Fusarium wilt of banana	5
2.3 Resistance against Fusarium wilt	9
2.3.1 Structural host defense	9
2.3.2 Biochemical host defense	11
2.4 Resistance inducers	13
2.4.1 Micro-organisms products	13
2.4.2 Plant extracts and plant products	15
2.4.3 Carbohydrates	17
2.4.4 Others	17
2.4.5 Commercially available products	18
2.5 Chitosan	20
2.6 Biocidal activity of chitosan	22
2.6.1 Antibacterial activity of chitosan	22
2.6.2 Antifungal activity of chitosan	23
2.7 Elicitation activity of chitosan	26
2.7.1 Signal perception	28
2.7.2 Signal transduction	29
2.7.3 Chitosan response genes	32
2.7.4 Chitosan response proteins	33
2.7.5 Secondary metabolites accumulation response	37
2.7.6 Structural response	39
2.7.7 Post harvest response	41
2.8 Effect of chitosan on Plant Diseases Control	42
2.8.1 Bacterial diseases	42
2.8.2 Virus diseases	43
2.8.3 Fungal diseases	45
2.8.3.1 Role as seed treatment agent	45
2.8.3.2 Role as a foliar treatment agent	49

	2.8.3.3	Role as a soil amendment	53
3		<b>IN VITRO ANTIFUNGAL ACTIVITY OF CHITOSAN AGAINST <i>FUSARIUM OXYSPORUM</i> F. SP. <i>CUBENSE</i></b>	54
	3.1	Introduction	54
	3.2	Materials and Methods	55
	3.2.1	Preparation of chitosan	55
	3.2.2	Pathogen and culture	56
	3.2.3	Effects of chitosan on mycelial radial growth	56
	3.2.4	Effects of chitosan on sporulation	57
	3.2.5	Effects of chitosan on spore germination	58
	3.2.6	Effects of chitosan on mycelial growth in broth culture	58
	3.2.7	Assessment by light microscopy	59
	3.2.8	Experimental design and statistical analysis	60
	3.3	Results	60
	3.3.1	Effects of chitosan on mycelial radial growth	60
	3.3.2	Effects of chitosan on sporulation	64
	3.3.3	Effects of chitosan on spore germination	65
	3.3.4	Effects of chitosan on mycelial growth in broth culture	66
	3.3.5	Assessment by light microscopy	66
	3.4	Discussion	68
4		<b>EFFECT OF CHITOSAN ON PHYSIOLOGICAL AND GROWTH OF BANANA SEEDLINGS</b>	75
	4.1	Introduction	75
	4.2	Materials and Methods	76
	4.2.1	Plant Culture and Chitosan Application	76
	4.2.2	Determine a safe chitosan concentration	77
	4.2.3	Effect of the suitable concentration of chitosan on growth and physiological of banana seedlings	77
	4.2.3.1	Net Photosynthesis	78
	4.2.3.2	Stomatal conductance	78
	4.2.3.3	Chlorophyll content	78
	4.2.3.4	Growth Responses	79
	4.3	Results	81
	4.3.1	Effect of different concentration of chitosan on banana seedlings	81
	4.3.2	Effect of chitosan on physiology of banana seedlings	83
	4.3.3	Effect of chitosan on growth of banana seedlings	85
	4.4	Discussion	86
5		<b>EFFECT OF CHITOSAN ON TOTAL PRODUCTION OF INDUCIBLE COMPOUNDS ASSOCIATED WITH INDUCED RESISTANCE IN BANANA SEEDLINGS</b>	89
	5.1	Introduction	89
	5.2	Materials and Methods	91
	5.2.1	Plant culture and chitosan application	91



5.2.2	Effect of chitosan on biochemical content of banana seedlings	91
5.2.2.1	Protein assay	91
5.2.2.2	Peroxidase assay	92
5.2.2.3	Phenylalanine ammonialyase assay	92
5.2.2.4	Chitinase activity assay	93
5.2.2.5	$\beta$ -1,3-Glucanase activity assay	94
5.2.7.2	Statistical Analysis	94
5.3	Results	95
5.4	Discussion	99
<b>6</b>	<b>THE EFFICACY OF CHITOSAN IN SUPPRESSING FUSARIUM WILT INCIDENCE IN BANANAS</b>	<b>104</b>
6.1	Introduction	104
6.2	Materials and Methods	105
6.2.1	Experimental Design	105
6.2.2	Inoculum Preparation	106
6.2.3	Plant Materials and Treatments	106
6.2.4	Disease Assessment:	107
6.2.5	Effect of chitosan on production of inducible compounds associated with host defence mechanisms	111
6.2.5.1	Protein assay	112
6.2.5.2	Peroxidase assay	112
6.2.5.3	Phenylalanine ammonialyase assay	112
6.2.5.4	Lignin assay	112
6.2.5.5	Chitinase activity assay	113
6.2.5.6	$\beta$ -1,3-Glucanase activity assay	113
6.2.5.7	Histological response	113
6.2.6	Growth assessment	115
6.3	Results	116
6.3.1	Disease Assessment	116
6.3.2	Effect of chitosan on production of inducible compounds associated with host defence mechanisms	119
6.3.3	Plant growth and vigour assessment	126
6.4	Discussion	130
<b>7</b>	<b>GENERAL DISCUSSION AND CONCLUSION</b>	<b>139</b>
	<b>REFERENCES</b>	<b>145</b>
	<b>APPENDICES</b>	<b>183</b>
	<b>BIODATA OF STUDENT</b>	<b>198</b>
	<b>LIST OF BUBLICATIONS</b>	<b>199</b>