UNIVERSITI PUTRA MALAYSIA

INDUCTION OF SUPPRESSIVE SOIL IN THE MANAGEMENT OF FUSARIUM WILT ON BANANA SEEDLINGS

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INDUCTION OF SUPPRESSIVE SOIL IN THE MANAGEMENT OF *FUSARIUM* WILT ON BANANA SEEDLINGS

By

ADELINE TING SU YIEN

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Agricultural Science in the Faculty of Agriculture Universiti Putra Malaysia

April 2001

For my Beloved ones:

Pa, Ma, Eve, Jarrod,
and, Steve;

"Yesterday, it was a wish,

Today, it is a meaningful wonder,

Tomorrow, it will be an inspiration,

and always will be."

Thank You for Everything.

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

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Chairman: **Professor Sariah Meon, Ph.D.**

Faculty: **Agriculture**

This study determined the potential of using artificially 'induced' suppressive soil to suppress the development of *Fusarium* wilt on susceptible banana seedlings (cultivar Berangan). *Trichoderma harzianum* (UPM 40) was selected as the microbial antagonist, and calcium nitrate ($Ca(NO_3)_2$) as the soil amendment. Both biotic and abiotic components, respectively, were incorporated into the soil to mimic the contents of naturally existing *Fusarium* suppressive soils. The potential of *T. harzianum* as a biocontrol agent was confirmed from the series of antagonism tests, with positive results in lysis, antibiosis and mycoparasitism tests. *In vitro* tests determined that *T. harzianum* required early establishment prior to challenge with *Fusarium oxysporum* f. sp. *cubense* race 4 (FocR4), to ensure effective antagonistic activity. Both *T. harzianum* and FocR4 tolerated pH 5-8, and Ca^{2+} concentrations within 5–750 ppm. Soil pH was not affected by $Ca(NO_3)_2$ application, indicating

compatibility of inoculating *T. harzianum* together with $Ca(NO_3)_2$ application When tested on Berangan seedlings in the glasshouse, treatment with $Ca(NO_3)_2$ alone provided better disease suppression compared to treatment with both *T. harzianum* and $Ca(NO_3)_2$, and treatment with *T. harzianum* alone. Treatment with $Ca(NO_3)_2$ alone recorded low disease incidence (DI) of 51% as compared to 59% and 69% from combined treatments and *T. harzianum* alone, respectively, 8 weeks after inoculation. Calcium reduced the population of FocR4, promoted plant growth, and induced host resistance through increased peroxidase and polyphenoloxidase activity, and phenol content. Increased in enzymatic activities and phenol content was related to extensive cell wall lignification as revealed by histological observations, resulting in resistance to FocR4 hyphal penetration. The formation of Ca-pectate also contributed to host resistance. Biocontrol efficiency of *T. harzianum* was dependent on soil environment, as the glasshouse trial did not suppress disease incidence, contrary to its antagonistic effect in *in vitro* tests. *T. harzianum* did not induce host resistance, instead, predisposed the seedlings to infection by increasing root growth and infection sites. Disease suppression achieved through treatment with $Ca(NO_3)_2$ was dependent on Ca^{2+} availability in the soil and Ca^{2+} content in the plant tissues. A more frequent application using suitable rates is then suggested as follow-up studies.

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

INDUKSI TANAH PENINDAS DALAM PENGURUSAN PENYAKIT LAYU *FUSARIUM* PADA ANAK POKOK PISANG

Oleh

ADELINE TING SU YIEN

April 2001

Pengerusi: **Profesor Sariah Meon, Ph.D.**

Fakulti: **Pertanian**

Kajian ini menentukan potensi menggunakan tanah penindas 'buatan' untuk menindas kejadian penyakit layu *Fusarium* pada anak pokok pisang yang peka (kultivar Berangan). *Trichoderma harzianum* (UPM 40) dipilih sebagai mikrobial antagonistik, manakala kalsium nitrat ($Ca(NO_3)_2$) dipilih sebagai bahan pemulih tanah. Kedua-dua komponen biotik dan abiotik ini ditambah ke dalam tanah untuk meniru komposisi sebenar tanah penindas *Fusarium* yang sediakala. Potensi menggunakan *T. harzianum* sebagai agen kawalan biologi terbukti dari siri ujian antagonistik yang dijalankan, dengan keputusan positif dalam ujian lisis, antibiosis dan parasitism Ujian *in vitro* telah mengesahkan pentingnya *T. harzianum* diinokulat lebih awal sebelum didedahkan kepada *Fusarium oxysporum* f. sp. *cubense* ras 4 (FocR4), supaya efisiensi aktiviti antagonistiknya terjamin. *T. harzianum* dan FocR4 diperhatikan mempunyai toleransi terhadap pH 5-8, serta kepekatan Ca^{2+} 5-750 bsj. Aplikasi $Ca(NO_3)_2$ tidak mempengaruhi pH tanah, sekaligus membuktikan

kesesuaian menginokulat *T. harzianum* bersama rawatan $Ca(NO_3)_2$. Apabila diuji pada anak pokok pisang Berangan, rawatan dengan $Ca(NO_3)_2$ sahaja menunjukkan potensi paling baik untuk menindas insiden penyakit *Fusarium*, berbanding rawatan dengan kedua-dua *T. harzianum* dan $Ca(NO_3)_2$, dan rawatan menggunakan *T. harzianum* sahaja. Rawatan dengan $Ca(NO_3)_2$, mencatatkan insiden penyakit yang rendah sebanyak 51% berbanding 59% dan 69% oleh rawatan kombinasi dan *T. harzianum* sahaja, 8 minggu selepas rawatan. Kalsium juga mengurangkan populasi FocR4 dalam tanah, mengalakkan pertumbuhan anak pokok pisang, dan mengalakkan sistem pertahanan teraruh melalui peningkatan aktiviti enzim *peroxidase, polyphenoloxidase* dan kandungan fenol. Peningkatan aktiviti enzim ini dikaitkan dengan lignifikasi pada dinding sel sebagaimana yang diperhatikan dari ujian histologi, yang meningkatkan keresistanan kepada penembusan hifa FocR4. Pembentukan Ca-pektat dalam sel juga memperkukuhkan lagi ketahanan hos terhadap serangan penyakit. Efisiensi *T. harzianum* sebagai agen kawalan biologi terhadap FocR4 dipengaruhi oleh keadaan tanah, kerana ujian di rumah kaca membuktikan *T. harzianum* tidak menindas insiden penyakit *Fusarium*, yang berlawanan dengan keputusan dari ujian *in vitro*. *T. harzianum* juga tidak mengalakkan ketahanan teraruh, malah mengalakkan kejadian penyakit melalui pertumbuhan akar dan tapak jangkitan. Kesan penindasan penyakit dipengaruhi oleh kedapatan Ca^{2+} dalam tanah dan kandungan Ca^{2+} dalam tisu tumbuhan. Dengan itu, kawalan yang lebih berkesan dijangka dicapai jika kekerapan aplikasinya ditambah, dengan menggunakan kadar yang sesuai, dan penentuan ini memerlukan kajian yang selanjutnya.

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To my dearest Pa, Ma, Eve and Jarrod, thank you for your love, support and encouragement. And, for my dear Steve, thank you for always inspiring, and putting colours into my life.

I certify that an Examination Committee met on 19th April 2001 to conduct the final examination of Adeline Ting Su Yien on her Master of Agricultural Science thesis entitled "Induction of Suppressive Soil in the Management of *Fusarium* Wilt on Banana Seedlings" 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: 04 MAY 2001

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Agricultural Science.

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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.

ADELINE TING SU YIEN

Date: 03.05.2001.

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LIST OF ABBREVIATIONS

DOA	Department of Agriculture
FAMA	Federal Agriculture Marketing Authority (FAMA)
FAO	Food and Agriculture Organization
INIBAP	International Network for the Improvement of Banana and Plantain
MARDI	Malaysian Agricultural Research and Development Institute
SIRIM	Standards and Industrial Research Institute of Malaysia

CHAPTER I

INTRODUCTION

The banana industry is a growing fruit industry in most countries worldwide, due to its increasing market demand and relatively low production costs. Most of the bananas produced are mainly for fresh consumption, eaten as dessert fruit or, as staple food because of its high starch content (Valmayor, 1987). Bananas are also commonly used in beer brewing, in vinegar production, in confectioneries to flavour cakes, puddings and muffins, and also as fibre material, wrappers or vegetables (Thurston, 1984; Valmayor, 1987).

As one of the most important fruit crops in many agricultural countries, bananas are produced extensively in Asia (India, Philippines, Thailand, Indonesia, Taiwan), Africa and, South and Central America (Honduras, Panama, Costa Rica, Guatemala) (Hassan and Pantastico, 1990). In Malaysia, it is the second most important fruit crop, accounting for 20 % of the total hectarage of fruit plantations (Yaacob, 1991). However, land used for banana cultivation has declined over the years from 40 000 ha in 1993 to 39 000 ha in 2000 (Loh, 2000).

The trading of banana grew significantly in the early 1870's, beginning with the trading of the Gros Michel (AAA) variety (Ploetz, 1994). This first commercially cultivated variety was considered as an "ideal variety" because of its large fruit, smooth skin texture, and cream coloured flesh that is moderately firm, slightly aromatic and sweet. Most importantly, it has excellent keeping quality and produces high yields (Hassan and Pantastico, 1990). Thus, the Gros Michel variety was extensively cultivated in new plantations, or in plantations of another banana variety known as the Silk variety (AAB) (Snyder and Smith, 1981).

The emergence of *Fusarium* wilt disease (Panama disease), in the late 1890's, threatened to diminish the banana industry. This disease caused severe losses in fruit yield, and death of plants. In just over 50 years since its first occurrence, it has destroyed more than 40 000 ha of banana plantations in Central and South America (Su et al., 1986).

Initially, only race 1 of the pathogen, *Fusarium oxysporum* f. sp. *cubense* (FocR1) was identified to be pathogenic towards Gros Michel and Silk varieties (Snyder and Smith, 1981). Race 2 (FocR2) and 3 (FocR3) only infect plantain (cooking) varieties, like Bluggoe (ABB), and *Heliconia* spp., respectively (Su et al , 1986).

Most of the Gros Michel plantations infested with FocR1 then, were successfully replanted with the resistant Cavendish variety. However, resistance to disease development soon vanished with the emergence of race 4 (FocR4), which caused destruction in the Cavendish plantations.

FocR4 spread rapidly and vastly. By 1955, banana varieties of Williams (AAA 'Cavendish') in Australia, have succumbed to FocR4 (Ploetz, 1994). In 1974, Cavendish varieties in the Philippines were severely infected (Snyder and Smith, 1981), and by 1977, FocR4 were reportedly recovered from numerous soils in Taiwan and Canary Islands.

The sudden emergence of FocR4 was believed due to independent mutation that may have occurred in the different Asian regions, as suggested by Su et al. (1986). However, Snyder and Smith (1981) claimed that FocR4 was just one of the many races of Foc, which originated from the Southeast Asian region, which remained undetected, as the discovery of some races of Foc in Vietnam that was undiscovered in other parts of the world. Furthermore, banana is a native of the Indo-Malaya countries, and has long established its existence, together with its diversified pathogens.