UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF AND SCREENING FOR ANTAGONISTIC ACTIVITY OF SELECTED MICROORGANISMS AGAINST fusarium solani ON BLACK PEPPER

FRANKLIN RAGAI KUNDAT

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By

FRANKLIN RAGAI KUNDAT

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Doctor of Philosophy

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DEDICATION

To my late father Julian Nyanggau Kundat, who passed away in 2013: Thank you dad, for your words of inspiration and encouragement in the pursuit of excellence.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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FRANKLIN RAGAI KUNDAT

December 2014

Chairperson : Professor Dato Nik Muhamad Ab. Majid, PhD

Faculty : Institute of Tropical Forestry and Forest Product

Due to environmental concerns of chemical fertilizers and pesticides usage, biological control is emerging as a promising alternative to fulfill the nutrient demand and disease control of black pepper. The general objective of this study was to evaluate the potential of microbes isolated from soil of a rehabilitated forest floor as biological contol agents (BCAs) in inducing root growth and suppressing the development of yellow disease caused by *Fusarium solani* on early growth of black pepper (var. Kuching). The microbial strains isolated from the soil of rehabilitated forest floor were evaluated for their *in vitro* antagonistic potential against *Fusarium* solani. Three microbial isolates, *Trichoderma atroviride* (UPM23F) showed the best performance followed by *Bacillus pumilus* (UPM38EB) and *Bacillus cereus* (UPM41EB) respectively, resulting in a significant inhibition of radial growth of *F.solani*. The highest colonization (4.77×10^4**)** of black pepper rhizoplane was achieved by *T. atroviride*. *B. pumilus* showed the highest colonization (9.01×10^4**)** in internal tissues of root tips. Their symbiotic associations with the host plants enhanced root growth as shown by increment in root length, root biomass, both fresh and dry. The study on microbial inoculants and nitrogen (N) demonstrated the ability of microbial inoculants in improving N uptake and thereby increase N use efficiency of applied chemical fertilizers. Assessment on the plant-microbe interaction showed that the microbial isolates induced production of inducible compounds such as peroxidase (PO), polyphenoloxidase (PPO), and phenylalamine ammonia lyase (PAL). The efficacies of microbial isolates were tested against *F. solani.* Two timing of inoculations were evaluated: inoculating soils at planting and inoculating soils 14 days before planting. Inoculation with *T. atroviride* (UPM23F) singly was most effective followed by inoculation with mixture of *T. atroviride* + *B. pumilus* (UPM23F+UPM38EB) based on variables such as delay in onset of symptoms, lower percentages (33.33%) in disease severity (DS) and higher percentages (83.80%) in disease reduction (DR). The extent of foliar yellowing was less severe in cuttings inoculated with microbial inoculants as compared to the control. Inoculation of soils prior to planting with microbial inoculants yielded the best results. This study has shown that the microbial isolates tested were effective against *F. solani* and have plant growth promoting properties and potential to be developed as BCAs. The understanding of biocontrol mechanisms involved in disease suppression would help

optimize their biocontrol efficiency. Therefore, further studies in relation to formulation, application frequency and techniques, are essential to maximize the potential of *T. atroviride* (UPM23F) and *B. pumilus* (UPM38EB) as BCAs against *Fusarium* yellow disease of black pepper.

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

PENCIRIAN DAN PENYARINGAN UNTUK AKTIVITI ANTAGONISTIK BAGI MIKROORGANISMA TERPILIH TERHADAP *Fusarium solani* PADA LADA HITAM

Oleh

FRANKLIN RAGAI KUNDAT

Disember 2014

Pengerusi : Professor Dato Dr. Nik Muhamad Ab. Majid, PhD

Fakulti : Institut Perhutanan Tropika dan Produk Hutan

Akibat daripada kesedaran terhadap persekitaran tentang penggunaan baja kimia dan racun perosak, kawalan biologi muncul sebagai altenatif yang memenuhi keperluan nutrien dan kawalan penyakit lada hitam. Objektif am kajian ini adalah untuk mengkaji potensi mikrob yang diisolat daripada tanah hutan terpulihara, sebagai agen kawalan biologi untuk merangsang tumbesaran tanaman dan merencat perkembangan penyakit kuning yang disebabkan oleh *Fusarium solani* pada pertumbuhan awal lada hitam (var. Kuching). Strain-strain mikrob yang telah diisolat daripada tanah hutan terpulihara dikaji secara *in vitro* untuk potensi antagonistiknya terhadap *Fusarium solani*. Tiga isolat mikrob tersebut, *Trichoderma atroviride* (UPM23F) menunjukkan prestasi terbaik diikuti oleh *Bacillus pumilus* (UPM38EB) dan *Bacillus cereus* (UPM41EB), menghasilkan perencatan yang beerti terhadap pertumbuhan radial *F.solani*. Kolonisasi tertinggi (4.77×10^4**)** pada rhizoplan lada hitam adalah oleh *T. atroviride*. *B. pumilus* menunjukkan kolonisasi tertinggi (9.01×10^4**)** di dalam tisu akar. Perhubungan simbiotik mikrob tersebut dengan tanaman perumah merangsang pertumbuhan akar seperti mana yang ditunjukkan oleh peningkatan dalam panjang akar, berat basah dan berat kering akar. Kajian tentang inokulum mikrob dan nitrogen (N) telah menunjukkan keupayaan inokulum mikrob dalam meningkatkan penyerapan N tanaman perumah dan seterusnya meningkatkan kecekapan penggunaan N bagi baja kimia yang diberikan. Penilaian terhadap interaksi mikrob dan tanaman perumah menunjukkan isolat–isolat mikrob menghasilkan keresistanan teraruh melalui penghasilan kompaun teraruh seperti peroksidase (PO), polifenolosidase (PPO), dan fenilalamin ammonia liase (PAL). Keberkesanan isolat–isolat mikrob telah diuji terhadap *F.solani*. Dua masa penginokulasian turut dikaji: inokulasi tanah semasa penanaman dan inokulasi tanah 14 hari sebelum penanaman. Inokulasi dengan *T. atroviride* (UPM23F) sahaja adalah paling berkesan diikuti oleh inokulasi gabungan *T. atroviride + B. pumilus* (UPM23F+UPM38EB) berdasarkan parameter–parameter seperti penangguhan kemunculan simptom, peratusan yang rendah (33.33%) dalam keterukan penyakit, dan peratusan yang tinggi (83.80%) dalam pengurangan penyakit (DR). Kadar penguningan daun adalah kurang teruk pada keratan yang diinokulasi dengan inokulum mikrob berbanding rawatan kawalan. Penginokulasian tanah dengan inokulum mikrob semasa penanaman memberikan keputusan yang terbaik. Kajian

ini telah menunjukkan bahawa isolat–isolat mikrob yang dikaji adalah berkesan terhadap *F.solani* dan mempunyai ciri–ciri penggalak tumbesaran tanaman, yang mana berpontensi dimajukan sebagai agen kawalan biologi (BCAs). Pemahaman terhadap mekanisma kawalan biologi yang terlibat di dalam perencatan penyakit akan membantu dalam mengoptimumkan keberkesanan kawalan biologi isolate-isolat mikrob tersebut. Maka dengan itu, kajian lanjut berkaitan formulasi, frekuensi dan teknik aplikasi adalah amat diperlukan untuk memaksimakan potensi *T. atroviride* (UPM23F) and *B. pumilus* (UPM38EB) sebagai agen kawalan biologi untuk penyakit kuning *Fusarium* pada lada hitam.

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I certify that a Thesis Examination Committee has met on 2nd December 2014 to conduct the final examination of Franklin Ragai Kundat on his thesis entitled " Characterization of and Screening for Antagonistic Activity of Selected Microorganisms Against *Fusarium Solani* on Black Pepper " 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 Thesis Examination Committee were as follows:

Nur Ashikin Psyquay Abdullah, Ph.D.
Associate Professor
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia
(Chairman)

Kamaruzaman Sijam, Ph.D.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Radziah Othman, Ph.D.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Muhammad Abid, Ph.D.
Professor
Faculty of Agricultural Sciences and Technology
Bahauddin Zakariya University
Multan Pakistan
(External Examiner)

_____

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the Doctor of Philopsopy. The members of the Supervisory Committee are as follows:

Nik Muhamad Abd. Majid, Ph.D.
Professor
Institute of Tropical Forestry and Forest Product
Universiti Putra Malaysia
(Chairman)

Ahmed Osumanu Haruna, Ph.D.
Associate Professor
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia
Bintulu Sarawak Campus
(Member)

Wong Sing King, Ph.D.
Senior Lecturer
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia
Bintulu SarawakCampus
(Member)

_____

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Signature: _____
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Committee: _____

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Name of Member
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Committee: _____

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Name of Member
of Supervisory
Committee: _____

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
BCA	Biological Control Agent
CRD	Complete Randomized Design
DI	Disease Incidence
DS	Disease Severity
DR	Disease Reduction
PAL	Phenylalamine Ammonia Lyase
PDA	Potato Dextrose Agar
PIRG %	Percentage Inhibition of Radial Growth
PO	Peroxidase
PPO	Polyphenol oxidase
RCBD	Randomized Complete Block Design

CHAPTER 1

INTRODUCTION

The major emphasis on black pepper fertilization is still confined to N, P, and K (Pillai et al. 1987; Sadanandan, 1994). In Sarawak, foliar application of 0.7% urea at weekly intervals, totaling nine sprays, were found to increase yield by 22% (Anon., 1995). Farmers used to apply chemical fertilizers as they are cheaper, easily available, and required in lesser quantity compared with their organic counterparts disregarding available and their deleterious effects on soil environment (Chamle et al., 2010). The placement and dosage of inorganic fertilizers are also decisive in some cases (Sharangi and Sahu, 2009). In spite of this, inorganic fertilizers alone are no more reliable to ensure productivity and quality. Biological management such as application of beneficial microbes is better alternative in terms of sustainability and environmental concerns (Tena and Beyene, 2010).

Beneficial microbes have the potential to contribute to sustainable plant growth and they generally function in three different ways: synthesizing particular compounds for the plants, facilitating the uptake of certain nutrients from the soil, and increase resistance of plant to diseases. Direct growth promotion includes symbiotic and non symbiotic beneficial microbes through production of plant hormones such as auxins, cytokinins, gibberellins, ethylene and abscisic acid. Beneficial microbes also help in solubilization of mineral phosphates and other nutrients, enhance resistance to stress, stabilize soil aggregates, and improve soil structure and organic matter content. Beneficial microbes retain more soil organic N and other nutrients in the plant soil system, thus reducing the need for N and P and enhancing release of the nutrients.

Indirect plant growth promotion includes the deleterious effects of phytopathogenic organisms. This can be achieved by the production of siderophore. Biological controls of soil-borne plant pathogens and the synthesis of antibiotics have been reported in several microbial species. Another mechanism by which beneficial microbes can inhibit phytopathogens is the production of hydrogencyanide (HCN) and/ or fungal cell wall degrading enzymes such as, chitinase and ß-1, 3-glucanase.

Yellow disease of black pepper has long been known to affect black pepper plantation in Sarawak. Typical symptoms of yellow disease are thick yellow leaves with a stiff droop, and noticeable defoliation around the vines. These symptoms may occur both in young and old vines. Usually, the diseases start in scattered patches and gradually increase in size. As the disease advances, the affected area is surrounded by a number of diseased plants in various stages of discoloration. Leaf discoloration usually starts at the bottom of the vines, and spreads to the top, although in many cases symptoms may occur all over the vines.

As the disease advances, the total number of leaves reduces due to defoliation. The main roots lose their feeder roots, leading to dieback and eventually death of vines. The symptoms are more pronounced when the soil moisture is depleted. Results from previous studies indicated that yellow disease is caused by several factors such as infection by nematodes and fungus (Bridge 1978, Mustika, 1984), soil nutrient deficiency (Wahid 1976, de Ward 1979), and low soil moisture (Mustika, 1984). It

has been speculated that yellows disease is caused by nematode-fungus complex (Bridge, 1978) involving *R. similis, Fusarium spp*. and possibly other fungi. There is clearth of evidence to support the hypothesis. At present, there are no effective control measures for pepper yellow disease.

It is therefore, important to develop cost effective methods to meet the nutrient demand and disease control of black pepper. The manipulations of the microbiological communities in the rhizosphere of crop plants to increase yield and the biological control of diseases have been extensively studied in field and greenhouse crops (Menzies and Ehret, 1997). Formulations have been developed and tested for disease suppression and for promoting the growth of plants. Previous studies found that introduction of microbial inoculants has shown significant results, both as plant growth promoter in several crops, increase in the development of the root system and in prevention against certain root diseases of greenhouse crops.

The general objective of this study was to evaluate the potential of microbes isolated from soil of a rehabilitated forest floor, as BCAs in inducing growth and suppressing yellow disease development during the early growth stage of black pepper. The mechanism of disease suppression either through induction of host resistance, increased plant vigour or direct antagonism towards *Fusarium solani*, was also investigated. The specific objectives were to:

1. Identify potential biological control agent (BCAs) through in vitro screening for antagonistic activity towards *Fusarium solani*

2. Determine the influence of BCAs on root colonization, N uptake and use efficiency, and production of inducible compounds of black pepper

3. Assess the BCAs role as control against *Fusarium solani* in black pepper.

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BIODATA OF STUDENT

Franklin Ragai Kundat was born on the June 14, 1973, in Betong Division, Sarawak, Malaysia. He received his secondary education at SMB ST. Thomas, Kuching Division, Sarawak, Malaysia and completed in 1990. He continued his study at Universiti Putra Malaysia where he pursued Diploma in Agriculture at Bintulu Campus, Sarawak. Three years later, he pursued Bachelor of Horticultural Science in Serdang Campus, Selangor, Malaysia and earned his Bachelor degree in 1998. Upon completing his first degree, he enrolled for his Master's degree program at the same university and earned Master of Agricultural Science (majoring biological control) in 2002. He was employed at Universiti Teknologi Mara (UiTM) Kota Kinabalu, Sabah Campus as a lecturer before he was appointed as a lecturer at Universiti Putra Malaysia Bintulu Sarawak Campus in 2004. In 2009, he enrolled for his Doctor of Philosophy program at UPM. He is married to Sanau Sampang and blessed with three children, Fibbie Santa Ragai, Farrell Lang Ragai and Favian Bana Ragai.

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