UNIVERSITI PUTRA MALAYSIA

ANTIFUNGAL POTENTIAL OF Melaleuca alternifolia (Maiden & Betche) Cheel AGAINST FUNGAL PATHOGEN Fusarium oxysporum f.sp. cubense TROPICAL RACE 4

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By

PAVITRA A/P PARAMALINGAM

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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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February 2022

Chairman : Associate Professor Noor Baity binti Saidi, PhD
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Fusarium wilt or Panama disease is recognised as one of the most destructive banana diseases in the world that is caused by *Fusarium oxysporum* f. sp. cubense, Tropical Race 4 (TR4). To date, only commercial fungicides are present to treat this disease. Plant extracts with antimicrobial properties attributable to their spectrum of secondary metabolites are actively being pursued. In this study, the inhibitory activities of five essential oils (*Syzygium aromaticum*, *Cinnamomum verum*, *Alpinia conchigera*, *Eucalyptus globulus* and *Melaleuca alternifolia*) and lima hydrosols (*Eucalyptus globulus*, *Cinnamomum verum*, *Zingiber zerumbet*, *Ficus carica* and *Melaleuca alternifolia*) on TR4 were investigated in vitro through agar well diffusion assay. Compared to fungicide, *M. alternifolia* oil and hydrosol were the most effective in suppressing the mycelial growth with percentage inhibition radial growth (PIRG) recorded at 69% and 45.32%, respectively. Broth macradilution assay revealed the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *M. alternifolia* oil at 200 ppm and hydrosol at 50% v/v. Besides, spore germination assay revealed 0% inhibition of fungal spore germination at 300 ppm of oil and 3.3% inhibition at 100% hydrosol, respectively. From an in vivo study, *M. alternifolia* extracts significantly ($p \leq 0.05$) delayed Fusarium wilt symptom development in greenhouse-grown Berangan and Cavendish plantlets at all concentrations compared to control plantlets. The GC-MS analysis of *M. alternifolia* essential oil identified thirty-one bioactive compounds such as terpinen-4-ol, eucalyptol, à-terpineol and benzene, and 1-methyl-3-1-methylethyl. On the other hand, the LC-MS analysis of *M. alternifolia* hydrosol identified eighteen bioactive compounds including dihydro-jasmonic acid, methyl ester, L-galactono-1,4- lactone and diisopropyl sulfate. Hence, terpinen-4-ol from essential oil and Unknown compound 1 from hydrosol are suggested to be the best candidate that possesses fungicide properties against TR4. These findings indicate that *M. alternifolia* extracts could be used as natural alternatives to synthetic fungicides to control TR4.

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

POTENSI ANTIKULAT *Melaleuca alternifolia* (Maiden & Betche) Cheel TERHADAP PATOGEN KULAT *Fusarium oxysporum f.sp. cubense* TROPICAL RACE 4

Oleh

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"Fusarium wilt" atau penyakit Panama dikenali sebagai salah satu penyakit pisang yang paling serius di dunia yang disebabkan oleh *Fusarium oxysporum f. sp. cubense*, Tropical Race 4 (TR4). Sehingga kini, hanya racun kulat komersial yang boleh merawat penyakit ini. Ekstrak tumbuhan dengan sifat antimikroba yang disebabkan oleh spektrum metabolit sekundernya sedang giat dikajikan. Dalam kajian ini, aktiviti penghambatan lima minyak pati (*Syzygium aromaticum, Cinnamomum verum, Alpinia conchigera, Eucalyptus globulus* dan *Melaleuca alternifolia*) dan lima hidrosol (*Eucalyptus globulus, Cinnamomum verum, Zingiber zerumbet, Ficus carica* dan *Melaleuca alternifolia*) diselidiki secara in vitro melalui ujian penyebaran sumur agar. Berbanding dengan racun kulat, minyak dan hidrosol *M. alternifolia* adalah yang paling berkesan terhadap pengurangan pertumbuhan miselium dengan "percentage radial growth" (PIRG) masing-masing mencatatkan 69% dan 45.32%. Ujian makradilution broth menunjukkan "minimum inhibition concentration" (MIC) dan "minimum fungicidal concentration" (MFC) minyak *M. alternifolia* pada 200 ppm dan hidrosol pada 50% v/v. Selain itu, ujian percambahan spora menunjukkan 0% perencatan percambahan spora pada 300 ppm minyak dan 3.3% perencatan pada 100% hidrosol. Dari kajian in vivo, ekstrak *M. alternifolia* mengurangkan perkembangan gejala "Fusarium wilt" secara ketara ($p < 0.05$) di perkebunan Berangan dan Cavendish yang ditanam di rumah hijau pada semua kepekatan berbanding dengan tanaman kawalan. Analisis GC-MS minyak pati *M. alternifolia* mengenal pasti tiga puluh satu sebatian bioaktif seperti terpinen-4-ol, eucalyptol, à-terpineol dan benzene, dan 1-methyl-3-1-methylethyl. Sebaliknya, analisis LC-MS terhadap hidrosol *M. alternifolia* mengenal pasti lapan belas sebatian bioaktif termasuk asid dihidro-jasmonik, metil ester, L-galactono-1,4- lakton dan diisopropil sulfat. Oleh itu, terpinen 4-ol daripada minyak pati dan sebatian 'Unknown 1'daripada hidrosol dicadangkan sebagai calon terbaik yang mempunyai sikap antikulat terhadap TR4. Penemuan ini menunjukkan bahawa ekstrak *M. alternifolia* dapat digunakan sebagai alternatif semula jadi terhadap racun kulat sintetik untuk mengawal TR4.

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This thesis was submitted to the Senate of the 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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α	Alpha
β	Beta
°C	Degree celcius
Σ	Mean total
%	Percentage
µL	Microleter
µm	Micrometer
cm	Centimeter
m	Meter
mm	Milli meter
mg	Milli gram
ppm	Parts per million
psi	Pounds per square inch
V	Volume
AST	Antimicrobial susceptibility testing
CLSI	Clinical and laboratory standards institute
ddH_2O	Distilled water
DPPH	diphenyl 2-picrylhyorazyl
DSI	Disease severity index
ESI	Electrospray ionization
Foc	*Fusarium oxysporium* f. sp. *cubense*
FT	Fourier transform
GC	Gas chromatography
HPLC	High performance liquid chromatohraphy
HPTLC	High performance thin layer chromatography
IL	Interleukin

IPPC	International plant protection convention
IR	Infrared
LC	Liquid chromatography
LSI	Leaf symptom index
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration
MS	Mass spectrometre
NMR	Nuclear magnetic resonance
NO	Nitric oxide
PDA	Potato dextrose agar
PDB	Potato dextrose broth
Q-TOF	Quad time of flight
RDI	Rhizome discoloration index
ROS	Reactive oxygen species
TLC	Thin layer chromatography
TNF	Tumour necrosis
TR4	Tropical race 4
UV	Ultra violet
VCG	Vegetative compatibility group

CHAPTER 1

INTRODUCTION

Bananas constitute a significant source of economic growth, food security, income and nutrition for many countries in the world (Dale et al., 2017). In Malaysia, bananas are the most planted fruit in 2019, occupying more than 30 thousand hectares of land (DOA, 2019). However, the banana industry has been facing dramatic losses due to a disease known as Fusarium wilt or Panama disease (Drenth & Guest, 2016). The disease is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*). There are four recognized races of *Foc* that pose increased risks to banana supplies globally by affecting food security and income generation (Dita et al., 2018). Among the *Foc* races, TR4 is the most virulent and considered the greatest threat to banana production (Zhang et al., 2018; Dale et al., 2017). TR4 has a wide host range and is extremely difficult to manage because of its ability to survive in the soil for an extended period (Ploetz, 2015).

Fusarium wilt disease is mainly controlled using physical and chemical methods (Pegg et al., 2019). However, the physical control such as eradication, exclusion and soil health management are not efficient because of insufficient emphasis (Li et al., 2021). On the other hand, chemical fungicides could adversely affect soil and human health and have limited efficiency due to the pathogenic variability of the fungus (Siamak & Zheng, 2018; Xue et al., 2015; Latz et al., 2016).

Thus, biological control in managing Fusarium wilt has gained interest recently, mainly because of economic, environmental, and safety concerns of chemical fungicide usage (Bubici et al., 2019; Bazioli et al., 2017). At the same time, resistance trait is also not present in many commercial banana cultivars (Isquierdo et al., 2021; Pegg et al., 2019). The application of beneficial microbes and their metabolites and active compounds from plant extracts in bioformulation have been shown to be effective in managing plant diseases (Abbas et al., 2020; Vishwakarma et al., 2020; Comite et al., 2021).

The search for natural products with novel uses is pursued actively nowadays, particularly plant extracts or essential oils with anti-microbial properties, attributable to their spectrum of secondary metabolites (Saravanakumar et al., 2015). Plant extracts from *Syzygium aromaticum*, *Acalypha indica*, *Ocimum tenuiflorum*, *Salvia rosmarinus*, *Thymus vulgaris*, *Cinnamomum verum* and *Piper betle* have been shown to be effective against *Foc*, including TR4, by suppressing the growth of the fungus or delaying the disease symptom (Isianto and Emilda, 2011; Huang et al., 2012; Monteiro et al., 2013; Megane & Kamble, 2014; Gnanasekaran et al., 2015).

Different plant compounds carry different profiles of secondary metabolites that vary in activities. The amount and inhibitory potential of active compounds from plants are also influenced significantly by geographical origin and environmental factors (Liu et al.,

2016; Kumar et al., 2017). Due to lack of curative effect and toxicity, a new prototype biological control is needed to address TR4 infection. Besides, role of plant extracts against TR4 is not widely discovered and studied yet. Hence, this urged the evaluation of Malaysian medicinal plants as a source of potential antifungal agent against TR4. Malaysia has a rich collection of unique medicinal plants, most of which are endemic species.

The plants contain diverse bioactive compounds with potent anti-inflammatory and anti-microbial activities (Abu Bakar et al., 2018). Local plant species, namely *Melaleuca alternifolia*, *Ficus carica*, *Cinnamomum verum*, *Eucalyptus globulus*, *Zingiber zerumbet* and *Alpinia conchigera* are known to possess such bioactive compounds, where they showed good anti-microbial activity against *Plasmodium, babesia, Pseudomonas aeruginosa, Aspergillus niger, Botrytis cinerea* and *Rhizopus spp* (Ibrahim et al., 2009; Yu et al., 2015; Rodney et al., 2015; Abdel-Gaber et al., 2020). Therefore, it was hypothesized that extracts from *M. alternifolia, F. carica, C. verum, E. globulus, Z. zerumbet* and *A. conchigera* can exhibit antifungal activity against TR4.

The work described in this thesis was designed to achieve the following objectives:

1. To determine the antifungal potential of essential oils from *Syzygium aromaticum*, *Cinnamomum verum*, *Alpinia conchigera*, *Eucalyptus globulus* and *Melaleuca alternifolia* and hydrosols from *Syzygium aromaticum, Eucalyptus globulus, Zingiber zerumbet, Ficus carica* and *Melaleuca alternifolia* against TR4 via in vitro assays.

    1.1 To investigate the minimum inhibitory concentration (MIC) of *Melaleuca alternifolia* (Tea tree) oil and hydrosol against TR4 by broth microdilution assay.

    1.2 To study the impact of Melaleuca alternifolia oil and hydrosol against TR4 by spore germination assay.

2. To determine the effect of different concentrations of *Melaleuca alternifolia* essential oil and hydrosol on Fusarium wilt disease in banana plantlets under greenhouse conditions.

3. To identify the bioactive compounds in the essential oil and hydrosol of *Melaleuca alternifolia* using high-throughput screening.

    3.1 To identify bioactive compounds of *Melaleuca alternifolia* oil using Gas chromatography-mass spectrometry (GC-MS) analysis.

    3.2 To identidy bioactive compounds of *Melaleuca alternifolia* hydrosol using Liquid chromatography-mass spectrometry (LC-MS) analysis.

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