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EFFECT OF AQUEOUS NEEM LEAVES EXTRACT IN CONTROLLING Fusarium WILT BANANA (Musa spp.)

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EFFECT OF AQUEOUS NEEM LEAVES EXTRACT IN CONTROLLING *Fusarium* WILT BANANA (*Musa* spp.)

By

UNG YI

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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

EFFECT OF AQUEOUS NEEM LEAVES EXTRACT IN CONTROLLING *Fusarium* WILT OF BANANA (*Musa* spp.)

By

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Chair : Assoc. Prof. Siti Zaharah Sakimin, PhD
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Neem leaf extracts (NLE) have frequently been applied to inhibit plant diseases and for the development of bio-fertilizer that leading to the commercial exploitation of this tree in agriculture. However, previous studies had indicated contradictory outcomes when it was applied as antifungal disease treatment and as bio-fertilizer when applied through the soil for several crops, including banana. Therefore, the present investigation was undertaken (i) to assess the growth of *Fusarium oxysporum* f. sp. c*ubense* (Foc) fungus using a suitable media for control of pH in-vitro condition and (ii) to examine the occurrence and severity of diseases caused by Foc on banana "Cavendish" treated with NLE at the optimum soil acidity in the glasshouse condition. In 1A *in-vitro* experiment, different NLE rate from 0 to 10 ml was added into 250 ml of potato dextrose agar (PDA) media to grow the Foc fungus the while in the 1B experiment, the Foc were growth in different pH level (5, 5.5 as control, 6 and 7) of PDA media under complete randomized design (CRD) experimental design. In 1A experiment, colony radial extension decreased while the percentage of inhibition of radial growth (PIRG, %) increased significantly with increasing NLE rate for 7 days after incubation (DAI). However, no significant difference of all parameters was recorded for both 8 ml (8:250) and 10 ml (10:250) of NLE. Besides that, both pH 5 and 7 of PDA media also had the same effective significant effect in all parameters than under any other pH of the PDA media in the 1B experiment. Due to the planting condition of banana plants and according to significant differences, in Experiment 2, the best level of 8 ml aqueous NLE per 250 ml media (or at the ratio of 8:250 or 4:125) from Experiment 1A was applied to *Fusarium*-inoculated Cavendish banana (*Musa* spp. AAA group cv. 'Grand Nain') under the best soil media pH condition (optimum pH 7) from Experiment 1B. The 40 ml *Fusarium* fungus solution with the population of 2.50×10^8 spores ml^{-1} was poured onto the soil. The solution of NLE treatment was applied after one month of *Fusarium* application. The treatments in Experiment 2 were arranged by randomized complete block design (RCBD). Banana plants associated fungus were detrimental significantly and showed wilting symptoms. Foc resulting in high

disease severity (leaves and rhizome) and incident of *Fusarium* wilt (%). Moreover, it was observed that the application of extract improves significantly in plant height, pseudo-stem diameter, root size and distribution, plant biomass production as well as soil physiochemical properties and tend to resist *Fusarium* wilt diseases. Thus, the major finding of this study shows that the application of NLE solution promotes better growth of cavendish banana plants and tend to withstand *Fusarium* wilt infection under optimum pH 7 media. Based on the results, aqueous NLE (at the ratio of 4:125) was recommended to be applied in the field as a treatment to improve Cavendish banana growth and delay *Fusarium* diseases damage to plants under pH7 soil condition.

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

POTENSI EXTRAK AIR NEEM SEBAGAI PENGAWALAN PENYAKIT *Fusarium* PADA TANAMAN PISANG (*Musa* spp.)

Oleh

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Daun neem extract (NLE) sering digunakan oleh petani untuk mengatasi pelbagai masalah penyakit tanaman dan mempunyai potensi yang tinggi untuk pembangunan komersial kerana permintaan baja organic semasa telah meningkat secara drastic. Walau bagaimanapun, kajian dan penyelidikan yang sedia masih kurang bukti untuk mengenal pasti keberkesanan penggunaan daun neem extract sebagai rawatan penyakit dan sebagai baja organic terhadap pertumbuhan pelbagai penanaman termasuk pisang. Sehubung dengan itu, berikut adalah objektif penyelidikan ini bagi menjawab permasalahan tersebut iaitu (i) mengkaji pertumbuhan *Fusarium oxysporum* f.sp. c*ubense* (Foc) fungi dalam keadaan *in-vitro* menggunakan media yang sesuai di bawah keadaan pH yang beza dan (ii) menentukan kesan NLE terhadap pertumbuhan pokok pisang 'Cavendish' yang diinokulasi dengan Foc dan pengurangan penyakit layu *Fusarium* atas keasidan tanah yang optimum dalam rumah kaca. Dalam Kajian 1A, NLE dengan kepekatan yang berbeza dari 0 hingga 10 ml telah ditambah dalam potato dextrose agar (PDA) untuk tumbuh Foc fungi manakala dalam Kajian 1B, Foc tumbuh pada PDA media dengan kadar pH yang beza (5, 5.5-control, 6 dan 7) di bawah reka bentuk eksperimen rawak lengkap (CRD). Peningkatan NLE dari 0 ml hingga 10 ml dalam Kajian 1A telah meningkatkan secara ketara terhadap peratusan perencatan pertumbuhan jejari (PIRG, %) Foc tetapi kecikan koloni jejari Foc dari hari pertama hingga hari ketujuh selepas inkubasi (DAI). Walau bagaimanapun, aplikasi antara 8 ml (8:250) dan 10 ml (10:250) NLE tidak menunjukan significant yang beza untuk semua parameter. Selain daripada itu, keputusan Kajian 1B menunjukkan bahawa pH 5 dan 7 memberi kesan signifikan yang sama terhadap koloni jejari, kadar pertumbuhan dan PIRG Foc. Menurut keadaan penanaman pokok pisang termasuk kesan signifikan, dalam Kajian 2 kepekatan terbaik 8 ml air NLE per 250 ml media (kadar 8:250 atau 4:125) dari Kajian 1A telah digunakan atas *Fusarium* inokulasi pokok pisang Cavendish (*Musa* spp. AAA group cv. 'Grand Nain') dengan pH media terbaik (optimum pH 7) dari Kajian 1B. Media tanah diinokulasi dengan kadar 40 ml *Fusarium* pada kepekatan 2.50 x 10^8 spora ml^{-1} serta diaplikasi

dengan rawatan NLE sebulan selepas inokulasi *Fusarium* fungus. Rawatan dalam Eksperimen 2 disusun dengan reka bentuk blok lengkap secara rawak (RCBD). Pokok pisang diinokulasikan dengan fungus menunjuk tanda-tanda layu. Keputusan mendapati bahawa keparahan (severity) penyakit (daun dan sulur) dan insiden layu *Fusarium* (%) meningkat dengan inokulasi Foc. Selain daripada itu, aplikasi NLE menunjukkan peningkatkan yang ketara bagi parameter ketinggian pokok, diameter batang, ukuran dan pengedaran akar (luas permukaan akar, diameter akar dan isipadu akar), berat pokok (berat segar akar, berat kering akar dan nisbah pucuk akar) serta sifat fizikokimia tanah (kapasiti pertukaran kation , nitrogen, fosforus, kalium, kalsium dan magnesium) dan mungkin berkesan dalam melawan penyakit layu *Fusarium*. Oleh itu, penemuan utama daripada hasil kajian ini membuktikan bahawa NLE memberi faedah atas penumbuhan pokok dan berpotensi meningkatkan kerintangan pokok pisang cavendish terhadap serangan penyakit layu *Fusarium* atas optimum pH7 media. Secara keseluruhannya, air NLE (4:125) disarankan untuk diaplikasi di ladang sebagai rawatan untuk melambatkan penyakit *Fusarium* yang mengakibantkan kerosakan teruk pada pokok dan memperbaiki pertumbuhan pokok pisang Cavendish di bawah keadaan tanah pH7.

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

$	Dollar sign
%	Percentage
±	Plus-minus sign
0.1N	0.1 Normality
A.	*Azadirachta*
ANOVA	Analysis of Variance
AA	Auto Analyzer
AAS	Atomic Absorption Spectrometer
ABA	Abscisic Acid
A. Juss	Adrien-Henri de Jussieu (author abbreviation)
BBMV	Banana Bract Mosaic Virus
BBTV	Banana Bunchy Top Virus
BIO	Bioorganic
BSV	Banana Streak Disease
cv.	Cultivar
Ca	Calcium
CEC	Cation Exchange Capacity
CFU	Colony Forming Unit
cm	Centimetre
cmol	Centimole
CMV	Cucumber Mosaic Virus
CRD	Complete Randomized Design
Cu	Copper
CZA	Czapek-Dox Agar

℃	Degree celcius
DAI	Days After Incubation
DF	Degree of Freedom
DOA	Department of Agriculture
DSI	Diseases Severity Index
EC	Electrical conductivity
EID	Emerging infectious disease
et al.	And friends
F.	Fusarium
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistical Database
Fe	Iron
Foc	*Fusarium oxysporum* f.sp. *cubense*
f. sp.	Forma specialis
g	Gram
GA3	Gibberellic Acid
GLM	General Linear Models
GML	Ground Magnesium Limestone
H+	Hydrogen Ion.
ha	Hectare
HCl	Hydrocholoric Acid
H2O2	Hydrogen Peroxide
HPLC	High Performance Liquid Chromatographic
HSD	High Significant Different
H2SO4	Sulfuric Acid
IAA	Indole-3-acetic acid

Im	Immune
IU	International unit
kg	Kilogram
K	Potassium
kPa	Kilopascals
K2SO4	Potassium Sulphate
L	Litre
LSD	Least Significant Different
LSI	Leaf Symptom Index
M	Molarity
m	Meter
mm	Millimeter
Mardi	Malaysian Agricultural Research and Development Institute
Mg	Magnesium
mg	Milligram
ml	Millilitre
MMEA	Maize Meal Extract Agar
Mn	Manganese
Mr	Moderately resistance
Ms	Moderately susceptible
µ	Micro
N	Nitrogen
nm	Nanometre
ns	Not significant
NaOCl	Sodium Hypochlorite
NaOH	Sodium Hydroxide

NAP	National Agriculture Policy
NH4F	Ammonium Fluoride
NH4OAc	Ammonium Acetate
NL	Neem Leaves
NLE	Neem Leaves Extract
NaOCl	Sodium Hypochlorite
P	Phosphorus
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
pH	Measurement of Acidity / Alkalinity
PIRG	Percentage of Inhibition of Radial Growth
RDI	Rhizome Discoloration Index
RCBD	Randomized complete block design
RM	Ringgit Malaysia
Rs	Resistance
R/S	Root/shoot ratio
s	Second
SAS	Statistical analysis system
Sc	Susceptible
Si	Silicon
sp.	Species (single)
spp.	Species (plural)
SSR	Self-sufficiency ratio
st	-first
TR4	Tropical Race 4
th	Suffixes (other than -first, -second and -third)

UPM	Universiti Putra Malaysia
USD	United states dollar
Vs	Very susceptible
v/v	Volume/Volume
Zn	Zinc

CHAPTER 1

INTRODUCTION

Banana (*Musa spp.*) also known as Pisang in Malaysia is one of the most well-known fruits in the world. However, this world's most popular fruit might soon go extinct due to outbreaks of catastrophic wilt diseases induced by *Fusarium oxysporum* f. sp. c*ubense* (Foc). *Fusarium* wilt disease symptoms come in various forms such as yellowing of the leaf, wilting of banana trees, discoloration of the vascular system. The lethal strain of Foc led to huge banana production losses all over the world especially Cavendish plantations in China, Indonesia, Philippines including Malaysia and causing heavy economic losses of over 75 million USD (around RM 300 million) mark. According to Masdek *et al*. (2003) and Nasdir (2003) this destructive fungus destroying more than 5,000 ha of banana annually while losses in banana plantations of both Malaysia and Indonesia country exceed 8 million of plants. Disease's control is thus urgent to eliminating this pathogenic-fungus and prevent the spread of disease.

The suppressive *Fusarium* fungus is not effective by chemical (Stover, 1962, Lakshmanan *et al*., 1987; Herbert and Marx, 1990) and biological control strategies. Preventive means with disease-resistant cultivars and disease-free propagation material is the best strategy (Saravanan *et al.* 2003; Cao *et al.* 2005; Nel *et al*. 2007; Lian *et al*. 2009). However, efforts to develop effective, reliable and environmentally friendly biocontrol strategies should not be neglected as it remains an important organic strategy against crop diseases. Numerous biological control technique such as inoculating beneficial microbial organisms (Alabouvette, 1986; Larkin *et al*., 1996; Larkin and Fravel, 1998; Ploetz *et al*., 2003), bioorganic product application (Runia, 2014; Xue *et al*., 2015) and soil amendment (Nasir *et al*., 2003) have been reported to shows slight suppression in controlling *Fusarium* diseases on different agriculture crops. At present, there are still very few studies that have focused on the antifungal activities of both Malaysia neem extract and Malaysia land condition against the *Fusarium* pathogen, especially on local Cavendish banana crops.

The researcher of Malaysia has given high attention and priority to the neem tree (*Azadirachta indica* A.Juss.) powerful products. Rich nutrient content in neem leaves extracts (NLE) improving fertility of soil and plants' nutrients uptake. Meanwhile, this extract also helps in controlling several parasitic fungi due to their active ingredient, azadirachtin which responsible for stopping the growth cycle of microorganism without any side effect on crops. However, under favorable environmental conditions, fungal spores still germinate and spread easily in the agriculture field even in the present of the fungicide.

Host, pathogen, and environment in the "disease triangle", are the three components that determined the potential risk of an agriculture crop in Emerging Infectious Disease (EID). Thus, changing the media pH level can suppress the

pathogen directly or indirectly through impact on this soil pathogen activity and on host colonization by converting the disease-conducive media to disease-suppressive media (Kloepper *et al*., 1980). Thus, the pH level that optimum for the agriculture crops but not for the disease's pathogens should be chosen as it could make the crops strong enough to resist the pathogens. However, while the general principles are theorized, there is a lack of detailed scientific knowledge and prove especially on Cavendish plants in Malaysia under both laboratory and field performance.

Currently, the rapid spread of *Fusarium* diseases in Malaysia had caused serious economic losses problem in local banana agriculture (Husain and William, 2011). Besides that, acidic topsoil (pH 4.2 to pH 4.8) in most of the land areas in Malaysia, may even result in more serious *Fusarium* diseases incident (Gordon *et al*., 2019; Orr and Nelson, 2018). Acidic soils also cause significant losses in banana production as it does not suitable for crops growth and were low in disease resistance. Thus, in view of the above problems, the main objective of this study was to systematically study the effects of different aqueous neem leaves extract rate (NLE) and different pH level of media in controlling *Fusarium* (Foc) growth, soil physicochemical properties, growth and physiological changes of Cavendish banana. The specific objectives of the present study were:

i. To study the effects of different aqueous NLE rate applications on the *Fusarium* growth under *in-vitro* condition.
ii. To determine the effects of different pH levels of growth media on the *Fusarium* growth under *in-vitro* condition.
iii. To determine the effect of aqueous NLE on controlling *Fusarium* wilt, soil physicochemical properties, growth performance and physiology changes of Cavendish banana under optimized soil pH condition.

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