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IDENTIFICATION AND BIOCONTROL POTENTIALS OF ENTOMOPATHOGENIC FUNGI AND THEIR ENDOPHYTIC EFFECTS AGAINST Bemisia tabaci Gennadius ON TOMATO, Solanum lycopersicum Linn.

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By

IBRAHIM SANI

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

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DEDICATION

I dedicate this piece of work to Almighty Allah and our beloved Prophet Muhammad (Allah's peace and blessing be upon him) as well as his companions and the rest of Muslims Ummah, including my loving parent Malam Sani Aliyu Kankara and Malama Suwaiba Sani Kankara, my beloved wife Nafisa Jino and my children Al'amin and Sani (Aiman).

Abstract thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the of the requirement for Degree of Doctor Philosophy

IDENTIFICATION AND BIOCONTROL POTENTIALS OF ENTOMOPATHOGENIC FUNGI AND THEIR ENDOPHYTIC EFFECTS AGAINST *Bemisia tabaci* Gennadius ON TOMATO *Solanum lycopersicum* Linn.

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March 2022

Chairman : Syari Jamian, PhD
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Tomato, *Solanum lycopersicum* is a plant-vegetable fruit that is widely grown throughout the world due to its nutritive and health benefit. It is a common and popular vegetable in Malaysia, produced and managed largely by smallholders' farmers, and provides thousands of employments for the peoples. However, insect pests including whitefly, *Bemisia tabac*i are the major constraints in tomato productions. The application of chemicals is the most efficient option to protect tomatoes against pests and diseases, despite various adverse effects reported on non-target organisms, including beneficial insects (pollinators), livestock and humans. In recent years, researchers have shown an increasing interest in the possibility of using biological control agents including entomopathogenic fungi (EPF) as an alternative to chemical control measures. In addition to direct application to control insect pests, EPF can colonize plant tissues and grow endophytically, benefiting their host plant without causing any symptoms or negative effects. Entomopathogenic fungi were isolated from soil using the insect bait method with mealworm beetle (*Tenebrio molitor*) and from infected *B. tabaci* infesting vegetables. All the isolates were identified based on morphological characterization and molecular identification using Internal Transcribed Spacer (ITS) region. The *in vitro* entomocidal activity of the three *Metarhizium anisopliae* isolates was evaluated against *B. tabaci* at different incubation temperatures (15, 20, 25, and 30ºC) using a concentration of conidia, $1x10^7$conidia/mL. Meanwhile, the virulence of two isolated fungal species, *Cordyceps javanica* and *Purpureocillium lilacinum* were evaluated against *B. tabaci* under laboratory and glasshouse conditions. Furthermore, the effect of tomato seed treated with EPF on plant growth, mortality, and population of *B. tabaci* were evaluated using the seed inoculation method. A total of 12 isolates of Entomopathogenic fungi (EPF) were identified out of *M. anisopliae* (seven isolates) and *P. lilacinum* (two isolates) found from a soil sample, while species *C. javanica* (two isolates) and *P. lilacinum* (one isolate) isolated from infected *B. tabaci*. The percentage mortality of the three isolates of *M. anisopliae* (Ma-2b, Ma-9a and Ma-15) tested against *B. tabaci* with an average mortality rate 36.1% - 97.7% at different temperatures (15, 20, 25, and 30ºC) at 7 days post-treatment. The isolates Ma-15 caused the highest percentage

mortality of 97.7% and analysis of LT_{50} and LT_{90} shows an increase in mortality (1.6 and 3.9 days respectively), at 30º. Moreover, the experiment conducted with *C. javanica* and *P. lilacinum* indicated that each of the isolate significantly ($p< 0.05$) reduce the population of adults and nymphs *B. tabaci* under laboratory and glasshouse conditions. Our study also revealed that tomato seed treated with *C. javanica* and *P. lilacinum* allowed their recovery 60 days after inoculation (DAI). Both endophytic isolates also reduced the population and adult emergence of *B. tabaci* compared to control treatment. Findings from this study recommend that locally isolated entomopathogenic fungi have the potential to be developed as biopesticides in the future to control whiteflies on tomato plants. The use of entomopathogenic fungi as one of the elements of Integrated Pest Management (IPM) can reduce dependence on the use of chemical pesticides, being environmentally friendly and turn ensuring food safety.

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

PENGENALAN DAN POTENSI KULAT ENTOMOPATOGEN SEBAGAI AGEN KAWALAN BIOLOGI DAN KESAN ENDOFITIKNYA TERHADAP *Bemisia tabaci* (GENNADIUS) PADA TANAMAN TOMATO

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Tomato, *Solanum lycopersicum* merupakan tanaman sayuran jenis berbuah dan banyak ditanam di seluruh dunia kerana khasiat dan kebaikan untuk kesihatan. Tomato adalah tanaman yang popular di Malaysia, dihasilkan dan diuruskan oleh pekebun kecil dan menyediakan peluang pekerjaan untuk masyarakat. Walau bagaimanapun, serangga perosak termasuk lalat putih, *Bemisia tabaci* adalah salah satu cabaran utama pengeluaran tomato. Penggunaan racun kimia adalah pilihan paling berkesan untuk melindungi tomato daripada perosak dan penyakit, walau bagaimanapun pelbagai kesan buruk dilaporkan ke atas organisma bukan sasaran, termasuk serangga berfaedah (pendebunga), ternakan dan manusia. Sejak beberapa tahun kebelakangan ini, para penyelidik telah menunjukkan minat yang tinggi terhadap penggunaan agen kawalan biologi termasuk kulat entomopatogen (EPF) sebagai alternatif bagi menggantikan kawalan kimia. Tambahan pula melalui aplikasi secara langsung untuk mengawal serangga perosak, EPF dapat menembusi tisu tumbuhan dan tumbuh secara endofitik, ia memberi manfaat kepada perumah tanaman tanpa menyebabkan simptom atau kesan negatif. Kulat entomopatogenik dipencilkan dari tanah menggunakan kaedah pengumpanan serangga dengan menggunakan kumbang 'mealworm' (*Tenebrio molitor*) dan pemencilan daripada *B. tabaci* yang dijangkiti penyakit pada tanaman sayuran. Semua koleksi mikrob yang dipencilkan dikenalpasti melalui ciri morfologi dan pengenalan molekul menggunakan jujukan Internal Transcribed Spacer (ITS). Sejumlah tiga pencilan *Metarhizium anisopliae* melalui aktiviti *in vitro* telah dinilai terhadap *B. tabaci* pada suhu pengeraman yang berbeza (15, 20, 25, and 30ºC) menggunakan kepekatan spora 1 x 10^7 conidia/mL. Sementara itu, kesan kedua - dua spesies kulat yang telah dipencilkan, *Condyceps javanica* dan *Purpureocillum lilacinum* telah dinilai terhadap *B. tabaci* di dalam makmal dan rumah kaca. Tambahan pula, kesan rawatan biji benih tomato dengan menggunakan EPF terhadap pertumbuhan tanaman, kematian, dan populasi *B. tabaci* telah dikaji dengan menggunakan kaedah inokulasi biji benih. Sejumlah 12 isolat kulat entomopatogen (EPF) telah dikenalpasti daripada spesies *M. anisopliae* (tujuh pencilan) dan *P. lilacinum* (dua pencilan) menggunakan sampel dari tanah, manakala spesies C. *javanica (dua pencilan)* dan *P. lilacinum (satu pencilan)*

didapati daripada B. *tabaci* yang dijangkiti. Peratus kematian bagi ketiga-tiga pencilan daripada *M. anisopliae* (Ma-2b, Ma-9a dan Ma-15) terhadap *B. tabaci* dengan purata kematian 36.1% - 97.7% yang diuji pada suhu yang berbeza (15, 20, 25 dan 30ºC) selepas hari ketujuh pemerhatian selepas rawatan. Pencilan Ma-15 merekodkan kematian *B. tabaci* tertinggi dan analisa LT_{50} dan LT_{90} menunjukkan pertambahan kematian (1.68 dan 3.98 hari) masing-masing pada suhu inokulasi 30ºC. Tambahan lagi, eksperimen yang dijalankan dengan menggunakan *C. javanica* dan *P. lilacinum* menunjukkan bahawa setiap pencilan dapat mengurangkan populasi serangga dewasa dan nimfa *B. tabaci* dengan perbezaan bererti ($P<0.05$) di dalam makmal dan rumah kaca. Keputusan kajian juga menunjukkan bahawa biji benih tomato yang dirawat dengan pencilan kulat *C. javanica* dan *P. lilacinum* membolehkan pemulihan pokok selama 60 hari selepas inokulasi (DAI). Kedua-dua pencilan endofitik juga mengurangkan populasi dan kemunculan serangga dewasa *B. tabaci* berbanding dengan rawatan kawalan. Penemuan dari kajian ini mengesyorkan bahawa kulat entomopatogen pencilan tempatan berpotensi untuk dibangunkan sebagai biopestisid pada masa hadapan untuk mengawal lalat putih pada tanaman tomato. Penggunaan kulat entomopatogenik sebagai salah satu elemen Pengurusan Perosak Bersepadu (PPB) dapat mengurangkan kebergantungan terhadap penggunaan racun kimia, bersifat mesra alam dan seterusnya menjamin keselamatan makanan.

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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:

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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

ANOVA	Analysis of variance
BCA	Biological control agents
Bp	Base pair
Cm	Centimetre
DAI	Days after Inoculation
DAT	Days after Treatment
DsRNA	Double-stranded RNA
EPF	Entomopathogenic fungi
EPN	Entomopathogenic nematode
g	Gram
GPS	Global Positioning System
ITS	Internal Transcribe Spacer
IPM	Integrated Pest Management
IRAC	Insect Resistance Action Committee
HPR	Host plant resistance
Hrs	Hours
ha^{-1}	Hectare
Kg	Kilogram
L	Litre
MED	Mediterranean
MEAM1	Middle East-Asia Minor 1
Mt-COI	Mitochondrial cytochrome oxidase subunit I
MOA	Ministry of Agriculture and Agro-Based Industry Malaysia

m	Meter
Mg	Milligram
mm	Millimetres
PCR	Polymerase chain Reaction
PDA	Potato Dextrose Agar
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Tomato, *Solanum lycopersicum*, is one of Malaysia's most common and popular vegetables, growing largely in the Cameron Highlands in Pahang and Kundasang in Sabah states (Rahim et al., 2017). In recent years, the demand for tomatoes in Malaysia and its export markets has shown an increasing trend due to certain factors and government promotional programs (Radam et al., 2015). However, insect pests are one of the major constraints in tomato productions and all parts of the plant are susceptible to attacks, including leaves, stems, flowers, roots, and fruits. These insect pests include aphids, beetles, caterpillars, mites, thrips, and whiteflies (Panyasiri et al., 2007; Brezeanu et al., 2014; Afreen et al., 2017). Chemical pesticides are the most extensively employed to manage insect pests of vegetables in Malaysia, despite their detrimental ramification on non-target organisms, humans, and the environment (Mispan et al., 2015).

However, many studies have reported several drawbacks attached to chemical pesticides which include considerable pollution, health problems in humans and animals, pest resistance development, and destruction of natural enemies (Abdelghany, 2015). As a result, there is an urgent need to develop alternatives that are safe, effective, and biodegradable. In recent years, Integrated Pest Management (IPM) has been recognized as an alternative to chemical control techniques and entomopathogenic fungi (EPF) is one of the major components of IPM. EPF are natural enemies present in the insect population and have long been recognized as efficient biological control agents in IPM techniques (Jiang et al., 2020). There are several benefits associated with EPF when compared with chemical pesticides, this includes endophytic capability, specificity to host, cost-effectiveness, and eco-friendly (Mantzoukas & Eliopoulos, 2020).

EPF species from numerous genera have been shown to cause natural mortality of several sap-sucking pests including the *Bemisia tabaci* and more than 20 species were identified as effective against this pest (Bugti et al., 2018; Mascarin et al., 2013; Rios-Velasco et al., 2014; Sain et al., 2019). Species such as *Ashersonia* spp., *Beauveria bassiana, Cordyceps javanica, Isaria fumosoroseus, Lecanicillium lecanii, Metarhizium anisopliae,* and *Purpureocillium lilacinum,* are the most prominent EPF that have been used as *B. tabaci* biocontrol agents (BCA) (Abdel-Raheem & Ahmed Al-Keridis, 2016; Borisade, 2015; Panyasiri et al., 2007; Shah et al., 2020; Sun et al., 2021; Zhang et al., 2017). The EPF has also been demonstrated to infect and kill all stages of the *B. tabaci* life cycle (del Prado et al., 2008).

Insect pests usually control with EPF by either inundated or inoculative application of fungal propagules (Liu et al., 2017). In addition to direct applications, EPF has recently been discovered to colonize plants and grow endophytically, improving seedlings' height and causing significant mortality among pest infesting plants (Dash et al., 2020; Wei et

al., 2020). For example, the endophytic colonization of tomatoes by *B. bassiana* can significantly provide a positive influence on plant growth and protection against whitefly in tomato plants (Barra-Bucarei et al., 2020; Wei et al., 2020).

Of the 1556 species belonging to 161 genera of whiteflies identified in the world, *B. tabaci* is one of the most economically important pests of vegetable and horticultural crops worldwide (Alessandro et al., 2016; Perring et al., 2018). Many species of the *B. tabaci* complex are recognized to be an important polyphagous pest of more than 600 host plants and some new hosts are also being recorded while many additional hosts are not yet formally recorded (Oliveira et al., 2001; Shadmany et al., 2019). The insect feeds on several solanaceous and ornamental crops including brinjal, chili, cotton, okra, potato, tomato, and tobacco (Khan & Wan 2015; Kunjwal & Srivastava, 2018).

More recently, a serious infestation of whiteflies species on economically important vegetables has been recorded both in the lowland and highland areas of Malaysia (Shadmany et al., 2019). It was first recorded as an unimportant agricultural pest in Malaysia but has currently become a serious insect pest to many vegetables and greenhouse crops, including tomato, brinjal, and chilli (Saad et al., 2015). Therefore, this research is aimed at isolating indigenous isolates of EPF and evaluating their efficacy against whitefly *B. tabaci* on the tomato plant.

1.2 Statement of the problems

With the increasing rate of vegetable production, insect pest infestation such as whitefly, *Bemisia tabaci* Gennadus, Mealybugs, *Pseudococcus* spp, and Diamondback moth (DBM) *Plutella xylostella* L. has become a primary concern and major constraint that can significantly reduce yield and threat to food security. The whitefly has currently become a serious insect pest to many vegetables and greenhouse crops, with tomato (*Solanum lycopersicum* L.), being the most susceptible to attack (Afreen et al., 2017). However, synthetic chemical pesticides are the mainstay and most widely used for their control, despite their toxicity and hazardous effects on non-target organisms, including beneficial insects (pollinators), livestock, and humans. A biological control agent such as EPF has been developed as an alternative against the conventional use of chemical pesticides in an IPM system to effectively control *B. tabaci.*

Several studies have been conducted to demonstrate the effectiveness of EPF against whitefly, *B. tabaci* infesting tomato. Species such as *M. anisopliae, B. bassiana, Aschersonia* spp, *C. javanica,* and *P. lilacinum* have been found to causes significant mortality on all life stages of the *B. tabaci,* under laboratory and greenhouse conditions (Abdel-Raheem & Al-Keridis, 2016; Sain et al., 2019; Sun et al., 2021; Zhang et al., 2017).

Many publications from Malaysia have proven the potential of indigenous EPF isolates against *B. tabaci. Metarhizium anisopliae* isolate (PR1) has been reported to have a pathogenic effect on *B. tabaci,* causing 97 percent mortality under osmotic conditions

(Islam et al., 2016). Meanwhile, Rahim et al., (2013), reported the infectivity of a recently isolated *I. fumosorosea* strains to all life stages of *B. tabaci* infesting eggplants in the UPM glasshouse. A similar experiment was carried out with 10 *P. fumosoroseus* isolates against *B. tabaci* egg and nymph (Eslamizadeh et al., 2015).

Most of these EPF species were identified from the soil. However, it is extremely challenging to isolates new species and strains of EPF from the soil in particular regions due to human activities that result in frequent genetic connections among organisms inhabiting soils (Dong et al., 2016). Moreover, the discovery of isolates from the insect host, the isolate characterization, and the virulence test are the first steps in producing novel EPF as BCA (Dayanti et al., 2018). To our knowledge, there is no other EPF species isolated from *B. tabaci* apart from *I. fumosorosea* in Malaysia (Eslamizadeh et al., 2013).

In addition to direct application to control *B. tabaci*, EPF can colonize plant tissues and grow endophytically, benefiting their host plant by increasing growth and protection against *B. tabaci* without causing any symptoms or negative effects. However, little or no information can be found on the effects of inoculated EPF endophytes on the host plants and insect herbivores in Malaysia.

The use of EPF as an artificial endophyte in tomatoes could reduce damage caused by insect pests especially whitefly, *B. tabaci* that has recently become one of the major threats to tomato production in Malaysia.

1.3 Significance of the study

Among the insect pests, *B. tabaci* is known to be the most serious insect pest attacking vegetables in Malaysia. Of all the vegetable crops attacking by whitefly, tomato is the most seriously injured crop on the leaf. Both nymphs and adults cause damage to tomatoes leading to a considerable reduction of market values and financial losses to producers. Chemical pesticides are the mainstay and most used for the control of insect pests of vegetables, despite their effects on non-target organisms. In addition, the cost of insecticides and proper application equipment is beyond the economic means of many resource-poor farmers who grow the crop.

Reducing tomato loss to insect pests through improved insect pest control strategies is a good first step towards attaining the target of Malaysia vegetables' sustainable production and growth. Therefore, advancement in agricultural research with a specific focus to control insect pests with microbial control agents is crucial for achieving consistent vegetable production and sustainable production for food security and safety in Malaysia.

1.4 Objectives of the Study

The main objectives of the study are:

1. To isolate and identify entomopathogenic fungi from soil and infected *B. tabaci*
2. To investigate the effect of germination, radial growth, and sporulation of *Metarhizium anisopliae* and their virulence to whitefly (*B. tabaci*), at different temperatures under laboratory condition
3. To evaluate the efficacy of entomopathogenic fungi against whitefly, *B. tabaci* under laboratory and glasshouse condition
4. To asses the colonization of *C. javanica* and *P. lilacinum* in tomato plant through seed treatment and examine the effect of these entomopathogenic fungi on plant growth
5. To examine the effects of endophytic *C. javanica* and *P. lilacinum* on mortality, population, and adult emergence of *B. tabaci.*

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