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EVALUATION OF Andrographis paniculata Burm. F. EXTRACTS AGAINST Bemisia tabaci GENNADIUS

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EVALUATION OF *Andrographis paniculata* Burm. F. EXTRACTS AGAINST *Bemisia tabaci* GENNADIUS

By

REZA TANHA NAJAFABADI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

January 2015

To those that devoted their lives to science, nothing can give more happiness than making discoveries, but these cups of joy are full only when the results of their studies find practical application.

Louis Paster

DEDICATIONS

I Lovingly Dedicate This Thesis to My Parents for Their Immeasurable Supports in All Steps of My Life. Specially my wife and my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time.

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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Chair: Professor Dzolkhifli Bin Omar, PhD

Faculty: Agriculture

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is one of the most destructive pests of vegetables and ornamental crops in the world. The development of resistance to most synthetic insecticides by this insect pest has necessitated a search for alternative methods of controlling it. Some botanical pesticides have been shown to provide satisfactory alternatives to chemical pesticides. *Andrographis paniculata*, an annual herb of the family Acanthaceae is primarily used for its medicinal properties, although insecticidal applications of this plant against insect pests of agricultural and medical importance have also been reported. This study aimed to evaluate the efficiency of various solvents in extracting the active compounds of *A. paniculata*, to determine the quality and quantity of the compounds and active ingredients in *A. paniculata* extract and to evaluate the toxicity of *A. paniculata* leaf extract against *B. tabaci.* Leaves of *A. paniculata* collected from Melaka, Malaysia were extracted with methanol, chloroform, and ethanol using Soxhlet extractor. Methanol was found to be the best solvent for the extraction of active compounds, especially andrographolide, from *A. paniculata* leaves. Chloroform gave the lowest yield of active compounds. Phytochemical screening of bioactive compounds in *A. paniculata* leaf extracts showed methanol extracts contained alkaloids, saponins, flavonoids, tannins, terpenoids and steroids. The levels of these bioactive compounds were generally low in chloroform and ethanol leafs extracts. The quantity andrographolide in methanolic *A. paniculata* leaf extract showed the highest amount (92.32 ppm) followed by ethanol (41.17 ppm) and chloroform (14.85 ppm). With regard to the toxicity of three solvent extracts (methanol, ethanol, and chloroform) of *A. paniculata* leaves against the adult and nymphal stages of *B. tabaci*, the most important finding was that *A. paniculata* leaf extract can be used as a bioinsecticide to control second instar nymphs and adults of *B. tabaci*. The LC_{50} values for methanol, chloroform and ethanol extracts of *A. paniculata* against second instar

nymphs of *B. tabaci* were 39.48, 120.05, and 54.17 ppm, respectively. Among the three tested solvents, the crude methanol extract was found to have the most effective toxicity against second instar nymphs of *B. tabaci*. The toxicity of the three solvent extracts of *A. paniculata* was also tested against adults of *B. tabaci* after 96 hours of exposure. At this time point, the observed LC_{50} ranged from 43.68 ppm for the methanol extract to 172.7 ppm for the chloroform extract. The LC_{50} values for methanol, chloroform and ethanol extracts of *A. paniculata* against adult of *B. tabaci* were 43.68, 172.70, and 70.07 ppm, respectively. The larvicidal and adulticidal activity of *A. paniculata* against *B. tabaci* has not been previously studied in any detail, and this is the first report of its larvicidal and adulticidal activity against this pest.

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yang diuji, ekstrak metanol didapati mempunyai ketoksikan paling efektif terhadap instar kedua nimfa, *B. tabaci*. Ketoksikan ketiga-tiga pelarut untuk ekstrak *A. paniculata* diuji terhadap *B. tabaci* dewasa selepas pendedahan 96 jam menunjukkan nilai LC50 43.68, 172.70, dan 70.07 ppm untuk metanol, kloroform dan etanol ekstrak masing-masing. Aktiviti kebolehan membunuh larva dan *A. paniculata* terhadap *B. tabaci* belum diuji secara terpininci sebelum ini, dan ini adalah laporan pertama bagi aktiviti membunuh larva dan dewasa terhadap serangga ini.

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This thesis was submitted to the Senate of Universiti Putra Malaysia 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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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CRD	Completely Randomized Design
DBM	Diamond back Moth
EPA	The U.S. Environmental Protection Agency
FOA	Food and Agriculture Organization
GC-MS	Gas Chromatography and Mass Spectroscopy
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
IRAC	Insecticide Resistance Action Committee
IRM	Insecticide Resistance Management
IPM	Integrated Pest Management
LCL	Less-Than-Container Load
LC-MS	Liquid Chromatography and mass spectroscopy
LC_{50}	Median Lethal Concentration
LC_{90}	Lethal Concentration 90
LD_{50}	Median Lethal Dose
LD_{90}	Lethal Dose 90
LC	Liquid Chromatography
MECC	Micellar Electrokinetic Capillary Chromatography
MEKC	Microemulsion Electrokinetic Chromatographic
MHA	Muller Hinton Agar
nAChR	Nicotinic Acetylcholine Receptor
NSKE	Neem Seed Kernel Extract
PH	Parker-Hannifin
PPM	Parts Per Million
RH	Relative Humidity
RF	resistance factors
RP	Reversed-Phase

SAS	Statistical Analysis System
TLC	Thin Layer Chromatography
UCL	Ultra Chromatography Liquid
UPLC	Ultra Performance Liquid Chromatography
UV	Ultraviolet Visible
w/w	Weight for weight

CHAPTER 1

INTRODUCTION

Bemisia tabaci is one of the most important sap-sucking pests, affecting a diverse range of crops that includes fruits, vegetables, and fibre and ornamental crops. It causes damage through direct feeding, promoting sooty mold growth, and, most importantly, by vectoring some of the key plant viruses. The species has long confounded whitefly systematists because many whiteflies were described and at the same time synonymised due to considerable overlap in their morphological characteristics (Perring, 2001). Consequently, consensus was reached that *B. tabaci* is a cryptic species complex (Brown et al., 1995; De Barro et al., 2011a) representing at least some 28 species (Dinsdale et al., 2010; Hu et al., 2011). The species of this complex vary in terms of some features, including rate of development, expression of resistance, breadth and efficacy of virus transmission, attraction by natural enemies, host utilisation, damage to the physiological host, and endosymbionts (Kirk et al., 2000; Oliveira et al., 2001; Horowitz et al., 2007). Among these species, a few, namely the B and Q biotypes, are regarded as invasive and harbour far more potential to cause huge crop losses than endemic biotypes. For instance, following the introduction of the invasive B biotype to the United States in the mid-1980s, *B. tabaci* has cost more than $2 billion in crop loss, crop damage, and pest control (Toscano et al., 1998). Non-chemical control of this pest is difficult to achieve, especially in cases in which the species vectors plant viruses. Thus, chemical control remains the primary method of control of *B. tabaci* (Medina-Ortega, 2011).

While most botanical insecticides fail to adequately compete against the newest generation of synthetic insecticides, their application still has merit in some scenarios. Such scenarios include application in industrialised countries in cases where human and animal health is paramount, in niche markets such as certified organic production, and most importantly, in developing countries, especially in tropical and subtropical zones (Isman, 2008). *Andrographis paniculata*, an annual herb of the family Acanthaceae (Kumar et al., 2012), is primarily used for its medicinal properties; however, use of this herb as an insecticide against insect pests of agricultural and medical importance has also been reported.

Andrographis paniculata was introduced to Malaysia (Valdiani et al., 2012) and is now being cultivated in some states for its medicinal properties. In fact, it is one of the most commonly used medicinal herbs in Malaysia (Hanapi et al., 2010). While the species has been researched for its medicinal applications in Malaysia (Mustaffa et al., 2011; Arifullah et al., 2013), studies regarding the insecticidal activity of this herb in Malaysia are non-existent. Its insecticidal, antifertility,

antifeedant and repellent activity against important agricultural pests of the orders Lepidoptera, Hemiptera, and Coleoptera orders have been tested (Qader et al., 2011). To the best of our knowledge, there have been no studies regarding its effect on whiteflies, including the sweet potato whitefly or *B. tabaci*. Recently, an invasive biotype of *B. tabaci*, the Q biotype, was reported in the Cameron Highlands and Kundasang in Malaysia (Shadmany et al., 2013). This biotype has a well-established reputation for enormous capacity to resist multiple classes of insecticides. Interestingly, a Q-type population from Cameron Highlands that was bioassayed against various insecticides showed very high levels of resistance to some of the tested materials (Shadmany et al., 2013). Because chemical control is still used as the primary method of control of this pest and because of the high potential of *B. tabaci* for the development of resistance to chemical agents, it is imperative to develop more environment-friendly chemical control agents, preferably with different modes of action, to better manage the pest and delay the onset of resistance. As mentioned above, the search for effective botanicals is justifiable in developing countries like Malaysia as it most likely carries a lower poisoning risk compared with conventional insecticides (Isman, 2008). Consequently, this study aims to evaluate the efficiency of various solvents in extracting the active compounds of *A. paniculata*, to determine the quality and quantity of the compounds and active ingredients in *A. paniculata* extract and to evaluate the toxicity of *A. paniculata* leaf extract against *B. tabaci*.

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