UNIVERSITI PUTRA MALAYSIA

BIOACTIVITY OF AGLAIA SPP. EXTRACTS ON Callosobruchus maculatus FABRICIUS AND Spodoptera litura FABRICIUS

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By

MAZNAH BINTI MUNING

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

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DEDICATIONS

I dedicate this thesis to my parents (Muning Bin Hj. Mazid and Kulai Binti Hj. Zainal), for their prayers and always believe in me. To my siblings (Maimun, Mazlan, Marani, Marleanah, Munilawati, Muhaini and Muyiddin) and my husband (Erwan) for their encouragement and supports during all hard time that I faced. To my friends (Aidah, Alisah, Diana, Faezah, Lindsay, Marida, Roslina and Zahora) who always be there for me, during my up and downs. I'm feeling so blessed to have all of you, thank you Allah S.W.T.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

BIOACTIVITY OF *AGLAIA* SPP. EXTRACTS ON *Callosobruchus maculatus* FABRICIUS AND *Sopodoptera litura* FABRICIUS

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April 2014

Chairperson: Prof. Ahmad Said Sajap, PhD
Faculty: Forestry

It has been proven that the Meliaceae family contains biologically active compounds and insecticidal properties. Some species in the Meliaceae family belong to the *Aglaia* genus which have potential compounds especially triterpenoid compounds to control insect pests. However, many *Aglaia* species have yet to be tested on important local pests. Hence, this study is carried out to ascertain potential compounds from the plant leaf extracts of two species of *Aglaia*, namely *Aglaia odoratissima* and *Aglaia variisquama* and to study the insecticidal activities of both species against C*allosobruchus maculatus* and *Spodoptera litura.* The plant leaf extracts were extracted using hexane, chloroform, ethyl acetate, and methanol. Mortality of *C. maculatus* showed significant difference ($P<0.05$) for solvent and concentration used in *A. odoratissima* and *A. variisquama* extract. *Aglaia odoratissima* extract of 10% methanol recorded 70±9.2% and 73±7.2% mortality after 24 hours and 48 hours of treatment respectively. Similar to *A. odoratissima,* 10% methanol of *A. variisquama* extracts showed highest mortality with 51±3.8% after 24 hours of treatment and 69±6.8% mortality recorded after 48 hours of the treatment. There were significant difference ($P<0.05$) recorded for concentration using for both species and 10% concentration was the most effective in reducing eggs laid by *C. maculatus* after treatments with only four to 14 eggs produced. Furthermore, total eggs produced and hatched also showed significant difference for concentration used with 10% concentration showed effectiveness of all solvent for both species. The tests recorded 50% reduction of eggs production and eggs hatched compared to their control. Repellency activity showed significant different for duration (hour) of *C.maculatus* exposed to treatment with 50% of repellency was recorded after five hour of treatment for both species. Bioassay against *S.litura* showed *A. variisquama* recorded high mortality in preliminary tests with 50.00±5.1 mortality. After further studies were done, concentration used showed significant difference ($P<0.05$) for mortality, growth inhibitory and antifeedant test against *S. litura* larval. Toxicity test showed all solvents were effective against *S. litura* larva at 1% concentration with highest mortality being recorded for chloroform solvent with 48±2.3% mortality. Growth inhibitory bioassay also shows effectiveness for all solvents at 1% concentration especially methanol solvent with almost 50% reduction in larval weight compared to its control. Extract of 1% of hexane, chloroform and methanol were found to be effective with over 20% different of leaves weight consumed recorded compared to their control. Gas chromatograph mass spectrum

analysis on *A. odoratissima* and *A. variisquama* had shown both species contained highly with terpens especially sesquiterpenoid and triterpenoid group. Both groups have been studied and some of the compounds in both groups have promising bioactivity against insect especially triterpenoid. Result against *C. maculatus* and *S. litura* can be concluded that *A.odoratissima* and *A. variisquama* have potential as botanical insecticide and showed high amounts of potential sesquiterpenoid and triterpenoid.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Master Sains

AKTIVITI BIO EKSTRAK-EKSTRAK *AGLAIA* SPP. TERHADAP *Callosobruchus maculatus* FABRICIUS DAN *Spodoptera litura* FABRICIUS

Oleh

MAZNAH BINTI MUNING

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Keluarga Meliaceae telah dibuktikan mengandungi kompound biologi aktif dan bahan-bahan yang bersifat racun serangga. Sebahagian spesis dalam keluarga Meliaceae merupakan genus *Aglaia* yang mana mengandungi kompound berpotensi terutamanya kompound triterpenoid untuk mengawal serangga perosak. Walaubagaimanapun, kebanyakan spesis *Aglaia* ini masih belum diuji ke atas serangga perosak tempatan. Oleh itu, kajian ini dijalankan untuk menentukan kompund yang berpotensi daripada ektrak daun bagi dua spesis *Aglaia* iaitu *Aglaia odoratissima* dan *Aglaia variisquama* serta untuk mengkaji aktiviti serangga bagi kedua-dua spesis tersebut terhadap *Callosobruchus maculatus* dan *Spodoptera litura*. Ekstrak daun tersebut diekstrak menggunakan heksana, kloroform, etil asetat dan metanol. Kematian *C. maculatus* menunjukkan perbezaan signifikan ($P<0.05$) bagi pelarut dan kepekatan yang di gunakan oleh ekstrak *A. odoratissima* dan *A. variisquama*. Ekstrak 10% metanol bagi *A. odoratissima* masing-masing mencatatkan 70±9.2% dan 73±7.2% kematian seleapas 24 jam dan 48 jam rawatan.Sama seperti ekstrak *A.odoratissima,* ekstrak 10% metanol bagi *A. variisquama* menunjukkan kematian yang tingg dengan 51±3.8% selepas 24 jam rawatan dan 69±6.8% kematian direkodkan selepas 48 jam rawatan. Terdapa juga perbezaan signifikan ($P<0.05$) direkodkan bagi kepekatan digunakan bagi kedua-dua spesis dan kepekatan 10% adalah paling berkesan menggurangkan telur yang diletakkan oleh *C. maculatus* selepas rawatan dengan hanya empat hingga 14 biji telur dihasilkan. Tambahan lagi, jumalah telur yang dihasilkan dan yang menetas juga menunjukkan perbezaa yang signifikan bagi kepekatan yang digunakan dengan kepekatan 10% menunjukkan keberkesanan bagi semua pelarut bagi kedua-dua spesis. Ujian merekodkan pengguranagan 50% bagi telur yang dihasilkan dan menetas. dibandingkan dengan kawalan mereka. Aktiviti pengusir menunjukkan perbezaa signifikan bagi tempoh (jam) *C. maculatus* didedahkan kepada rawatan dengan 50% pengusiran direkodkan selepas lima jam rawatan bagi kedua-dua spesis. Ujian keatas *S. litura* menunjukkan *A. odoratissima* merekodkan kematian yang tinggi dalam ujian awal dengan 50.00±5.1 kematian. Setelah kajian lanjut dijalankan, kepekatan yang digunakan menunjukkan perbezaan signifikan ($P<0.05$) bagi ujian kematian, penghalang tumbesaran dan antifeedant terhadap larva *S. litura*. Ujian ketoksikan juga menunjukkan keberkesana bagi kesemua pelarut pada kepekatan 1%

terutamanya pelarut metanol dengan hampir 50% pengurangan berat larva berbanding kawalannya. Satu peratus ekstrak hesana, kloroform dan metanol didapati berkesan dengan lebih 20% perbezaan berat daun yang dimakan direkodkan berbanding kawalan mereka. Analisis gas kromatograf mas spektrum pada *A. odoratissima* dan *A. variisquama* telah menunjukkan kedua-dua spesies mengandungi banyak terpens terutamanya kumpulan sesquiterpenoid dan triterpenoid. Kedua-dua kumpulan tersebut telah dikaji dan beberapa kompound dalam kedua-dua kumpulan menunjukkan aktiviti bio keatas serangga terutamanya triterpenoid. Keputusan terhadap *C. maculatus* and *S. litura* boleh disimpulkan bahawa *A. odoratissima* dan *A. variisquama* mempunyai poetnsi sebagai racun serangga botani dan menunjukkan jumlah sesquiterpenoid dan trterpenoid yang banyak.

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I certify that a Thesis Examination Committee has met on 28 April 2014 to conduct the final examination of Maznah binti Muning on her thesis entitled "Bioactivity of *Aglaia* spp. Extracts on *Callosobruchus maculatus* Fabricius and *Spodoptera litura* Fabricius" 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 Master of Science.

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This thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AD	After decade
BC	Before century
DDT	Dichloro-diphenyl-trichloroethane
EPPO	European and Mediterranean Plant Protection Organization
FRIM	Rorest Research Institute of Malaysia
GC-MS	Gas Chromatograph Mass Spectrum
MARDI	Malaysia Agricultural Research and Development Institute
NIST	National Institute of Standards and Technology
UPM	Universiti Putra Malaysia

CHAPTER 1

INTRODUCTION

1.1 General background

Public awareness towards safety or environmental friendly insecticides has increased to the point where users have become more interested in using safe insecticides than chemically based insecticides because it's less toxic to human and environment (Dayan *et. al.*, 2009, Rani *et. al.*, 2009, Seffrin *et. al.*, 2009, Isman, 2008, Isman, 2005, and Makanjuola, 1989). This negative impact of chemical insecticides had lead to search the new and harmless alternative like botanical insecticides to control insect pest (Akthar *at. el.*, 2008, Isman, 2006, Akthar and Isman, 2004, and Carpinella *et. al.*, 2003). According to Scott *et al.* (2003), the search for the new insecticides also affected by several factors, such as the banning of several chemically based insecticides and also the development of public perception and awareness towards the negative impact of chemically based insecticides.

Currently, botanical insecticides are studied widely and researchers have become more interested in studying new potential compounds and plants, as lots of new secondary compounds have been found to be potent towards pests, especially plants from the Meliaceae family (Perez *et. al.*, 2010, Koul *et. al.* 2003, Ng *et. al*, 2003, Li, 1999, Isman, 1997 and Champagne *et. al.* 1993). Gregger (2001) reported Meliaceae plants have been shown to contain biologically active compounds that can be sources of plant derived insecticides and supported by Donald *et al.* (1993) that reported almost 32 species of plants from the Meliaceae family have an inhibitory potential towards insect pest. One well known Meliaceae species was *Azadiractha indica* that had been successfully used on insect pest (Zounos *et al.*, 1999, Koul *et al.*, 1996, Lowery *et. al.*, 1993 and Mordue *et al.*, 1993). Besides the *A. indica* species, the genus of *Aglaia* from the Meliaceae family has also attracted the attention of researchers (Harneti *et. al.*, 2012, Dreyer *et. al*, 2001, Greger *et. al.* 2001). The genus of *Aglaia* can be found widely in the tropical rain forests of Southeast Asia (Salim *et. al.*2007, Muellner *et. al.* 2005, Bringmann *et. al.* 2003, Nugroho *et. al.* 1997 and Fuzzati *et. al.* 1996). According to Joycharat (2008), this genus has more than 100 woody species and ranging from small trees up to trees 40m. *Aglaia* genus have reported contains with potential compounds towards insects pest especially triterpenoids, flavanoids and liminoids (Duong and Proksch, 2007, Joycharat *at. al.* 2008).

Callosobruchus maculatus (Fabricius) is a major pest for several stored products especially *Vigna virata* (also known as mung beans). *Callosobruchus maculatus* infests mung beans by laying eggs on the bean's surface, consequently decreasing their economic value. The problem becomes worse when the larvae grow inside the beans and consume almost 25% of the bean, before emerging through a 'window' on the bean's surface (David, 2008). The impact of this *C. maculatus* infestation includes the physical loss of the stored product, through larval consumption, and

contamination of the stored product through dead bodies and waste products. Chemical insecticides have been used to control and prevent its infestation, but the negative impact of insecticides, through toxicity to humans, beetle's resistance, and environmental concerns, has forced the search for new plant based insecticides to solve these problems (Kestenholz *et. al.*, 2007, Ogunleye and Adefemi, 2007, and Rahman and Talukder, 2006, Boeke *et. al.*, 2004 and Kim *et. al.*, 2003).

Unlike *C. maculatus*, *S. litura* or also known as a army worm is a leaf eating caterpillar. During larval stage, *S. litura* a serious pest, usually found in Africa and Asia as reported by Ellis (2005). This insect pest is a well known feeder for over 100 host plants, most of them are vegetables (Ellis, 2005). According to CABI and EPPO report, *S. litura* larvae are able to completely defoliate the leaves of their hosts in a short period of time, in a group during their larval stages. Female moths of *S. litura* usually lay their eggs at night and produce almost 300 eggs and leave the egg batch on the surface of host's leaves (Bishara, 1934). Eggs of *S. litura* usually hatch after four days and the newly hatched larvae prefer to stay on the lower leaf's surface during the day and feed at night. During their last two instars, they will seek shelter during the day under the lowest leaves or in the soil at the base of the host plant (Brown and Dewhurst, 1975). Several findings have shown that *S. litura* larvae are resistante to chemical insecticides (Ravishankarand and Venkatesha, 2010, Tong *et al.*, 2013 and Ahmad *et al*., 2008), such as carbamate and organophosphate.

1.2 Justification of Study

1.2.1 Botanical Insecticides as Alternative Insecticides

Postel (1987) mentioned that almost 400 000 and two million insecticide poisoning cases occur throughout the world every year and most of the cases reported were among farmers in developing countries, and 10 000 to 40 000 of them result in death. The risk of cancer and long term effects on human health not only affect farmers who are active users of insecticide but also public community directly through consumption of contaminated vegetables or indirectly by using contaminated water resources.

Hence, botanical insecticides have to be discovered and developed to reduce reliance on chemical insecticides (Dayan *at el.* 2009). *Melia volkensii, Melia azedarach and Azadirachta indica* is a potential insecticide and according to Isman (1999), *A. indica* had lead researchers to discover other new botanical insecticides especially from the Meliaceae family and *Aglaia* genus. Meliaceae plants have gained researcher attention as the species in this family have been found to contain promising compounds such as triterpenoids, liminods, benzofuran and flavanoids which can control insect pest (Murray, 2006, Li, 1999 and Silva *et. al.* 1984). Some research on *Aglaia* genus (Meliaceae) has found existence of triterpenoid and benzofuran which can effectively control some lepidopteran (Koul *et. al*, 2005, Isman *et. al.*, 2005, Nugroho *et. al.*, 1997). According to Isman (2006), there were four major types of botanical used for insect control such as pyrethrum, rotenone, neem and essential oils with others species with limitied use and this phenomena lead to discovery of new

botanical insecticides.

1.2.2 Minimize Insect Pest Resistance

The phenomena of insect pest resistance to chemical insecticides has multiplied and intensified considerably in recent years. In the early 1950s Georghiou (1986) reported a rare case of insect resistance. However, with the since increasing use of chemical insecticide, the resistance of insect pest has also increased. Once the pest species is resistant to one class of insecticide, the insect will evolve its resistance more rapidly to other groups of chemicals which have a similar mode of action or similar metabolic pathways (Tong, 2013). Georghiou and Mellon (1983) mentioned that over 400 insect species have developed resistance to one or more insecticides and Tong *et. al.* (2013) reported that *S. litura* have developed resistance towards organophosphate, carbamate and pyrethroids. Therefore there is an urgent need to develop and investigate *Aglaia* genus and its potential to replace chemical insecticides usage and reduce insect resistance. According to Satasook *et. al.* (1994), over 40 species of *Aglaia* showed high insecticidal activities against insect pest.

1.3 Objectives

The main objective of this study was to study the potential of *A. odoratissma* and *A. variisquama* leaves extracts towards *C. maculatus* and *S. litura* as a plant derived insecticide. The specific objectives of the studies are as follows:

i. to study insecticidal activities of *A. odoratissma* and *A. variisquama* leaves towards *C. maculatus* and *S. litura*
ii. to determine the potential chemical compound of *A. odoratissma* and *A. variisquama* leaves that effected *C. maculatus* and *S. litura*

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