UNIVERSITI PUTRA MALAYSIA

BIOACTIVITY OF AZADIRACHTA EXCELSA JACK. EXTRACTIVES ON SELECTED INSECTS

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BIOACTIVITY OF *AZADIRACHTA EXCELSA* Jack. EXTRACTIVES ON SELECTED INSECTS

By

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Dedicated especially to,

Mom and Dad,

My brother Dominic,

and

My beloved Christopher,

who constantly inspired and prayed for me

throughout the completion of this thesis.

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LIST OF ABBREVIATIONS

ANOVA = Analysis of Variance

AWPA = American Wood Preservers' Association

AZA = Azadirachtin

BRCs = Brain-ring Gland Complexes

CA = Corpora Allata

DMRT = Duncan's Multiple Range Test

IGR = Insect Growth Regulator

IR = Infrared

JPSM = Jabatan Perhutanan Semenanjung Malaysia
(Forestry Department Peninsular Malaysia)

LHW = Light Hardwood

MARDI = Malaysian Agriculture Research and Development Institute

MAPPS = Malaysian Plant Protection Society

M.C. = Moisture Content

MHW = Medium Hardwood

MSTAT-C = Microcomputer Statistical Program

MTIB = Malaysian Timber Industry Board

PROC GLM = Procedure Generalised Linear Model

PTTH = Prthoracicotropic Hormone

R_f = Retention Factor

RH = Relative Humidity

RRIM = Rubber Research Institute Malaysia

SAS = Statistical Analysis System

S.E. = Standard Error

(continued).....

LIST OF ABBREVIATIONS (continued)

TLC	=	Thin Layer Chromatography
UPM	=	Universiti Putra Malaysia (formerly known as Universiti Pertanian Malaysia)
UV	=	Ultraviolet
ν_{max}	=	Lambda max
w/w	=	Weight per weight

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Azadirachta excelsa Jack. is a fast growing timber species that has been newly chosen as a potential forest plantation species to overcome timber deficit for domestic consumption in future. This species like *Azadirachta indica* is suspected to contain biologically active compounds that is detrimental on insects. Many studies have been done on the *A. indica*, a relative species to *A. excelsa*, however, studies on *A.excelsa* are still in preliminary stages. Therefore, the aim of this study was to evaluate the effect of *A.excelsa* crude extractives towards three selected insect pests, (i.e. *Spodoptera litura*, *Callosobruchus chinensis* and *Coptotermes curvignathus*). A preliminary test to evaluate the susceptibility of *A.excelsa* wood towards termites, compared to *Koompasia malaccensis*, *Hevea brasiliensis* and *Pinus caribaea* was also conducted.

In the first part of the study, the *A.excelsa* plant extracts were extracted from different plant parts (i.e. leaf, bark, trunk, twigs and branch) using a range of polar to non-polar solvents, namely hexane, petroleum ether, toluene, ethyl acetate and methanol. The plant extractives were sampled and analysed through Thin Layer Chromatography (TLC) and Infrared Spectrocopy (IR). In the second part of the study, bioassay tests were conducted on the selected insects. The insects were exposed to the plant extractives prepared at three concentration levels (i.e. 250 ppm, 500 ppm and 1000 ppm) and the solvents (0 ppm) as the control, by feeding the insects with treated food source. The *S. litura* larvae were fed with castor leaf discs soaked in the respective extractives. The *C. chinensis* adults were exposed to mungbean seeds that had been soaked in the plant extrctives while the *C. curvignathus* were fed with treated filter paper. In the last part of this study, the *A. excelsa* wood together with the other three wood species mentioned earlier, were exposed to the termites both in the laboratory and field.

The TLC and IR results on the *A. excelsa* extractives showed that extractives from the different plant parts contained more non-polar chemical compounds. Among the five plant parts, the leaf contained the highest number of chemical compounds. The functional groups that were identified in the methanol and hexane extracts of leaf, bark and twigs, were hydroxyl, carboxyl, aromatic and unsaturated aldehydes.

The bioassay on *S. litura* showed that the larvae consumed less leaf discs that were treated with higher concentration of extractive. Mortality mostly occured at the early larval stages. Although less mortality occured at the lower concentration, but the moulting and larval periods were extended to twice the normal period and thus the larvae consumed more leaf discs. However, the growth parameters (i.e. head capsule measurement and pupal weight) at the last instar stage was not effected by the treatments. Generally, bark extract caused a higher mortality and less food consumption compared to the leaf extract.

The *A. excelsa* crude extract was not a good protectant on mungbean seeds. Results obtained from the treatments show that the oviposition rate and percentage adult emergence were not significant.

The termites bioassay test showed that *A.excelsa* extractives caused high termite mortality coupled with less consumption on filter paper treated with bark extracts. Choice feeding test on wood blocks from four different species showed that *A.excelsa* was the least preferred wood species compared to the other species, both in the field and laboratory trials.

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

BIOAKTIVITI EKSTRAK TUMBUHAN *AZADIRACHTA EXCELSA* JACK. TERHADAP SERANGGA-SERANGGA TERPILIH

Oleh

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Azadirachta excelsa Jack. merupakan spesis pokok kayu cepat tumbuh yang baru dipilih sebagai spesis tanaman ladang hutan yang berpotensi untuk mengatasi masalah kekurangan bekalan kayu bagi kegunaan tempatan pada masa akan datang. Spesis ini seperti *Azadirachta indica*, disyaki mengandungi komponen biokimia aktif di dalam ekstrak tumbuhannya yang boleh mengawal serangga perosak. Banyak kajian telah dijalankan terhadap spesis *A. indica*, yang merupakan spesis serupa dengan *A. excelsa*. Walau bagaimanapun, kajian - kajian terhadap *A. excelsa* masih lagi pada peringkat awal. Tujuan kajian ini dijalankan adalah untuk menilai kesan ekstraktif mentah *A. excelsa* terhadap spesis serangga-serangga perosak terpilih, iaitu *Spodoptera litura, Callosobruchus chinensis* dan *Coptotermes curvignathus*. Kajian awal untuk menguji kerentanan kayu *A.excelsa* terhadap anai-anai, dibandingkan dengan *Koompasia malaccencis*, *Hevea brasiliensis* dan *Pinus caribaea* telah juga dijalankan.

Pada awal kajian ini, ekstraktif tumbuhan *A. excelsa* telah diekstrak daripada bahagian-bahagian tumbuhan yang berlainan (i.e. daun, kulit kayu, batang kayu, ranting kayu dan cabang), dengan menggunakan pelarut julat polar hingga tidak polar. Antara pelarut yang digunakan adalah heksana, petroleum ether, toluene, etil asetat, dan metanol. Ekstraktif tumbuhan tersebut telah disampel dan dianalisa melalui "Thin Layer Chromatography" (TLC) dan "Infrared Spectroscopy" (IR). Dalam bahagian kedua kajian ini, ujian "bioassay" telah dijalankan terhadap serangga-serangga terpilih. Serangga-serangga tersebut telah didedahkan kepada ekstraktif tumbuhan yang telah dilarutkan kepada tiga kepekatan berbeza (i.e. 250 ppm, 500 ppm dan 1000 ppm) serta pelarut yang digunakan semasa ekstraksi sebagai kawalan (0 ppm), dengan memberikan makanan yang telah dirawat. Larva *S. litura* telah diberikan kepingan daun pokok jarah yang telah direndamkan ke dalam ekstraktif berkenaan. Serangga dewasa *C. chinensis* telah didedahkan kepada biji kacang hijau yang telah dirawat dengan ekstraktif tumbuhan *A. excelsa*, manakala *C. curvignathus* telah diberikan kertas turas yang telah dirawat sebagai sumber makanan. Pada bahagian terakhir kajian ini, kayu *A. excelsa* bersama dengan spesis kayu yang telah disebutkan lebih awal telah didedahkan kepada anai-anai di lapangan dan juga di makmal.

Keputusan analisa TLC dan IR terhadap ekstraktif *A. excelsa* menunjukkan bahawa ekstraktif daripada bahagian tumbuhan yang dikaji mengandungi lebih

banyak kompaun kimia yang tidak polar. Di antara kelima-lima bahagian tumbuhan, bahagian daun mengandungi bilangan komponen kimia yang paling banyak. Kumpulan berfungsi yang telah dikenalpasti di dalam ekstrak metanol dan heksana daripada daun, kulit kayu dan ranting adalah hidroksil, karboksil serta aldehid aromatik dan tidak tepu.

Ujian "bioassay" terhadap *S. litura* menunjukkan bahawa jumlah pemakanan daun bagi larva serangga ini kurang pada daun yang telah dirawat dengan ekstraktif pada kepekatan yang paling tinggi. Sungguhpun kematian larva rendah pada kepekatan yang paling rendah, namun jangkamasa pengelongosongan dan jangkamasa larva telah diunjurkan dua kali ganda lebih panjang daripada tempoh biasa. Oleh yang demikian, larva tersebut terus memakan daun yang diberikan. Walau bagaimanapun, ekstraktif tersebut tidak memberi kesan signifikan terhadap berat pupa dan juga saiz kulit kepala pada peringkat larva terakhir. Pada keseluruhannya, ekstrak kulit kayu menyebabkan kematian yang lebih tinggi dan pemakanan daun yang kurang berbanding dengan rawatan ekstraktif daun.

Ekstraktif mentah *A. excelsa* bukanlah pelindung biji kacang hijau yang baik. Keputusan yang diperolehi menunjukkan bahawa kadar peneluran dan peratus dewasa yang keluar adalah tidak signifikan.

Ujian "bioassay" terhadap anai-anai menunjukkan bahawa ekstraktif *A. excelsa* telah menyebabkan kematian anai-anai yang tinggi dan juga pemakanan yang rendah ke atas kertas turas yang dirawat dengan ekstrak kulit kayu . Kajian pemakanan pilihan (Choice-feeding Test) ke atas empat spesis kayu yang telah diberikan menunjukkan bahawa kayu *A. excelsa* adalah spesis yang kurang diminati berbanding dengan spesis kayu yang lain. Keputusan yang sama telah diperolehi dalam ujian lapangan dan juga makmal.

CHAPTER I

GENERAL INTRODUCTION

Many insect species are beneficial to human beings, especially as pollinators, source of food and also source of medicine. However, when insects compete with man for food and organic resources, while others spread diseases or cause discomfort they become pests. Particularly in agriculture and forestry, these insects constitute an important constraint to crop and timber production. Over the years, means have been developed to reduce drastically the harmful effects of these insect pests. To a large extent, this process has been achieved through trial and error, including most current control methods which are for the most part empirical.

Therefore, to alleviate these pest problems, extensive attempts were made using synthetic pesticides in order to control these insect pests. In Malaysia, for example, chemical insecticides have been widely used in combating insect pest outbreaks.

However, owing to the extensive use of chemical and synthetic insecticides, various drawbacks have been observed in man and the environment. These include toxicity to non - target organisms, development of pest resistance and environmental degradation (Schmutterer 1988, Anon.,1991). In developing countries, inadequate product knowledge, supply uncertainties and high prices cause inefficient pesticide use and also create additional socioeconomic problems between the rich and poor. Consequently, the search for new, preferably environmentally sound, effective and low-cost insecticides are thus needed. Due to this matter, interest in plant-based insecticides has grown rapidly during recent years.

Plant-based pesticides offer a safer, environmentally friendly and effective alternative to synthetic insecticides. Phytochemical extracts from the neem tree, *Azadirachta indica* A. Juss for instance, have been extensively studied in the last decade. They have shown several properties useful for the management of insect pests. These include repellency, feeding and oviposition deterrence, insect growth regulatory activity, low mammalian toxicity, and minimal persistence in the environment (reviewed in Schmutterer 1990, Koul and Isman 1991, Ascher 1993, Mordue and Blackwell 1993). Neem may also have less impact on natural enemies than most conventional insecticides (Hoelmer *et al.* 1990, Stark 1992, McCloskey *et al.* 1993, Lowery and Isman 1994). Larval feeding inhibition and insect growth regulation are due primarily to the tetranortriterpenoid compound azadirachtin (Isman *et al.* 1990), which is found in great concentrations in the seed kernels.