UNIVERSITI PUTRA MALAYSIA

MOLLUSCICIDAL ACTIVITY OF LEGUMES, YELLOW FLAME (*PELTOPHORUM PTEROCARPUM*) AND RAINTREE (*SAMANEA SAMAN*) ON FRESHWATER SNAILS; *INDOPLANORBIS EXUSTUS* (PULMONATA: PLANORBIDAE) AND *RADIX QUADRASI* (PULMONATA: LYMNAEIDAE)

AMAL IBRAHIM KHALIFA BILAL

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BY

AMAL IBRAHIM KHALIFA BILAL

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

April 2002

DEDICATION

This thesis is Dedicated to

My husband,

Khalid Marol Riak

My Daughters,

Moun Khalid Marol

Awut Khalid Marol

Your motivation, sacrifice and support during the period of my academic mission is appreciated.

My parents

Ibrahim Madiet Bilal &Mother Aza Sharaf Elddin Hussein & Elder sister

Haja

Your prayers and encouragements that made me whom I am today is very much acknowledge

My brothers and sisters

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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Chairman: **Associate Professor Jambari Hj Ali, Ph.D.**

Faculty: **Science and Environmental Studies**

Acute toxicity by static bioassay of ground dried leaves (medium age) of leguminoses, yellow flame (*Peltophorum pterocarpum*) and rain tree (*Samanea saman*), in the form of ground powder solution, crude water and methanol extract was determined against target freshwater snails, *Indoplanorbis exustus* (Planorbidae) and *Radix quadrasi* (Lymnaeidae), and also on non-target species, red tilapia, *Oreochromis niloticus,* and shrimp, *M*acrobrachium *lanchesteri* using static bioassay technique. The field-collected snails were examined for the infection of trematode larvae. *I. exustus* was found to be the host to the two types of trematodes larvae (cercaria), namely furcocercous cercariae, bifurcated cercaria (schistosoma) and gymnocephalus cercariae, non-bifurcated tail cercaria (fasciola), whereas, *R. quadrasi* was found to be the host to the various types of gymnocephalus cercariae. The toxicity results indicated that molluscicidal and piscicidal activity is not limited to any particular plant species and that the dried ground leaves powder, crude water, and methanol extract; of *P. pterocarpum* and *S. saman* are toxic to the target and non-target species. However, toxicity of the

crude methanol extracts of these plants exhibited the highest potency as compared to the crude water extract and dried ground leaves. The 24 h LC_{50} of crude water and methanol extract of *P. pterocarpum* against the target species was found to be within the standard range of World Health Organization (≤100 mg/l) of being molluscicidally active. Based on the 24h LC_{50} values, the results indicated that the potency of *P. pterocarpum* treatments on the target snail species follow this trend; crude methanol extract (50.7-55.6 mg/l) was the most potent, followed by crude water extract (64.9-72.7 mg/l) and the dried, ground powder (338.2-390.4 mg/l). Comparison test between *I. exustus* and *R. quadrasi* showed that *R. quadrasi* was more sensitivity to crude methanol extract of *P. pterocarpum* than the *S. saman*, with 24h LC_{50} value of 50.7 mg/l and 108 mg/l, respectively. Test carried out on the non-target species, shrimp, *M. lanchesteri* was observed to be virtually absence of the toxic effect when exposed at the concentrations that kill 50% of the target snail species. But, red tilapia, *O. niloticus* was more susceptible and LC_{50} was obtained at the lower concentrations than the concentration that caused 50% mortality of the target snail species. The relationship of 24h LC_{50} values to the different snail shell length of laboratory breed *R. quadrasi* and *I. exustus* was also investigated using crude methanol extract of *P. pterocarpum*. Results indicated that the relationship between different shell length of *R. quadrasi* and 24h LC_{50} was a positively correlated with r = 0.98, but the relationship was polynomial (quadratic) with the equation line of $Y= 0.63 x^2 –8.5x +42.7$. In the case of *I. exustus* there was positive correlation between its sensitivity and its sizes, and relationship was linear with regression line of Y=2.77 x +8.3 and r of 0.96.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan penganugerahan ijazah Master Sains

AKTIVITI MOLLUSID DARI LEGUM YELLOW FLAME (*PELTOPHORUM PTEROCARPUM*) DAN RAIN TREE (*SAMANAE SAMAN*) TERHADAP SIPUT SIPUT AIR TAWAR *INDOPLANORBIS EXUSTUS* (PULMONATA: PLANORBIDAE)DAN *RADIX QUADRASI*(PULMONATA: LYMNAEIDAE)

Oleh

AMAL IBRAHIM KHALIFA BILAL

April 2002

Pengerusi: **Profesor Madya Dr. Jambari Haji Ali**

Fakulti: **Sains dan Pengajian Alam Sekitar**

Ujian ketoksikan akut daun muda leguminosa batai laut (Peltaphorum pterocarpum) dan hujan-hujan (Samanae saman) yang dikeringkan telah ditentukan dalam bentuk larutan serbuk, ekstrak kasar air dan ekstrak metanol terhadapa dua jenis siput air tawar sasaran iaitu Indoplanorbis exutus (Planorbidae) dan Radix quadrasi (Lymnaeidae), sementara spesies bukan sasaran ialah tilapia merah (*Oreochromis niloticus*) dan udang (*Macrobrachium lanchesteri*) menggunakan teknik bioassai statik. Siput-siput yang dikutip dari lapangan telah diperiksa kandungan larva trematod (cercaria) iaitu ekor bercabang (schistosoma) dan tidak bercabang (fasciola). Sementara siput R.quadrasi pula menjadi perumah kepada pelbagai jenis cercaria ekor tidak bercabang (gymnocephalus cercariae). Keputusan toksisiti menunjukan aktiviti mollusisid dan pisisid tidak terhad kepada spesies tumbuhan tertentu. Dalam bentuk serbuk ekstrak air air dan metanol kasar P.pterocarpum dan S.saman juga adalah toksik kepada kedua-dua spesies siput sasaran dan organisma bukan sasaran. Ekstrak metanol

kasar didapati memiliki keupayaan keracunan yang paling tinggi jika dibandingkan dari ekstrak kasar air dan serbuk. Nilai LC_{50} 24 jam ekstrak metanol dan air kasar P.pterocarpum didapati mempunyai aktiviti mollusisid dalam linkungan kepiawaian WHO (<100 mg/l) bila diuji dengan spesies sasaran. Nilai LC_{50} 24 jam untuk P.pterocarpum menghasilkan tahap kekuatan keracunan seperti berikut; ekstrak metanol kasar (50.7-55.6 mg/l) iaitu paling kuat, diikuti oleh ekstrak air kasar (64.9-72.7 mg/l) dan serbuk (338.2-390.4 mg/l). Perbandingan antara kedua siput mendapati R.quadrasi adalah lebih sensitif terhadap ekstrak metanol kasar P.pterocarpum berbanding S.saman dengan nilai LC_{50} 24 jam masing-masing 50.7 mg/l dan 108 mg/l. Ujian terhadap spesies bukan sasaran mendapati udang (M.lanchesteri) tidak mengalami sebarang kesan keracunan apabila didedahkan kepada kepekatan yang membunuh 50% spesies sasaran siput. Walau bagaimanapun, tilapia merah (O.niloticus) adalah lebih sensitif dimana nilai LC_{50} telah diperolehi pada kepekatan yang lebih rendah daripada kepekatan yang membunuh 50% spesies sasaran siput. Perkaitan nilai LC_{50} 24 jam diantara keracunan ekstrak metanol mentah P.pterocarpum terhadap beberapa saiz siput R.quadrasi dan I.exustus (yang diternak dalam makmal) juga telah diuji. Keputusan korelasi positif terdapat diantara LC_{50} 24 jam dengan siput berbagai saiz dengan r=0.98 dan perhubungannya tidak linear dengan persamaan garis $Y= 0.63X^2 -8.5 x +42.7$. Bagi I.exustus, pula terdapat korelasi positif antara saiz dengan sensitivitinya dan perhubungannya adalah linear dengan garisan regressi Y=2.77 x +8.3 dan 0.96.

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

%	: Percentage
≤	: Less or equal to
>	: More potent
≥	: More or equal
µg/ml	: Microgram per litre
~	: Approximately
^0C	: Degree centigrade
cl	: Confidence limit.
cm	: Centimetre
g	: Gram
GST	: Glutathione- S- transferase
ha^{-1}	: Per hectar
h	: Hour
kg/ha	: Kilogram per hectar
LC_{50}	: Concentration of toxicant sufficient to kill fifty percent of the test animal within a given period.
LC_{90}	: Concentration of toxicant sufficient to kill ninety percent of the test animal within a given period.
m^{-2}	: Per meter square
mg	: Milligram
mg/kg	: Milligram per kilogram

mg/l	: Milligram per litre
ml	: Millilitre
mm	: Millimetre
NaPCP	: Sodium Pentachlorophenol
OECD	: Organization of Economic Cooperation and Development
PCP	: Pentachlorophenol
pH	: Hydrogen concentration
ppm	: Part per million
r	: Coefficient correlation
UKM	: Universiti Kebangsaan Malaysia
UPM	: Universiti Putra Malaysia
US$	: United States Dollar
WHO	: World Health Organization
X	: Times

CHAPTER ONE

INTRODUCTION

1. 0 General Introduction

Molluscicides are chemicals or toxic agents designed specifically to kill various types of molluscs (Cremlyn, 1978). Molluscs although harmless to human, some species affect man in several direct and indirect ways. Molluscs, such as snails and slugs, cause considerable damage to a wide range of agricultural and horticultural crops, as well as gardens. Snails and slugs can caused considerable damage to young establishing seedling and mature leaves (Temeharoen, 1992). Crops such as rice in Southeast Asia (Suryanto, 2000) and tobacco in Malawi (Meredith, 1983) suffered severe attack by snail and slugs. Damage may also occur by direct feeding of harvested product like holing of potato tubers by slug.

Some species of molluscs' especially freshwater snails are known as intermediary hosts of human and animals (birds and mammals) parasitic trematodes (such several species of infectious Helminths). Most of these snails belong to a variety of genera such as *Oncomelania, Biomphalaria, Lymnaea, Planorbis, Marisa, Physa, Polypylis* and *Bulinus* (Faust *et al.*, 1975).

In the control programs of snail borne diseases snails, synthetic molluscicides have been used to reduce snail populations as an attempt to interrupt the parasite's life cycle (El Khoby *et al.*, 1998). However, mollusciciding has to be a long-term commitment, if it is to have a lasting impact against the disease. Furthermore

treatment of the extensive areas where trematodes diseases is endemic would require immense quantities of molluscicides. The cost of such quantities, if the molluscicide is synthetic and has to be imported, is beyond the economic reach of developing countries. Considerable infrastructure and logistical problems exist in supplying any chemicals to rural areas where people depend on irrigated farms. The application of synthetic molluscicides requires training and understanding in calculating the correct dosages and, in some cases, to prevent hazards arising from inappropriate use. Plant molluscicides especially in the countries, which are rich in term of plant diversity like tropical rain forest of Malaysia offer a possible alternative, as they can be made readily available in rural areas and tend to be easier and safer to use. Perhaps most importantly, the simple preparation and application methods available for the plant products should enable rural communities to operate snail control programs themselves, after initial assistance from the scientist, and local personal involved in the primary health care.

1. 1. Problem Statement.

Plants play a very important role in human life. Besides being major source of food, plants have numerous other practical applications such as for shelters, flavourings, and preservatives. In addition fine chemicals derived from plants have also been widely used, in pharmaceuticals, in pest control and management and as dyes.

Studies on plant molluscicides in the control of amphibious and freshwater snail as intermediate host of trematode parasitic diseases have been conducted in

several endemic sites in Latin America, Africa, and some part of Asia (Lemma *et al.*, 1978; Marston and Hostettmann, 1985; Marston *et al.*, 1993; Brackenbury and Appleton, 1997a; Allen *et al.*, 1998; Rug and Ruppel, 2000; Al-Zanbagi *et al.*, 2001). Most of these studies entail the control of intermediate hosts particularly those linked with transmission of human schistosomiasis (bilharzia), which is a parasitic disease endemic throughout South America, Africa, and East Asia. It affects more than 250 million people in over 76 countries (D'Arcy and Harron, 1983).

In Malaysia and other part of Southeast Asian countries, studies on the control of snails as an intermediate host, have examined the use of biological control method, namely trematode antagonism (Lie, 1963; Lie, 1972; Jambari, 1976). Freshwater snails such as *I. exustus* and *R. quadrasi* have been studied only in general and importance of their role as parasitic hosts to trematodes has not been studied in details.

While chemotherapy with orally administered anti-trematodal or anti-helminthic drugs (oxamniquine and praziquantel) is a viable method for curing human, animal and avian infected with the parasites, the use of plants with molluscicidal properties is simple, inexpensive, and appropriate technology for local control of the intermediate host snail (Marston and Hostettmann, 1985).

Studies on the use of local available plant derived molluscicides in the control of harmful and medically important freshwater snails have not been extensively conducted in southeast Asia, where prevalence of fluke parasite of domestic ducks, village chicken, cattle and buffalo is highly associated with the production of

irrigated rice. Such studies are needed to refine the accuracy of utilization of locally available plant resources in the control and prevention of damage cause by snail borne diseases to domestic livestock and human. Knowledge gained from such studies will be very important in the development of effective control and management plan for harmful freshwater snail in general and *I. exustus* and *R. quadrasi* in particular.

1. 2. Objectives

The main objective of this study is to examine the molluscicidal activity of yellow flame (*P. pterocarpum)* and rain tree (*S. saman)* against the target medically important snail, *I. exustus* and *R. quadrasi* and the non-targeted species such as fish and shrimp, which are normally coexist with the snails in the paddy field ecosystem.

The specific objectives are to:

1. Evaluate the individual toxicity potentials of yellow flame and rain tree prepared as dried powder leaves, crude water and methanol extracts on freshwater snails *I. exustus* and *R. quadrasi.*
2. Investigate the potency of these plants on some non-target aquatic organisms such as red tilapia (*Oreochromis niloticus)* and shrimp (*Macrobrachium lanchesteri*).

CHAPTER TWO

LITERATURE REVIEW.

2. 0. Introduction

The importance of freshwater and amphibious snails as an intermediate host of pathogenic trematode parasites has been gradually recognized with the advent of the interest of biologist (Malek and Cheng, 1974; El Khoby *et al*., 1998). For the control of the trematodes infectious diseases, multifaceted approaches are desirable (El Khoby *et al.,* 1998), including control of the intermediate host snails.

Among freshwater and amphibious snails that are of medical, veterinary and economical importance in transmitting trematode diseases are *Biomphalaria* species which, are found in Africa, Saudi Arabia, Yemen, South Western Asia and the Caribbean (Brackenbury and Appleton, 1997a). The genus *Bulinus*, which is implicated to be an intermediate host of S*chistosoma haematobium,* is found in Africa, Middle East, and South Europ. *Indoplanorbis annandale,* a genus in subfamily Buliminae occurs in India, Thailand, the Malay Peninsular, and Sumatra. *I exustus* is the intermediate host for several trematodes species parasitic to livestock in Asia, among which are *Schistosoma spindale, S. indicum, S. nasale* (Brown, 1994), *Hypoderaum dingeri* (Lie *et al.*, 1974) and the amphistome *Gastrodiscus secudus* parasitic as an adult in the equines (Malek and Cheng, 1974). The species of *Drepanotrema*, member of Helisomatinae, genus *Planorbarious* are reported to be