LARVICIDAL ACTIVITY OF RAW SENTANG LEAVES AGAINST *AEDES AEGYPTI* (LINNEAUS)

by

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of the requirements for the degree
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CERTIFICATE

This is to certify that the dissertation entitled

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ABSTRACT

Sentang (*Azadirachta excelsa*) that have been reported to possess larvicidal properties mainly due to the nature of the specific chemical constituents found in the plant namely azadirachtin. The purpose of the study was to evaluate the toxicity of raw sentang leaves as larvicide against *Aedes aegypti* mosquitoes under laboratory conditions. Sentang leaves were grinded and stirred in water to make treatment of different ranging from 400 to 2000 ppm. Bioassays on the toxicity of the solutions were conducted out against the third instar larvae of *Aedes aegypti* for a period 21 days. The result of the first 6 days showed that the solution of higher concentration (1600 and 2000 ppm) caused high mortality compare to the lower concentration and control. At day 6, the percentage of cumulative mortality for the 1600 ppm is 28.0% and 2000 ppm is 42.4%. However, at day 12 onwards larvae of *Aedes aegypti* mosquitoe seems can survive in the treatment when compared to the control that shows highest mortality (93.6% cumulative mortality at day 21). It is suggested that sentang's leaves also provide some nutrient to the larvae for its survival and at the same time inhibit the growth and development of larvae. The results obtained from this study indicated a promising role of sentang as botanical larvicide against *Aedes aegypti* mosquitoes.

ABSTRAK

Sentang (*Azadirachta excelsa*) merupakan salah satu tumbuhan yang dilaporkan mempunyai aktiviti larvisid disebabkan bahan kimia yang terdapat di dalamnya, iaitu azadirachtin. Tujuan kajian ini dijalankan adalah untuk menilai ketoksikan bahan kimia yang terdapat di dalam daun sentang terhadap larva nyamuk *Aedes aegypti* di dalam persekitaran makmal. Untuk tujuan ini, suatu amaun daun sentang akan dilumatkan dengan air untuk menghasilkan larutan. Kepekatan yang digunakan ialah dari 400 ppm hingga 2000 ppm. Kesan ketoksikan daun sentang ini diuji terhadap instar ke tiga larva *Aedes aegypti* selama 21 hari. Keputusan menunjukkan bahawa untuk 6 hari pertama, pada kepekatan larutan yang lebih tinggi(1600 dan 2000 ppm), menyebabkan kematian yang tinggi jika dibandingkan dengan kepekatan yang lebih rendah dan kawalan. Pada hari ke 6, peratus kematian melonggok untuk kepekatan 1600 ppm ialah 28.0%, manakala 2000 ppm ialah 42.4%. Walaubagaimanapun, bermula dari hari ke 12, larva *Aedes aegypti* yang dirawat seakan dapat terus hidup apabila dibandingkan dengan eksperimen kawalan yang menunjukkan peratusan kematian yang lebih tinggi (96.6% kematian melonggok pada hari ke 21). Hasil kajian mencadangkan bahawa daun sentang juga memberikan nutrien untuk larvae terus hidup tetapi dalam masa yang sama merencat tumbesaran dan perkembangan larva untuk menjadi nyamuk dewasa. Hasil kajian yang diperoleh ini menunjukkan bahawa sememangnya daun sentang boleh bertindak sebagai larvisid terhadap nyamuk *Aedes aegypti.*

1.0 INTRODUCTION

Mosquitoes are familiar and annoying pests of humans and animals. Their rapid wing movement produces a distinctive high-pitch hum, and their bites cause red, itchy welts. Mosquitoes are more than nuisance, because they also serve as carriers (vectors) of several disease-causing agent. *Aedes aegypti* is generally known as a vector for dengue fever and yellow fever. Dengue fever has become an important public health problem as the number of reported cases continue to increase, especially with more severe forms of the disease, dengue haemorrhagic fever and dengue shock syndrome, or with unusual manifestations such as central nervous system involvement (Choochote *et al.*, 2004).

Over the last 5 decades the indiscriminate use of synthetic insecticides in agriculture and public health programs for the control of pest species has created multifarious problem viz. insecticide resistance, environmental pollutant, toxic hazards to human and other non target organisms. In attempt to overcome these problems, it is desirable to develop new measures with environmental friendly, safe and easily available at low cost. In view of this, studies on natural plants products as larvicides have indicate that they could provide possible alternatives to synthetic chemical insecticides (Thomas *et al.*, 2004).

In recent years, several reviews have been published which outline the use of the Neem tree, *Azadirachta indica* (Meliaceae) as a botanical insecticide. The mode of action of azadirachtin, the principal insecticidal constituent of Neem oil has only recently been elucidated (D. Casey Sclar, 1994). Azadirachta excelsa (sentang) is the species that closely related to neem, which has a more westerly distribution and grows in dryer areas.

Intermediate forms (hybrids) are believed to occur where the distribution of the species overlaps. The genus is closely related to *Melia*, in which it was formerly included (Joker, 2000).

It is generally accepted that the tetranotriterpenoid (also called limonoid) compound azadirachtin is responsible for the majority of biological effects observed in organisms exposed to Neem compounds. However, 25 different biologically active compounds have been isolated from Neem seeds. Other compounds present in Neem oil are responsible for some of the biological activity observed. Within the azadirachtin molecule, the decalin fragment is responsible for the insect growth regulation and development effects observed, while the hydroxy furan fragment causes the antifeedant effects more widely observed among target species. The insect growth regulatory (IGR) and antifeedant effects of azadirachtin are independent of each other, but both remain relative to concentration (Sclar, 1994).

Due to azadirachtin, sentang is seen has the potential as larvicide. The objective of this study is to investigate whether raw sentang leaves is adequate to give the larvicidal activity against larvae of *Aedes aegypti*. This paper reports the larvicidal activity of sentang against late third instar larvae of *Aedes aegypti*. This project proposes the use of non-infected and laboratory-breed *Aedes aegypti* under caged condition as a cost effective mosquito model in this study.

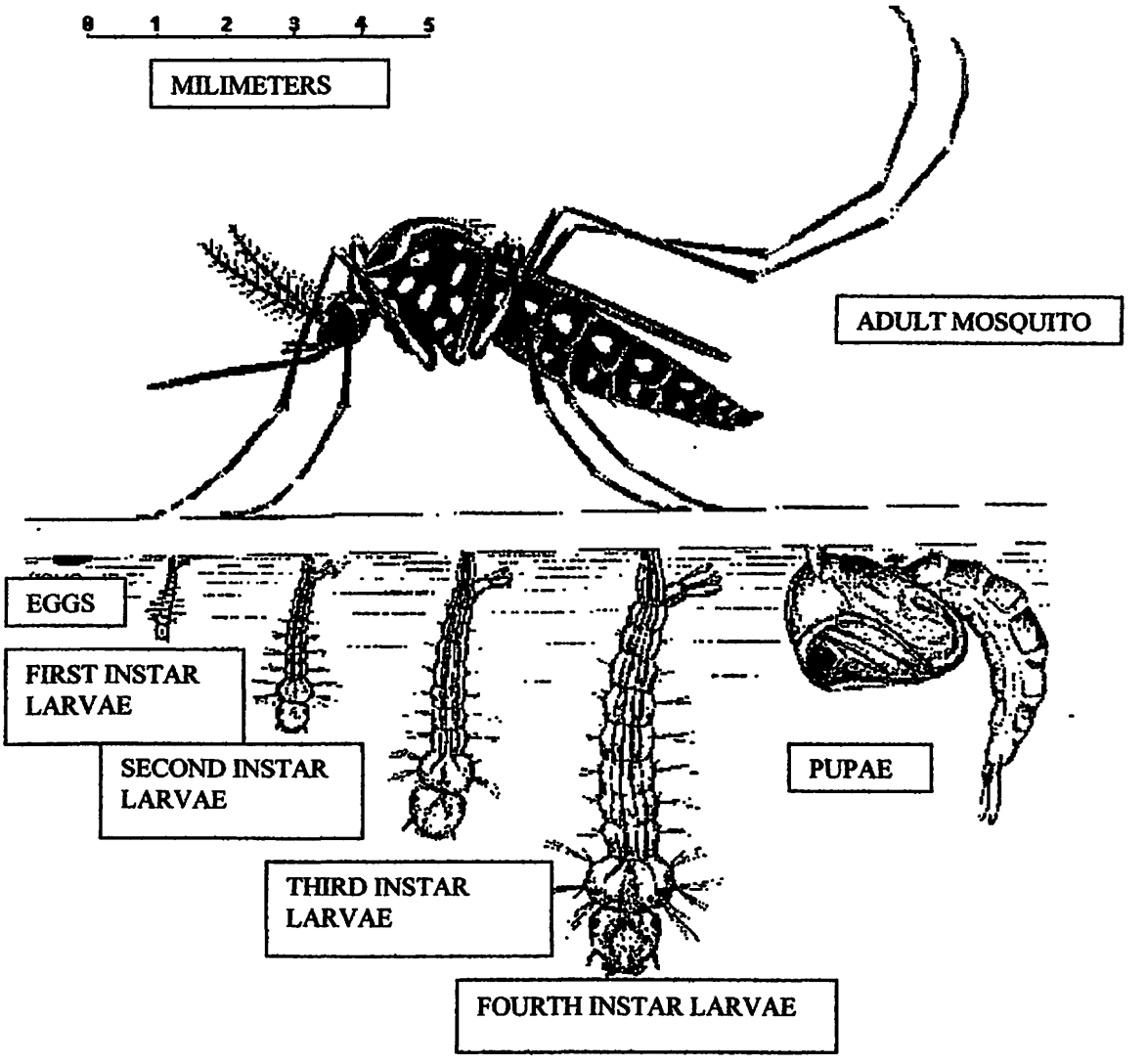
2.0 LITERATURE REVIEW

2.1 *Aedes aegypti* mosquitoes

The *Aedes aegypti* mosquito is the primary carrier for viruses that cause Dengue Fever and Yellow Fever. Often called the "Yellow Fever Mosquito", *Aedes Aegypti* is an early morning or late afternoon feeder, but will also bite at night under artificial illumination. Habitats are often found close to human areas and the mosquitoes will often bite indoors or in sheltered areas near the home. Human blood is preferred over other animals, with ankles as the favorite area. *Aedes aegypti* breed in artificial containers (flower pots, rain gutters, bird baths) and eggs can resist desiccation for up to one year. The eggs will hatch when flooded by deoxygenated water. *Aedes aegypti* fly only a few hundred yards from its breeding area (Beats *et al.*, 1993).

2.1.1 Life cycle of *Aedes aegypti*

Like all insects, mosquitoes hatch from eggs and go through several stages in their life cycle before becoming adults. The females lay their eggs in water and the larva and pupa stages live entirely in water. When the pupa changes into adults, they leave the water and become free-flying land insects. The life cycle of a mosquito can vary from one to several weeks depending upon the species (Figure 2.1).



Source from PRDU Reitoria (http://www.prdu.unicamp.br/dengue/mosquito.html)

Figure 2.1: Life cycle of *Aedes aegypti*

2.1.1.1 Eggs

Oviposition takes place in the afternoon in dark-colored water containing organic material (e.g., decaying leaves) in dark-colored containers with wide openings. Containers located in the shade are preferred. Females lay their eggs singly on the sides of the container at the water line in batches of 30-50. Egg laying occurs over a period of several days. Exposure to high humidity at the water line for 2-3 days is required for larvae to hatch from their eggs. However, if the eggs dry out before this development period, they will collapse and the embryos will die. If they remain unhatched above the water line, and the level of humidity is sufficient to permit larval-embryo development, eggs become "cured." This means they are resistant to desiccation and can survive for upwards of six or more months. They can also survive short periods of subfreezing weather. Later, when exposed to water, the eggs will hatch within a day or perhaps even within minutes. The eggs do not all hatch with a single inundation, however. Instead, they hatch in progressively smaller numbers through a succession of inundations (Reiter *et al.*, 1995).

2.1.1.2 Larvae

The 4 larval stages (instars) take 5-10 days for development (some texts say 5-7 days). The mosquito eggs hatch into larvae or "wigglers," which live at the surface of the water and breathe through an air tube or siphon. The larvae filter organic material through their mouth parts and grow to about 0.5 to 0.75 inches (1 to 2 cm) long; as they grow, they shed their skin (molt) several times. Mosquito larvae can swim and dive down from the surface when disturbed. The larvae live anywhere from days to several weeks depending on the water temperature and mosquito species (Freudenrich, 2005).

2.1.1.3 Pupae

Transformation from the pupal stage to the adult stage generally takes 2-3 days. Under the most favorable climatic and environmental conditions, the entire immature or aquatic cycle (i.e., from egg to adult) can occur in as little as 10 days (Reiter *et al.*, 1995).

2.1.1.4 Adults

Inside the pupal case, the pupa transforms into an adult. The adult uses air pressure to break the pupal case open, crawls to a protected area and rests while its external skeleton hardens, spreading its wings out to dry. Once this is complete, it can fly away and live on the land. One of the first things that adult mosquitoes do is seek a mate, mate and then feed. Male mosquitoes have short mouth parts and feed on plant nectar. In contrast, female mosquitoes have a long proboscis that they use to bite animals and humans and feed on their blood (the blood provides proteins that the females need to lay eggs). After they feed, females lay their eggs. Females continue this cycle and live anywhere from many days to weeks (longer over the winter); males usually live only a few days after mating. The life cycles of mosquitoes vary with the species and environmental conditions. The life span for adult mosquitoes is between 2 weeks to a month or more (Reiter *et al.*, 1995).

2.2 Larvicides

Larviciding is a general term for the process of killing mosquitoes by applying natural agents or commercial products designed to control larvae and pupae (collectively called larvicides) to aquatic habitats. Larvicide treatments can be applied from either the ground

or air. Larviciding was implemented as a malaria control procedure in the early 1900's and over the years has become prominent. Commercially available and experimental larvicides plus natural control agents available in Florida during FY 1994-95 are discussed below. Arbitrarily, they are categorized by their modes of entry/action on target/non-target organisms: Contact poisons, surface control agents, stomach poisons, and natural agents (Table 2.1) (FCCMC, 1998).

2.2.1 Contact poisons

As the name implies, this loosely defined group of compounds is effective when mosquito larvae or pupae come in contact with it. Chemicals are absorbed through the insects outer "skin" or cuticle and may be incidentally ingested or enter the body through other routes. Contact agents can be further subdivided into two sub-groups: 1) Toxins primarily affecting insect's nervous system and 2) toxins primarily affecting insect's endocrine system. The nervous system agents used for mosquito larvicides in Florida during FY 94-95 include many formulations of the organophosphate compound temephos, plus one formulation of natural pyrethrum. Endocrine system agents used in this period include many s-methoprene formulations and one diflubenzuron product (FCCMC, 1998).

2.2.2 Surface control agents

Larvicides in this category include oils and ethoxylated isostearyl alcohols. Oils were first used as effective anopheline larvicides for malaria control in Florida in the early 1900s.

Table 2.1: Major commercial larvicides used to control mosquito larvae in Florida

Name	Category	Trade Name	Formulation	Benefit	Risk/Disadvantage
Temephos	Cotact pesticide	Abate	G,EC	economical, highly effective chemical to use for a variety of mosquito species	not a selective insecticide
Pyrethrum	Contact pesticide	Pyrenone, pyronyl	ULV, EC	quickly dispatches both mosquito larvae and pupae	is toxic to fish, shrimp, crabs, and other aquatic organisms
s-methoprene	Contact pesticide	Altosid	G,B,P,LC	safe for workers and environmentally friendly, does not bioaccumulate.	applicator cannot detect a control failure until it is too late for additional larviciding
Oils	Surface control agent	Golden Bear 1111, BVA	mineral oil and inert ingredients, including surfactants, emulsifier.	good spreading characteristics	produces a thin "oil slick" on the water surface
Bacillus thuringiensis israelensis (Bti)	Stomach toxins	Aquabac, Bactimos, LarvX, Teknar, Dunks	WDG, AS, P,G,B	has a highly specific mode of action; minimal environmental concern and leaves no residues, and it is quickly biodegraded	-harmful if inhaled or absorbed through the skin -may cause minor eye irritations in humans
Bacillus sphaericus (Bs)	Stomach toxins	VectoLex	G, WDG	able to control mosquito larvae in highly organic aquatic environments, including sewage-waste lagoons, animal-waste ponds, and septic ditches	not regenerate in salt water, rendering its use impractical for control of saltwater mosquitoes
Lagenidium giganteum	Natural agent	Liginex AS	*L. giganteum* (California strain) mycelium, inert ingredients	very host specific, studies have demonstrated no mammalian or non-mammalian toxicity	is a living product, it has a shorter shelf life than chemical pesticides
Biological organisms	Natural agent	Nematodes, Protozoans, Algae, Copepods, Predacious Fish	-	host-specific- affords minimal disturbance to non-target species and to the environment.	The specificity also deters commercialization and application of some biocontrol agents

AS= aqueous suspension; B= Briquets; EC; emulsifiable concentrate; G Granules; LC= Liquid concentrate; P= Pellets; ULV= Ultra low Volume; WDG= Water-dispersible Granule

(FCCMC, 1998)

Commonly used larviciding oils kill larvae and pupae when inhaled into the tracheae along with air at the water's surface. With low dosages (1 gal. on per ac.), they can work very slowly, taking four to seven days to give a complete kill. Higher dosage rates are usually used (up to 5 gal. per ac.) to lower the killing time. Heavy oils can suffocate but only at very high dosage rates. This oil use is not common today as it was historically. The detrimental impacts of oil treatments may actually be intensified by the elimination of many mosquito predators such as notonectids and diving beetles. The larviciding oils are probably the least studied of the mosquito larvicides (FCCMC, 1998).

2.2.3 Stomach poisons

Mosquito control has two stomach toxins whose active ingredients are manufactured by bacteria. These control agents are often designated as bacterial larvicides. Their mode of action requires that they be ingested to be effective, which can make them more difficult to use than the contact toxins and surface control agents. Bacteria are single-celled parasitic or saprophytic microorganisms that exhibit both plant and animal properties and range from harmless and beneficial to intensely virulent and lethal (FCCMC, 1998).

2.2.4 Natural agents

This group of larvicides is comprised of biological organisms. The use of biological organisms to combat pest insects, such as mosquitoes, is termed biological control or biocontrol. Generally, this definition includes natural and genetically modified organisms and means that the agent must be alive and able to attack the mosquito. The overall premise is simple: biocontrol agents that affect mosquitoes naturally are reared artificially

and then released into the environment, usually in far greater numbers than they normally occur, and often in habitats that previously were devoid of them. Research on predators of mosquitoes, such as fish, dragonflies, and frogs is ongoing. Currently, there is no small- or large-scale program for application developments of these organisms. As the development of mosquito control technology moves forward, new biocontrol agents will be discovered. Ultimately, it is hoped that biological control will become an integral part of Florida's mosquito control programs (FCCMC, 1998).

2.3 Resistancy of mosquito larvae to larvicides

The continued use of insecticides likes DDT, malathion and temephos has resulted in the development of resistance in some strains of *Aedes aegypti* due to increased insecticidal selection pressure. Polson *et al.*, (2001) reported that there is some amount of reduced sensitivity to malathion and temephos in Caribbean populations of larval *Aedes aegypti* mosquitoes. Results showed that there were varying degrees of sensitivity to malathion and temephos among and within the countries from which the *Aedes aegypti* were taken. Besides that, in other research he also reported that resistance of *Aedes aegypti* to temephos appears to be incipient in Cambodia. Results showed that, when compared with the WHO diagnostic dosage of 0.02mg/l, the Phnom Penh population was resistant (LC95: 0.034mg/l).

2.4 *Azadirachta excelsa*

Sentang or its scientific name *Azadirachta excelsa* is from the family of Meliaceae. In Philippine, it also known as marango or "birdseye kalanthus" whereas in Thailand people called them tiam or sadao-tiam. In Sabah and Sarawak the common name for this plant is manggau and limpaga (Helen B. Florida & Priscilla B. de Mesa, 2001). The species is closely related to neem, *Azadirachta indica* A.Juss., which has a more westerly distribution and grows in dryer areas. Intermediate forms (hybrids) are believed to occur where the distribution of the species overlaps. The genus is closely related to *Melia*, in which it was formerly included (Joker, 2000).

Azadirachta excelsa growth naturally in a lowland humid forest species of South East Asian – Pacific region that mainly grows in old clearings or old secondary forest but also in primary dipterocarp forest. Native to Peninsular Malaysia, Sumatra, Borneo, Sulawesi, the Philippines, the Aru Islands and New Guinea. Widely planted, although rarely outside its native region. It is found up to about 350 m altitude and best growth is obtained in areas with annual rainfall of more than 2000 mm, mean annual temperature of 22-27°C and with a dry season of no more than 2 – 3 months. It does not tolerate cold or frost. Requires good quality soil, preferably sandy-loam soils with good drainage and aeration, with pH of 5.0-6.5. Growth rates on level land are better than on slopes or in mountainous areas. There are no breeding trials or known provenance trials for *A. excelsa*. Current planting material originates almost exclusively from unselected trees (Joker, 2000).

Deciduous tree up to 50 m tall, bole up to 125 cm in diameter, without buttresses. Leaves paripinnately compound, up to 60 (-90) cm long, with 7-11 pairs of leaflets. Leaflets asymmetrical, lanceolate to elliptical, up to 12.5 cm long and 3.5 cm, and wide (Plate 2.1). Flowers small greenishwhite in up to 70 cm long panicles. The fruit is a 1-seeded, oblong drupe, 2.5-3.2 cm long with fleshy mesocarp with white latex. Young fruits are green, turning yellow at maturity. The seeds are 20-25 mm long, 10-12 mm wide (Joker, 2000).

Sentang wood is valued for light construction, furniture, panelling and veneer. The young shoots and flowers are consumed as a vegetable. The tree is commonly planted along roadsides, and farm boundaries or in rubber plantations. Like neem, the seeds contain azadirachtin, which is used as an insecticide. In agroforestry, young plantations of *A. excelsa* are used for intercropping with rice, peanuts, mung beans, soybeans and vegetables (Dorthe Joker, 2000).

(a)

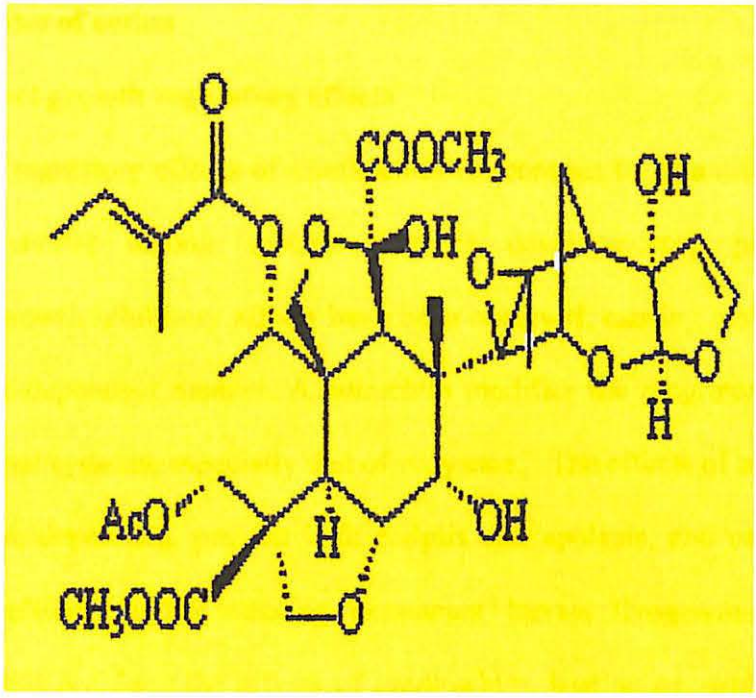
(b)

Plate 2.1: (a) The sentang tree and (b) leaves of sentang tree

2.4.1 Chemical composition and toxicity

All biologically active Neem compounds are suspected to be derived from one parent compound, the tetracyclic triterpenoid tirucallol. All other products formed are considered successive rearrangement and oxidation products of tirucallol. It is generally accepted that the tetranotriterpenoid (also called limonoid) compound azadirachtin (Figure 2.2) is responsible for the majority of biological effects observed in organisms exposed to Neem compounds. However, 25 different biologically active compounds have been isolated from Neem seeds. Other compounds present in Neem oil are responsible for some of the biological activity observed (Sclar, 1994).

The neem tree contains more than 100 bio-active ingredients and it is rich in proteins. Its bitter taste is due to an array of complex compounds called "limonoids". The most important bio-active principal is azadirachtin (repellent); other compounds are gedunin (anti-malarial), nimbin (anti-inflammatory, anti-pyretic), nimbidin (antibacterial), nimbidol (anti-malarial, anti-pyretic), quercentin (anti-malarial), salannun (repellent), and sodium nimbinate (spermicide). Young neem leaves contain 60% water, 23% carbohydrates, 7% proteins, more than 3% minerals, and 1% fat (Giger, 2001).



Source from MHHE
(http://www.mhhe.com/biosci/pae/botany/botany_map/articles/article_33.html)

Figure 2.2: Molecular structure of azadirachtin

2.4.2 Modes of action

2.4.2.1 Insect growth regulatory effects

The insect growth regulatory effects of azadirachtin (in contrast to its antifeedant effects) are remarkably similar among species. Various developmental, post-embryonic, reproductive and growth inhibitory affects have been observed, causing malformation and mortality in a dose-dependent manner. Azadirachtin modifies the programs of insects by influencing hormonal systems, especially that of ecdysone. The effects of azadirachtin are both dose and time dependent, prevent both ecdysis and apolysis, and can cause death before or during molting, possibly inducing "permanent" larvae. Exogenous application of growth hormones did not deter the effects of azadirachtin, leading researchers to suggest that the most probable site of action of azadirachtin is at the site of synthesis and release of prothoracicotropic hormone (PTTH) (Sclar, 1994).

The main action of azadirachtin appears to be at the release sites of PTTH from the corpora cardiaca. Azadirachtin appears to block the release of neurosecretory material from the corpora cardiaca resulting in a reduced turnover rate. This affects the rate of synthesis of PTTH by brain neurosecretory cells. Besides that, azadirachtin caused a significant depletion of immunoreactive ecdysteroids in *Tenebrio molitor* pupae. *Tenebrio molitor* has no PTTH glands and yet is still sensitive to the ecdysteroid antagonistic effect exhibited by exposure to azadirachtin. A possible explanation for this phenomenon could involve epidermal cells or oenocytes being affected as both are suggested as alternative sites of ecdysteriod production. It should be noted that all these effects are working in conjunction with blockages in JH (juvenile hormone) and allotropin titers, collectively

resulting in both molting and reproductive aberrations. It is assumed that azadirachtin has direct effects on a variety of tissues and organs. This suggests either a number of different modes of action or a specific toxic lesion to all cells which manifests itself more obviously in some cells than others (Sclar, 1994).

2.4.2.2 Antifeeding effects

The antifeedant effects of azadirachtin are well known. Both primary and secondary antifeedant effects have been observed in the case of azadirachtin. Primary effects include the process of chemoreception by the organism (e.g. sensory organs on mouthparts which stimulate the organism to begin feeding) whereas secondary processes are effects such as gut motility disorders due to topical application only. Inhibition of feeding behavior by azadirachtin results from blockage of input receptors for phagostimulants or by the stimulation of deterrent receptor cells or both. In a recent study by Yoshida and Toscano (1994), the relative consumption rate of *Heliothis virescens* larvae treated with azadirachtin was 25% of the control, attributing to the lowest assimilation efficiency of all natural insecticides tested. In another study, larvae of *Heliothis virescens* consumed less food, gained less weight, and were less efficient at converting ingested and digested food into biomass. Sensitivity between species to the antifeedant effects of azadirachtin are profound. Order Lepidoptera appear most sensitive to azadirachtin's antifeedant effects, with Coleoptera, Hemiptera and Homoptera being less sensitive (Sclar, 1994).

2.4.2.3 Other effects

It has been noted that the presence of azadirachtin alone is not as toxic as all Neem oil components present together. Other compounds present in Neem seed extracts besides

azadirachtin exhibit biological activity in myriad ways. Besides that, salinnin and nimbin, two other compounds present in Neem seed extracts, exhibit an entirely different mode of action than azadirachtin. Effects which may be exhibited by one or more compounds present in Neem seed extracts include: oviposition repellency, egg sterility, longevity, fitness and inhibition of chitin biosynthesis (Sclar, 1994).

3.0 MATERIALS AND METHODS

3.1 Materials

The materials and apparatus used in this research include sentang's leaves, eggs of *Aedes aegypti,* distilled water, grinder, glass jar, spatula, desk's lamp, syringe, pippete, scale and measuring cylinder.

3.2 Methods

3.2.1 Hatching eggs of *Aedes aegypti*

The non infected, *Aedes aegypti*, was used as test organism. Mosquito eggs were obtained from the Environmental Entomology Room, Environment Laboratory, School of Health Sciences, Universiti Sains Malaysia. The hatching of *Aedes aegypti* occurs in a plate. First, we put 200 ml distilled water in a dish. Then, the filter paper that contains the eggs was submerging into the water. The larvae were also given food (Plate 3.1). Development of larvae to 3rd and 4th instar were within three to four days. During this time, the plate of larvae were placed in the netting. Late 3rd instar larvae were used for all bioassay against *Aedes aegypti* (Plate 3.2).

3.2.2 Determine concentration

To decide the concentration, we have referred from the literature which the research is done by *Zaridah et al.* (2003). They have used concentrations of 200, 100, 50, 25 and 12.5 mg/L (ppm). But since they used the extract, we increase the concentration with 100X factor to get 20 000, 10 000, 5000, 2500 and 1250 series of concentration in ppm.

However, the solution cannot be observed because it is very greenish and cloudy (Plate 3.3). So, we reduce the concentration to 10X factor in order to make our solution is easily to be observed. Then, we get the 5 different concentration which are 400, 800, 1200, 1600 and 2000 ppm (Plate 3.4).

3.2.3 Preparation of solution

The fresh leaves of sentang are first clean with distilled water and allow to dry. Then we weigh the leaves according to the concentration that we have decided. After that, we grind the leaves and make 100 ml solution with distilled water (Plate 3.5). The solution was placed in a glass jar. Besides that, we also prepare the control and the benchmark. The control is made from 100 ml distilled water and the benchmark is made from 1.22 ppm abate.

3.2.4 Larvicidal test

Late third instar larvae of *Aedes aegypti* mosquito are collected from the plate. Then, 25 of them are introduced into each glass jar that has been labeled with control, benchmark, 400, 800, 1200, 1600 & 2000 ppm. Mortality of larvae was recorded after 24 hours exposure until 21 days. Besides mortality of larvae, we also observe other effect of sentang such us the behavior of the larvae. We use a lamp to help easier observation (Plate 3.6). Each experiment was replicated 5 times (Plate 3.7).

3.2.5 Analyses data

To analyze the data that we have obtain after 21 days, we used the Microsoft Excel program. First, the total number of mortality of larvae for the five replicates is calculated as percentage. Then, the percentage mortality of larvae is represent in the bar chart. For the each day, we look at the percentage mortality of larvae in the 5 different concentrations including the control and benchmark.

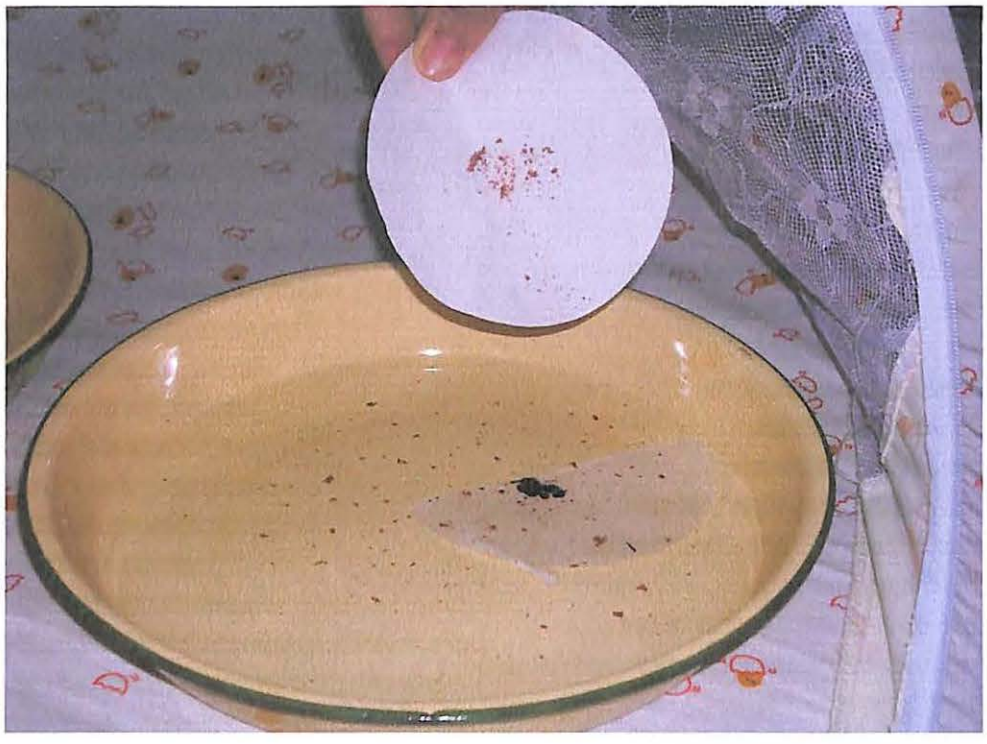


Plate 3.1: Submerge the eggs and feed them

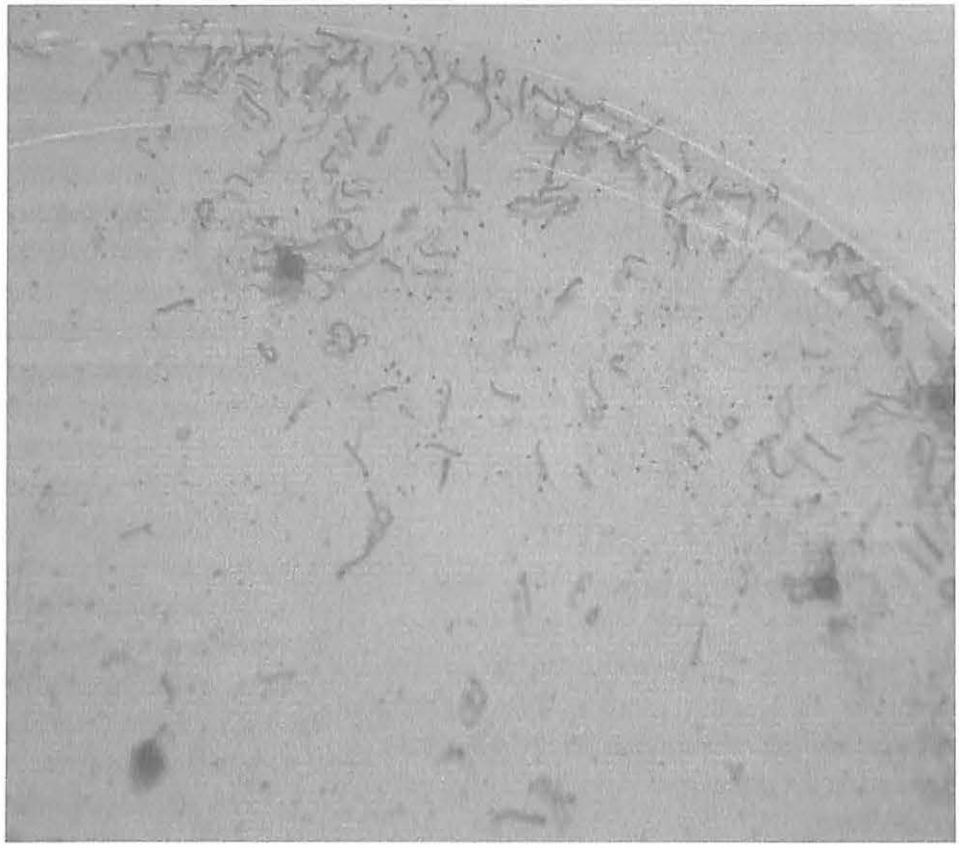


Plate 3.2: Third instar larvae of *Aedes aegypti*

Plate 3.3: Greenish and cloudy appearance of 20 000 ppm of concentration.