PESTICIDAL ACTION OF CERTAIN PLANT EXTRACTS AGAINST MOSQUITO VECTORS (CULICIDAE: DIPTERA)

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by

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CERTIFICATE

I certify that the thesis entitled "**Pesticidal action of certain plant extracts against mosquito vectors (Culicidae: Diptera)**" sumitted to the Mizoram University for the award of the degree of Doctor of Philosophy in Zoology by LALROTLUANGA is a record of research work carried out by him during the period from 2008 to 2012 under my guidance and supervision, and that this work has not formed the basis for the award of any degree, diploma, associateship, fellowship or other similars titles in this university or any other university or institution of higher learning.

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(N. SENTHIL KUMAR)

DECLARATION

I declare that the thesis entitled "**Pesticidal action of certain plant extracts against mosquito vectors (Culicidae: Diptera)**" submitted to the Mizoram University for the award of degree of Doctor of Philosophy in Biotechnology is a bonafide record of work carried out by me during the period from 2008 to 2012 under the guidance of **Dr. G. Gurusubramanian** (Supervisor), Department of Zoology and **Prof. N. Senthil Kumar** (Co-Supervisor), Department of Biotechnology and has not formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles in this University or other University or institution of higher learning.

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1. INTRODUCTION AND REVIEW OF LITERATURE

Mosquitoes are the most important single group of insects in terms of public health importance. Mosquitoes not only cause nuisance by their bites but also transmit deadly diseases. Among the approximately 4000 known mosquito species, less than 10% are regarded as efficient vectors of pathogenic agents of infectious diseases having high impact, both direct and indirect, on human welfare and health. Mosquito-transmitted diseases remain a major cause of the loss of human life worldwide with more than 700 million people suffering from these diseases annually (Taubes 1997). Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases (Fradin and Day 2002). Mosquitoes transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. Dengue fever incidence has increased fourfold since 1970 and nearly half the world's population is now at risk. In 1990, about 1.5 billion people lived in regions where the estimated risk of dengue transmission was greater than fifty percent (Hales *et al.* 2002). An outbreak of chikungunya virus infection emerged in the southwest Indian Ocean islands in 2005, spread out to India, and resulted in an ongoing outbreak that has involved more than 1.5 million patients, including travelers who have visited these areas (Taubitz *et al.* 2007). Despite its debilitating effects, lymphatic filariasis is given a very low control priority (Ramaiah *et al.* 2000).

The overall prevalence and health significance of malaria, lymphatic filariasis and dengue have made them top priorities for global elimination and control programmes (Kyelem *et al.* 2008; WHO 2008a; WHO 2008b). Half of entire human

population, an estimated 3.3 billion people, live in malaria risk areas around the world with about 250 million people infected annually. Malaria is believed to be responsible for approximately one million deaths per year, particularly among children under five year's old and pregnant women (WHO 2008a). Malaria is endemic in 109 countries, the majority located in the intertropical belt of Africa, Asia and Latin America. The highest malaria burden is found in Africa with an estimation of 212 million cases (86% globally) distributed in 45 countries. The other continents contribute the remainder of the 35 million cases (WHO 2008a). Effective control of malaria in many countries is aggravated by inadequate health infrastructure and overall poor socioeconomic conditions. The situation has become more complicated during the last 50 years with the increase in resistance to anti-malarial drugs used to combat the infections and development of insecticide resistance in *Anopheles* mosquitoes that serve as vectors (Fig. 1A) (Manguin *et al.* 2008). The impact upon this demographic group must be considered astounding. In addition to outright health effects, the economic disparities have been calculated in terms of average gross domestic product (GDP) to be five times lower and yield 2% less annual average growth of GDP in areas of endemic malaria (Sachs and Malaney 2002). By contrast, most of the wealthy, developed nations outside of Africa have had good success in eliminating malaria from within their borders (Hardin and Jackson 2009). The four human Plasmodia are exclusively transmitted by *Anopheles* mosquitoes, of which approximately 70 species (15% of all known anophelines) are considered to be of epidemiological significance (Manguin *et al.* 2008; Service and Townson 2002).

Anopheles barbirostris is considered an important vector of malaria and Brugian filariasis in Sulawesi, Flores and Timor (Atmosoedjono *et al.* 1977) (Plate 1). Mosquitoes traditionally identified as *An. barbirostris* are common and widely

distributed from India through mainland Southeast Asia and southward through Indonesia to Sulawesi, all of the Lesser Sunda island chain to Timor Island and possibly the eastern fringe of the Maluku (Mollucas) archipelago (Reid 1968; Harrison and Scanlon 1975). *An. barbirostris* is generally found in highland areas (Harrison and Scanlon 1975) but in western Timor it is considered a coastal species (Ndoen *et al.* 2010). A recent survey in northern Sumatra identified *An. barbirostris* as a potential vector of malaria (Syafruddin 2007). Females of *An. barbirostris* bite humans, but generally prefer to feed on other animals, especially bovids (Reid 1961). Feeding apparently takes place outdoors, but adult mosquitoes have been found resting inside houses and animal shelters as well as outside (Harrison and Scanlon 1975). Outdoor biting in peninsular Malaysia near the Thai border takes place throughout the night (Abu Hassan 2001). In view of the feeding preferences and behaviour of females, *An. barbirostris* probably plays little, if any, role in the transmission of malaria and filariasis in most areas where it occurs (Sinka *et al.* 2011). *An. barbirostris* is a confirmed vector of *P. falciparum* malaria in Sri Lanka (Amerasinghe *et al.* 1999). Both *P. vivax* and *P. falciparum* have been detected by ELISA in females of *An. barbirostris* in Bangladesh (Alam *et al.* 2010).

An. barbirostris is a swamp breeder, typically found in deep fresh water that is still or slow moving (Reid 1968). However, it is not uncommon in or near rice fields and is tolerant of relatively high levels of organic pollution including sewage, and can be found in ground pools with high concentrations of animal dung. Other habitats vary from sunlit to moderately shaded ground-water bodies, including river and stream margins and pools, ditches, moats, lakes, permanent and temporary ground pools, rice fields, wells, canals, marshes, rock pools, ponds, springs, swamps and

animal footprints. The habitats usually contain some vegetation (Harrison and Scanlon 1975).

After malaria, lymphatic filariasis is regarded the second most common global arthropod-borne infectious disease with an estimated burden of 128 million people infected over 78 endemic countries (WHO, 2008b). The predominance of lymphatic filariasis infections are found in humid tropical areas of Asia, Africa, the western Pacific and scattered areas of the Americas with an estimated 1.3 billion people at-risk for developing new active lymphatic filariasis infection annually (Fig. 1B) (WHO, 2008b). Southern and Southeast Asian regions have by far the greatest number of people (891 million) at-risk for lymphatic filariasis (68% globally), with 454 million people at-risk in India alone (WHO, 2008b). Tropical Africa represents the second largest number of people at-risk, estimated at 382 million in 2007 (30% globally) and 51 million cases which for most of them are deemed seriously afflicted with disabilities and disfigurement (WHO 2008b). Infection from *Wuchereria bancrofti*, although not fatal, is considered a leading cause of infirmity, permanent disability and chronic morbidity, often resulting in societal stigma of disfigured victims. *W. bancrofti* is mainly transmitted by *Culex* and *Anopheles* mosquito species for the nocturnally periodic form or by selected members in the genera *Aedes* (Manguin *et al.* 2008). *Culex quinquefasciatus* is an important vector of lymphatic filariasis and is known to carry and transmit *Wuchereria bancrofti* to some degree of efficacy in many regions of the world (Plate 1) (Bernhard *et al.* 2003). *Cx. quinquefasciatus* is one of the most widespread mosquitoes in the world. It is found throughout the most pan and subtropical Americas (Weinstein *et al.* 1997), the Neotropics, Afrotropics (White 1975), Indomalayan, Australasian (Lee *et al.* 1989), Eastern Asian regions, United Kingdom and parts of the Middle East (Bram 1967),

and New Zealand (Sandlant 2002). The females usually breeds in organically rich and polluted surface waters or artificial containers and the eggs are not desiccation resistant and are laid as rafts on the water surface (Weinstein *et al.* 1997). *Cx. quinquefasciatus* is a domesticated species which is often found living in close proximity to humans. The females are nocturnal biters, they will readily bite man indoors and out (Weinstein *et al.* 1997), but will also bite other animals, even amphibians (Holder *et al.* 1999; Lee *et al.* 1989).

Cx. quinquefasciatus is part of group b of the Pipiens group and belong to the subgenus *Culex* (Dobrotworsky 1965). It is a medium, light brown mosquito; the abdominal sternites of the females are pale scaled with a few dark scaled patches medially (Belkin 1957). It is also an important vector of West Nile Virus (WNV) in some areas of the world. *Cx. quinquefsciatus* is able to transmit Ross River Virus, Alfuy, Almpiwar, Corriparta, Dengue, Sindbis, Japanese Encephalitis virus (Reuben *et al.* 1994), Reticuloendotheliosis virus (Holder *et al.* 1999) and the protozoan, *Hepatozoon breini,* within the laboratory. *Cx. quinquefasciatus* is also a laboratory host to a wide variety of other arboviruses including Murray Valley Encephalitis (Weinstein *et al.* 1997), Edge Hill, Eubenangee, Getah, Kokobera, Koongol, Kowanyama, Kunjin, Mapputta, Stratford, Trubanaman, Wongal, Reovirus type 3 and Chikungunya viruses (Holder *et al.* 1999; Lee *et al.* 1989). It is a domestic pest in many urban areas and often comes indoors at night to bite (Holder *et al.* 1999). It is also a major vector of bird pox and the avian malaria-causing protozoa (Derraik and Stanley 2005), *P. relictum* (Laird 1996) and *P. cathemerium* (Lee *et al.* 1989).

Dengue is the most rapidly spreading mosquito-borne viral disease in the world. In the last 50 years, incidence has increased 30-fold with increasing geographic expansion to new countries and, in the present decade, from urban to rural

settings (Fig. 2). An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries (Manguin *et al.* 2010). Some 1.8 billion (more than 70%) of the population at risk for dengue worldwide live in member states of the WHO South-East Asia Region and Western Pacific Region, which bear nearly 75% of the current global disease burden due to dengue.

Aedes albopictus is an important vector of dengue (Plate 1). Other than dengue disease, *Ae. albipictus* can transmit pathogens and viruses, such as the West Nile virus, Yellow fever virus, St. Louis encephalitis, and Chikungunya fever (Hochedez *et al.* 2006). It was first described as 'the banded mosquito of Bengal' by Skuse in 1894 from Calcutta, India (Huang 1968). Adult males and females are covered with shiny black scales with distinct silver white bands on the palpus and tarsi. Its most striking characteristic is the band of silver scales forming a distinct stripe on the dorsal surface of the thorax and head. The Asian tiger mosquito is about 2 to 10 mm length with a striking white and black pattern (Novak 1992).

Aedes albopictus is a very aggressive daytime biter with peaks generally occurring during the early morning and late afternoon. It feeds on a number of hosts including man (indoors and outdoors), domestic and wild animals and birds. Its generalized feeding behavior contributes to its vector potential (Novak 1992). *Ae. albopictus* occurs throughout the Oriental Region from the tropics of Southeast Asia, the Pacific and Indian Ocean Islands, north through China and Japan and west to Madagascar. It also has expanded into the Hawaiian Islands, islands of the southern Pacific and in North and South America (Novak 1992). The Asian tiger mosquito is a container-inhabiting species which lays its eggs in any water-containing receptacle in urban, suburban, rural and forested areas. The primary immature habitats of this

species are artificial containers such as tires, flower pots, cemetery urns/vases, buckets, tin cans, rain gutters, ornamental ponds, drums, even the finger holes of an abandoned bowling ball have been reported (Gratz 2004). Larvae are also found in natural containers such as treeholes, bamboo pots, and leaf axils.

Human beings have used plant parts, products and secondary metabolites of plant origin in pest control since early historical times. Vector control has been practiced since the early 20th century. During the pre-DDT era, reduction of vector mosquitoes mainly depended on environmental management of breeding habitats, *i.e*., source reduction. During that period, some botanical insecticides used in different countries were Chrysanthemum, Pyrethrum, Derris, Quassia, Nicotine, Hellebore, Anabasine, Azadirachtin, d-limonene camphor, Turpentine (Rahuman *et al.* 2008). From the early 1950s, DDT and other synthetic organochloride and organophosphate insecticides were extensively used to interrupt transmission of vector borne diseases by reducing densities, human-vector contact and, in particular, the longevity of vector mosquitoes. In the mid-1970s, the resurgence of vector borne diseases, along with development of insecticide resistance in vector population, poor human acceptance of indoor house spraying and environmental concerns against the use of insecticides led to a rethinking in vector control strategies (WHO 2005).

Application of alternative methods in mosquito control as part of the Integrated Mosquito Management (IMM) has been gaining importance (Ghosh *et al.* 2012). Integrated Mosquito Management (IMM) is a decision-making process for the management of mosquito populations, involving a combination of methods and strategies for long-term maintenance of low levels of vectors. The purpose of IMM is to protect public health from diseases transmitted by mosquitoes, maintain healthy environment through proper use and disposal of pesticides and improve the overall

quality of life through practical and effective pest control strategies. The main approaches of IMM include: *(i)* Source reduction and habitat management by proper sanitation, water management in temporary and permanent water bodies, and channel irrigation. Vegetation management is also necessary to eliminate protection and food for mosquito larvae; *(ii)* Larviciding by application of dipteran specific bacteria, insect growth regulators, surface films and oils, expanded polystyrene beads, phytochemicals, organophosphates and organochlorides, *(iii)* Adulticiding by application of synthetic pyrethroids, organophosphates and synthetic or plant derived repellents, insecticide impregnated bed nets, genetic manipulations of vector species, etc., *(iv)* Use of mosquito density assessment in adult and larval condition and disease surveillance; and *(v)* Application of biological control methods by using entomophagous bacteria, fungi, microsporidians, predators and parasites. Of the above avenues of IMM, larviciding approach is the more proactive, proenvironment, target specific and safer approach than controlling adult mosquitoes. Vector control offers a viable alternative to reduce the spread of vector born diseases (Chowdhuri *et al.* 2009).

In addition to personal protection and educating the public, the most successful method of minimizing the incidence of mosquito-borne diseases is to eradicate and control the mosquito vectors, which is performed principally by systematic treatment of the breeding places through a combination of environmental management and application of larvicides that do not harm other organisms in the environment (Cetin *et al.* 2004; Corbel *et al.* 2004). Organophosphorus, carbamate, and pyrethroid insecticides are less persistent, as they break down quickly in the environment, and are therefore recommended as classical larvicides. Nevertheless, pyrethroids are very toxic to fish and should not be used where there are fish or

crustaceans (Rozendaal 1997). The most commonly used larvicides are the organophosphorus compounds such as temephos, fenthion, and chlorpyrifos, which are highly active against mosquito larvae and other aquatic insects. Temephos is the larvicide of choice for *Aedes aegypti* and *Anopheles* control, while fenthion and chlorpyrifos are used against *Culex* spp. (Das *et al.* 2007). Questions have been raised as to whether these larvicides would cause environmental pollution and hazards to human health and other nontarget organisms if recurrently applied. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations (Das *et al.* 2007). At present, the most successful measures to decrease the incidence of mosquito borne diseases are by personal protection and control of the vectors.

The use of plant derived compounds for mosquito control has been reported since 1933. Plants used in traditional medicine or recorded in ethnopharmacological literature provide a source of information for such investigation. Medicinal plants with larvicidal properties have paramount importance for the local control of mosquito (Debella *et al.* 2007). A large number of plant-derived substances posses physiological and behavioral activities against insect pests and may provide new sources of natural pesticides. Natural products have shown that it is possible to produce a great range of biological activities, including toxicity, repellent action, antifeedant and growth regulation properties (Huang and Ho 1998; Chiam *et al.* 1999). Plant terpenoids have dominated the subject of chemical ecology since they have been studied for their activities against a variety of insect models (Gonza´lez-Coloma *et al.* 1995). The great ecological interest of plants terpenoids has been reflected in several studies, finding strong toxicant effects that produce larval mortality, significant growth inhibition, repellent, and fumigant activity.

The presence of so-called secondary compounds, which have no known function in photosynthesis, growth or other aspects of plant physiology, give plant materials or their extracts their anti-insect activity. Secondary compounds include alkaloids, terpenoids, phenolics, flavonoids, chromenes and other minor chemicals. These chemicals may kill, retard or accelerate development or interfere with the life cycle of the insect in other ways (Bell *et al.* 1990). So, these chemicals can disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phagostimulants, anti-feedants or modify oviposition. Although compared with modern synthetics the plant substances are relatively weak. The botanical insecticides are generally pest-specific, readily biodegradable and usually lack toxicity to higher animals (Bowers 1992).

A major motivation to promote research on and use of pest control methods at low environmental cost is the demand of consumers searching for healthier products, a social behavior reflected in product registration laws that favor the use of low-cost insecticides having minimal environmental impact (Souza *et al.* 2008). Another reason is that botanicals have been a focus of interest of chemists and biologists because of their structural complexity, potency, and selectivity. Pyrethrum has been the most important botanical for almost two centuries (Isman 2000). Rotenone, ryanodine, veratridine, and azadirachtin, active ingredients of ryania (*Ryania speciosa*), sabadilla (*Schoenocaulon officinale*), and neem (*Azadirachta indica*), respectively, have been widely used for their effectiveness and low toxicity to mammals. The most vigorous development program in recent years has been for expanded uses of neem seed extract for Integrated Pest Management (Casida and Quistad 1998; Isman 2000).

In recent years, considerable attention has been directed to the research and application of insect growth regulators, juvenile hormone analogues, antijuvenile compounds (precocenes) and phytoecdysones in plant protection. These substances have been used successfully on a large scale in plant protection. The toxic constituents present in the plant represent the secondary metabolites and have only an insignificant role in primary physiological processes in plants that synthesize them (Singh 1993). It has been estimated that only 5 to 15% of the 2,50,000 to 7,50,000 existing species of plants have been surveyed for biologically active compounds and even this is an over estimate, as the investigated plants have been partially screened for a single or at best, few types of activity. Due to rapid deforestation, phytologists may have only few decades left for surveying and cataloguing the fast extinguishing flora (Varma and Dubey 1999).

In situations where mosquitoes have developed resistance to all conventional larvicides, consideration may be given to using larvicidal oils, bacterial larvicides such as *Bacillus* spp., or more expensive insect growth regulators as alternatives. However, the implementation of these agents in the field has failed to show satisfactory results in many parts of the world (Liu *et al.* 1996; Chevillon *et al.* 2001). These partial failures have prompted renewed interest in the search and development of better vector control strategies that destroy vectors over a wide range, but cause no harm to nontarget organisms and the environment. Thus one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes to bite human beings. Herbal products with proven potential as insecticide or repellent can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level. The use of indigenous plant products in vector control might be one

potentially alternative approach (Champakaew *et al.* 2007). These make it imperative to use phytochemicals in mosquito control (Anonymous 2003).

The efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts used, age of plant parts (young, mature or senescent), solvent used during extraction as well as upon the available vector species (Shaalan *et al.* 2005). Sukumar *et al.* (1991) have described the existence of variations in the level of effectiveness of phytochemical compounds on target mosquito species *vis-à-vis* plant parts from which these were extracted, responses in species and their developmental stages against the specified extract, solvent of extraction, geographical origin of the plant, photosensitivity of some of the compounds in the extract, effect on growth and reproduction. Changes in the larvicidal efficacy of the plant extracts can occur due to geographical origin of the plant; response in the different mosquito species and; due to variation in the species of plant examined and between plant parts used to study the larvicidal efficacy (Kishore *et al.* 2011). However, the principal objective of the present study is to report the changes in larvicidal potentiality of the plant extracts due to change of the particular solvent used during extraction.

It has been shown that the extraction of active biochemical from plants depends upon the polarity of the solvents used (Ghosh *et al.* 2012). Polar solvent will extract polar molecules and non-polar solvents extract non-polar molecules. This can be achieved by using many solvent systems ranging from hexane/ petroleum ether, the most non polar (polarity index of 0.1 that mainly extracts essential oil) to that of water, the most polar (polarity index of 10.2) that extracts biochemical with higher molecular weights such as proteins, glycans, *etc*. Chloroform and acetone are moderately polar (polarity index of 4.1) that mainly extracts steroids, alkaloids, etc. It

has been found that solvent with minimum polarity (hexane or petroleum ether) or that with maximum polarity (aqueous/ steam distillation) have been used in many studies (Ghosh *et al.* 2012). However, those biochemicals that were extracted using moderately polar solvents were also seen to give good results as reported by a few bioassays (Ghosh *et al.* 2012). Thus, different solvent types can significantly affect the potency of extracted plant compounds and there is difference in the chemo-profile of the plant species.

Kishore *et al.* (2011) reviewed the efficacy of phytochemicals against mosquito larvae according to their chemical nature and described the mosquito larvicidal potentiality of several plant derived secondary materials, such as, alkanes, alkenes, alkynes and simple aromatics, lactones, essential oils and fatty acids, terpenes, alkaloids, steroids, isoflavonoids, pterocarpans and lignans. Generally, the active toxic ingredients of plant extracts are secondary metabolites that are evolved to protect them from herbivores. The insects feed on these secondary metabolites potentially encountering toxic substances with relatively non-specific effects on a wide range of molecular targets. These targets range from proteins (enzymes, receptors, signalling molecules, ion-channels and structural proteins), nucleic acids, biomembranes, and other cellular components. This in turn, affects insect physiology in many different ways and at various receptor sites, the principal of which is abnormality in the nervous system (such as, in neurotransmitter synthesis, storage, release, binding, and re-uptake, receptor activation and function, enzymes involved in signal transduction pathway) (Rattan 2010). Such disruption also includes the blockage of calcium channels (by ryanodine), of nerve cell membrane action (by sabadilla), of octopamine receptors (thymol), hormonal balance disruption, mitotic poisioning (by azadizarachtin), disruption of the molecular events of morphogenesis

and alteration in the behaviour and memory of cholinergic system (by essential oil), etc. Of these, the most important activity is the inhibition of acetylcholinerase activity (AChE) as it is a key enzyme responsible for terminating the nerve impulse transmission through synaptic pathway (Pushpalata and Muthukrishnan 1999). The mode of action and site of effect for larvicidal phytochemicals has received little attention. Rey *et al.* (1999) found that botanical derivatives primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae.

Larvicidical activity of botanicals

Larvae from the three medically important mosquito genera *Aedes, Anopheles* and *Culex* are all susceptible to a greater or lesser extent to some phytochemicals. LC_{50} values for crude extracts were found to range widely from promising to very unpractical doses (Shaalan *et al.* 2005). The lowest and most promising dose for a crude extract was 0.69 mg/L recorded for a steam distilled extract of *Callitris glancophylla* against *Aedes aegypti* (Shaalan *et al.* 2005). These low doses are comparable to many synthetic insecticides. Most studies on phytochemicals focus on herbs and other medicinal plants because of the historical experiential knowledge and some scientific studies which have shown them to be particularly active against certain organisms.

A number of researchers have used plant products in the control of various mosquito species. The petroleum ether extracts of *Rhinacanthus nasutus*, *Derris elliptica*, *Trigonostemon reidioides*, *Homalomena aromatica*, *Stemona tuberose*, and *Acorus calamus* (Komalamisra *et al.* 2005); *Piper nigrum* (Rasheed *et al.* 2005); *Chenopodium album* and *Sonchus oleraceus* (Sharma *et al.* 2006); *Argemone*

mexicana (Sakthivadivel and Thilagavathy 2003); *Solanum xanthocarpum* (Mohan *et al.* 2006); *Ajuga remota* (Sharma *et al.* 2004); *Thymus capitatus* (Mansour *et al.* 2000); *Vitex negundo*, *Nerium oleander*, and *Syzygium jambolanum* (Pushpalatha and Muthukrishnan 1995); *Murraya koenigii*, *Coriandrum sativum*, *Ferula asafetida*, and *Trigonella foenum* (Harve and Kamath 2004); and *Eichhornia crassipes*, *Ageratum conyzoides*, *Cleome icosandra*, *Tagetes erectes*, and *Tridax procumbens* (Saxena *et al.* 1992); *Jatropha curcas*, *Pedilanthus tithymaloides*, *Phyllanthus amarus*, *Euphorbia hirta*, and *Euphorbia tirucalli* (Rahuman *et al.* 2008) have been tested against the larvae of *A. aegypti* and *C. quinquefasciatus*. Leaves of *Argemone mexicana*, *Jatropa curcas*, *Withania somnifera*, *Citrullus colocynthus*, *Aloe barbadensi*, *Cannabis sativa* have larvicidal properties against *An. stephensi* (Sakthivadivel and Daniel 2008; Maurya *et al.* 2007). *Myrtus communis*, *Oryganum syriacum*, *Mentha microcorphylla*, *Pistacia lentiscus*, *Lavandula stoechas*; *Eucalyptus globules*, *Solanum xanthocarpum* and *Thymus capitatus* have shown larvicidal actions against *Cx. pipiens* (Traboulsi *et al.* 2002; Mansour *et al.* 2000) (Table 1).

The acetone crude extract of *Fagonia indica* and *Arachis hypogaea* (Chaubal *et al.* 2005); *Murraya koenigii*, *Coriandrum sativum*, *Ferula asafoetida*, *Trigonella foenum* (Harve and Kamath 2004); *Acalypha indica*, *Achyranthes aspera*, *Leucas aspera*, *Morinda tinctoria* and *Ocimum sanctum* (Bagavan *et al.* 2008) were tested and found to be effective against mosquito larvae. The acetone extracts of *Tridax procumbens* have shown high larvicidal property against *An. subpictus* (Kamaraj *et al.* 2011); Saxena *et al.* (1992) also noticed potential larvicidal properties of *Ageratum conyzoides*, *Cleome icosandra* and *Tridax procumbens* against *Cx. quinquefasciatus*. *Ageratina adenophora* is also reported to have larvicidal activities

against *Ae. aegypti* and *Cx. quinquefasciatus* (Raj Mohan and Ramaswamy 2007). Acetone leaf extract of *Feronia lomonia* and *Millingtonia hortensis* have shown low lethal concentration against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* (Rahuman *et al.* 2000; Kaushik and Saini 2008). Anees (2008) reported the larvicidal properties of *Ocimun sanctum* against *Ae. aegypti* and *Cx. quinquefasciatus* (Table 2).

The crude chloroform extract of seeds of *Millettia dura* showed high activity against second-instar larvae of *A. aegypti* (Yenesew *et al.* 2003). Roots of *Plumbago zeylanica, P. dawei* and *P.stenophylla* (Maniafu *et al.* 2009); latex and stem bark of *Euphorbia tirucalli* (Yadav *et al.* 2002); flower of *Nyctanthes arbortristis* (Khatune *et al.* 2001); fruit peel of *Citrus sinensis* (Bagavan *et al.* 2009); leaf of *Aloe ngongensis* (Matasyoh *et al.* 2008); seed of *Millettia dura* (Yenesew *et al.* 2003); *Cassia obtusifolia* seed (Yang *et al.* 2003) were also reported to have larvicidal action against mosquito larvae (Table 3).

Methanol extracts of *Atlantia monophylla* leaf (Sivagnaname and Kalyanasundaram 2004); *Dysoxylum Malabaricum* leaf and leaf and seeds of *Melia azedarach* (Senthil Nathan *et al.* 2006); *Moringa oleifera* bark (Kamaraj and Rahuman 2010); *Ocimum gratissimum* leaf (Kamaraj and Rahuman 2010); *Solenostemma argel* aerial parts (Al-Doghairi 2004); root of *Solanum xanthocarpum* (Mohan et al 2006); leaf of *Chrysanthemum indicum* (Kamaraj *et al.* 2010); leaves of *Azadirachta indica* and *Rhazya stricta* (El Haq *et al.* 1999); leaves of *Trichosanthes anguina*, *Momordica charantia*, *Luffa acutangula*, *Benincasa cerifera* and *Citrullus vulgaris* (Prabakar and Jebanesan 2004); leaves of *Vitex negundo*, *V. trifolia*, *V. peduncularis*, *V. altissima* (Krishnan *et al.* 2007); *Centella asiatica* leaf (Rajkumar and Jebanesan 2005a); *Euphorbia tirucalli* latex and bark (Yadav *et al.* 2002); seed and leaf of *Eucalyptus globulus* (Sheeren 2006); *Atlantia monophylla* leaf

(Sivagnaname and Kalyanasundaram 2004); leaves of *Pavonia zeylanica* and *Acacia ferruginea* (Vahitha *et al.* 2002); leaves of *Coccinia indica*, *Cucumus sativus* and *Momordica charantia* (Rahuman and Venkatesan 2008); seed of *Cassia tora* (Jang *et al.* 2005); *Annona squamosa* leaf, fruit of *Gymnopetelum cochinchinensis*, *Caesalpinea* sp. Bark and *Piper* sp. stem (Das *et al.* 2007); *Chamaecytoparis obtuse* leaf (Jang *et al.* 2005) and *Acalypa alnifolia* leaf (Kovendan *et al.* 2012) have high larvidical activities against *Aedes*, *Anopheles* and *Culex* spp. (Table 4).

Mosquito larvicide test on aqueous extracts have also been done by many researchers. Seed of *Carica papaya*, fruit of *Murraya paniculata*, leaf of *Cleistanthus collinus*, *Hemidesmus indicus*, *Gymnema sylvestre*, *Eclipta prostrata* (Khanna and Kannabiran 2007); leaves of *Artimisia cina* and *Cleome droserifolia* (Aly and Badran 1986); fruit of *Piper retrofractum* (Chansang *et al.* 2005); leaf of *Solanum villosum* (Chowdhury *et al.* 2008); dried fruit of *Solanum nigrum* (Raghavendra *et al.* 2009) were tested against mosquito species and found to have potent larvicidal activities (Table 5).

Biochemical parameters

Several plant extracts were effective as potential acute or chronic insecticides, insect growth inhibitors or antifeedants against a variety of insect species (Shaalan *et al.* 2005; Kishore 2011; Ghosh *et al.* 2012). Such larval intoxication and growth regulator of immature and adult mosquitoes were found correlated with some biochemical changes in the tested species particularly in sugar, glycogen, lipid and protein contents (Senthilkumar *et al.* 2009; Preet and Sneha 2011).

The total protein, carbohydrate, and lipids were also found to be reduced along with certain amino acids in *An. stephensi* treated larvae suggesting that the

treatment lowered feeding, improper utilization of digested food, and interference with the hormones regulating the protein synthesis leading to reduced nutrient profiles. The reduction in total lipids was observed because of stress induced by the plant extracts (Senthilkumar *et al.* 2009).

Insect Growth regulators

During the past two decades, considerable progress has been made in the development of natural and synthetic compounds, which are capable of interfering with the process of growth, development and metamorphosis of the target mosquito species, which are known as insect growth regulators (IGRs). Two types of IGRs are available, one which inhibit the growth of larvae due to juvenile hormone like action and known as JH mimics or analogues and the other type of IGR compound which interfere with chitin production leading to moulting disturbances, resulting in death of the insect (Batra *et al.* 2005). IGRs differ widely from the commonly used insecticides as they exert their insecticidal effects through their influence on development, metamorphosis and reproduction of the target insects by disrupting the normal activity of the endocrine system. Compared to the conventional larvicides, the IGRs are known to be safer and selective in action (Mian and Mulla 1982).

Insect growth regulators (IGRs) affect hormonal control of mosquito growth and development. The main effect of IGRs is the inhibition of adult emergence, but reproduction and ecdysteroid production in surviving females are also affected (Fournet *et al.*1995). In general, IGRs have high levels of activity and efficacy against various species of mosquitoes in a variety of habitats (Mulla *et al.* 1989; Lee 2001). Additionally, it has been known that they have shown a good margin of safety to non-target biota including fish and birds. Residue and non-target studies indicated

that IGRs have no prolonged residues and are an environmentally safe compound with minimal impact on non-target organisms although they are not much safe to some aquatic insects (Miura and Takahashi 1975; Mulla *et al.* 1986). On the basis of these attributes, IGRs are likely to provide additional tools for mosquito control, supplementing microbial larvicides, pyrethroids and organophosphorus larvicides (Mulla *et al.* 1989).

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Adulticidal bioassay

Pest management techniques are many and varied: mechanical, cultural, biological, and chemical. Treatment of adult mosquitoes – adulticiding – is achieved entirely via pesticide applications targeted to adult mosquitoes. The process of adulticiding is a step wise process that often is considered the method of last resort in

an Integrated Pest Management (IPM) approach to mosquito control (Connelly 2012). Adult female of certain mosquito species have the ability to transmit diseases from an infected individual to a susceptible person. Vector control measures have, therefore, been established to control the transmission of the disease by targeting the carriers. Over the past few decades, the vector has however developed the ability to evade intervention measures, which target adult mosquitoes, thus, exacerbating the problem for vector control programmes (Sharp 1983). The current vector control technique involves the use of residual insecticides which are sprayed onto walls and roofs of houses. With concern for the quality and safety of life and the environment, the emphasis on controlling mosquito vectors has shifted steadily from the use of conventional chemicals toward alternative insecticides that are target-specific, biodegradable, and environmentally safe, and these are generally botanicals in origin. Although plants and their derivatives were used for controlling and eradicating mosquitoes and other domestic pests before the advent of synthetic organic chemicals, only few insecticides of plant origin have been found commercially available. Plant-derived bioproducts, however, still have encouraging results in the control of mosquito vectors if they are adequately effective and harmless to beneficial non-target organisms and the environment. Furthermore, the insect resistance to mosquitocidal botanical agents has not been documented (Shaalan *et al.* 2005).

Although communities are documented to traditionally use preparations of various plant organs as repellents through either topical application or fumigation no reports of plant extracts being used traditionally as mosquito adulticides have been found. The importance of ethnomedicinal plants lies not only in their chemotherapeutic value in traditional health care but also in their potential as sources of biologically active entities (Maharaj *et al.* 2011). Botanical phytochemicals with

mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, pupicidal, and adulticidal properties.

Almost, no research on the effect of phytochemicals on adult mosquitoes has been conducted (Shaalan *et al.* 2005). Results from a study by Perich *et al.* (1995) suggest that adulticidal action is a potential and unexploited field of research. The seed kernel of *Azadirachta indica*, the leaf of *Cymbopogon nardus* and the wood of *Fernandoa adenophylla* were also reported to have adulticidal properties against *Aedes aegypti* (Zaridah *et al.* 2006). Above 90% mortality was found in the ethyl acetate and methanol extract of all experimental plants at the concentrations of 500 µg/mL (Kamaraj and Rahuman 2010). The adult mortality was found in methanol extract of *Artemisia nilagirica*, with the LC_{50} and LC_{90} values of 205.78 and 459.51 ppm for *An. stephensi*, and 242.52 and 523.73 ppm for *A. aegypti*, respectively (Panneerselvam *et al.* 2012).

Maharaj *et al.* (2011) screened the adulticidal bioactivity of eighty plants of South America, of all the extracts analysed, the highest activity was observed in *Ptaeroxylon obliquum* (Ptaeroxylaceae) and *Pittosporum viridiflorum* (Pittosporaceae). Kamaraj and Rahuman (2010) determined the larvicidal and adulticidal activities of hexane, ethyl acetate and methanol extracts of *Momordica charantia*, *Moringa oleifera, Ocimum gratissimum, Ocimum tenuiflorum, Punica granatum* and *Tribulus terrestris against Cx. gelidus* and *Cx. quinquefasciatus.*

Rohani *et al.* (1997) has reported the efficacy of few Malaysian essential oils such as *Litsea lliptica, Polygonum minus* and *Piper aduncum* as potential mosquito adulticides while Sulaiman *et al.* (2001) has reported that essential oils of *Melaleuca cajuputi* and *Cymbopogon nardus* have adulticidal effects on *Aedes* mosquito at high-

rise flats in Kuala Lumpur. The compounds, 4 thiophenes,5-(but-3- ene-1-ynyl)-2,2-bithiophene, 5-(but-3-ene-1-ynyl)- 5′-methyl-2,2-bithiophene,2,2′,5′,2”-terthiophene, and 5-methyl-2,2′,5′,2”-terthiophene, isolated from floral extract of *Tagetes minuta* were largely responsible for the toxicity exhibited against the adults of *Ae. aegypti* and *An. stephensi*. Govindarajan and Sivakumar (2012b) also reported the adulticidal properties of *Cardiospermum halicacabum* against *Cx. quinquefasciatus*, *Ae. Aegypti* and *An. stephensi*.

Dua *et al.* (2010) also reported the adulticidal effects of *Lantana camara* against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. culifacies*, *An. fluvialitis* and *An. Stephensi.* Yongkhamcha and Indrapichate (2002) described the toxicity of mintweed, yam bean and celery seed extracts on *Ae. aegypti*. Chaiyasit *et al.* (2006) referred that essential oils derived from five plant species, celery (*Apium graveolens*), caraway (*Carum carvi*), zedoary (*Curcuma zedoaria*), long pepper (*Piper longum*), and Chinese star anise (*Illicium verum*), were subjected to investigation of adulticidal activity against mosquito vectors. Govindarajan and Sivakumar (2012a) also reported that adult mortality was found in methanol extract of *Andrographis paniculata* against the adults of *Cx. quinquefasciatus* and *Ae. aegypti* with the LC_{50} and LC_{90} values of 149.81, 172.37 ppm and 288.12, 321.01 ppm, respectively.

Repellent Bioassay

Repellents make humans unattractive to a mosquito so that it will avoid areas of the body that have been treated with the products and they do not kill mosquitoes. Quality repellents will provide protection from bites for a long period of time from just one application. The use of repellents to protect humans and his animals from bites of mosquitoes already has been accepted as part of an overall integrated

mosquito-borne disease control program (Chavasse and Yap 1997; Yap *et al.* 2000). The use of natural products such as plant extracts or oils was not common until now due to many reasons. Thus, it is now rather easy to find chemical compounds that have repellency properties and to use them as active ingredients in a repellent product (Amer and Mehlhorn 2006). Freeborn (1928) and Dover (1930) cited some insect repellent formulations consisting of a number of essential oils such as citronella, camphor, tar, pennyroyal, and castor oils that provided a long-lasting protection from insect bites.

Repellents have an important place in protecting man from the bites of insect pests. An effective repellent will be useful in reducing man-vector contact and in the interruption of disease transmission. Repellent compounds should be non-toxic, nonirritating and long lasting (Kalyanasundaram 1991). Majority of commercial repellents are prepared by using chemicals like allethrin, N-N-diehyl-m-toluamide (DEET), dimethyl phthalate (DMP) and N, N-diethyl mendelic acid amide (DEM). It has been reported that these chemical repellents are not safe for public use (Zadikoff 1979; Ronald *et al.* 1985). In the last few years, with the increase of public concern on the safety of many chemical products that were used previously as insecticides or insect repellents, several institutes and researchers started development of natural active ingredients especially from plant sources.

For a long time various folk remedies have been used to repel pests with extracts from plant. The most effective group of repellents includes amine and pyridines which are derivative of quaternary ammonium bases like cyclic amides and rosins. The oils of Citronella, an extract of *Andropogon* sps. and *Cymbopogon vardus* have been claimed to be particularly effective as mosquito repellent (Nakanishi 1997). Jilani *et al.* (1988) tried the repellent and growth-inhibiting effects of turmeric

oil (*Curcuma longa*), sweetflag oil (*Acorus calamus*), neem oil (*Azadirachta indica*), and Margosan-O (a commercial neem-based insecticide) on red flour beetles (Tenebrionidae). It was found that the repellency increased with increasing concentration of the oils and Margosan-O, while the turmeric oil or sweetflag oil repelled insects during the first 2 weeks, while some essential oils have been used as insect repellents since ancient ages such as citronella and ennyroyal. Repellents of plant origin do not pose hazards of toxicity to human and domestic animals and are easily biodegradable. Natural products are safe for human when compared to that of synthetic compounds (Sharma *et al.* 1993a; Sharma and Ansari 1994). Recently, extracts from neem (*Azadirachta indica* A.Juss) and citronella glass (*Cymbopogon nardu*s Rendle) have been known as such possible natural compounds. These repellent compounds have low level toxicity to human and animals and high repellent effect against insects (Schmutterer 1990; Sharma *et al.* 1993b; Lindsay *et al.* 1996; Lee *et al.* 2004).

The repellent properties of plants to mosquitoes and other pest insects were well known before the advent of synthetic chemicals. In southern India, leaves of *Vitex negundo* are burned to repel mosquitoes from houses (Curtis *et al.* 1989). The plant products have been used traditionally to repel or kill the mosquitoes in many parts of the world (Novak 1985). Forty essential oils extracted from Australian plants were evaluated against mosquitoes, march flies, and sand flies. The most effective of these were *Dacrydium franklini, Backhousia myrtifolia, Melaleuca bracteata,* and *Zieria smithii* (Penfold and Morrison 1952). Repellency properties of nepetalactone (cyclopentanoid monoterpene) isolated from the catnip plant, Nepeta cataria, against 17 species of insects were reported by Eisner (1964). Also, many monoterpenes were reported for their insect repellent properties such as α-pinenen, limonene, terpinolene,

citronellol, citronellal, camphor, rotundial, dolichodial,teucrein, and isoborneol (Takikawa *et al.* 1998; Eisner *et al.* 2000). Among 29 tested alkaloids obtained from *Delphinium, Consolida*, and *Aconitum* species, 21 compounds showed a promising insect repellent activity, while eight of them were not active. Hetisine had the highest activity while venulol showed the lowest (Ulubelen *et al.* 2001).

Protection against mosquito bites was reported for the genus *Azadirachta indica* (Sharma *et al.* 1993a; Sharma *et al.* 1993b; Sharma and Ansari 1994); *Cymbopogan* (Das and Ansari 2003); *Mentha* (Ansari *et al.* 1999); *Eucalyptus maculata citriodon* (Trigg 1996); *Tagetus* (Tyagi *et al.* 1994); and *Lantana camara* flowers. Ansari *et al*. (2005) also reported the repellent properties of pine oil *against An. culicifacies* and *Cx. quinquefasciatus*. Egunyomi *et al*. (2010) also reported the repellent activity of *A. indica*, *Cymbopogon citratus*, *Ocimum gratissimum*, *Ageratum conyzoides*, *Annona squamosa*, *Hyptis suaveolens*, *Tridax procumbens*, *Citrus sinensis*, *Lantana camara* and *Solanum nigrum* at 2mg/ml against *An. stephensi*.

The local communities adapt various methods to repel the insects/ mosquitoes. Application of smoke by burning the plant parts is one of the most common practices among the local inhabitants. Other types of applications are spraying the extracts by crushing and grinding the repellent plant parts, hanging and sprinkling the repellent plant leaves on the floor etc. The leaf of repellent plant is one of the commonly and extensively used plant parts to repel the insects and mosquitoes, followed by root, flower and remaining parts of repellent plants (Kishore *et al.* 2011).

The mizo people burn plants as mosquito repellent. Some of traditional repellent plants used in order to avoid mosquito and other insect bites have been listed (Table 7).

Oviposition deterrency

Oviposition is one of the most important events in the life cycle of mosquitoes. Mosquito population can be reduced by disrupting its oviposition (Xue *et al.* 2001). Among the different methods of mosquito control strategies, the use of botanical extracts for oviposition repellent test may be the least and unexplored strategy. As far as our literature survey could ascertain, little information is available on the oviposition deterrent activities of the plant extracts against *Ae. albopictus*.

Rajkumar and Jebanesan (2009) have tested that the oviposition deterrency of *Cassia obtusifolia* leaf extract against *An. stephensi*. Mehra and Haridar (2002) also described oviposition deterrency of *Cuscuta hyaline*. Rajkumar and Jebanesan (2005b) also tested the leaf extract of *Solanum trilobatum* against the gravid females of *An. stephensi*. *Melia azedarach* leaf extract is also reported to have ovipostional deterrency against *Ae. aegypti* (Coria *et al.* 2008). Autran *et al.* (2009) have reported that the essential oil from leaves and stems of *Piper marginatum* exhibited an oviposition deterrent effect against *Ae aegypti.* Elango *et al.* (2009) reported that the oviposition deterrency of acetone, ethyl acetate and methanol extracts of *Aegle marmelos*, *Andrographis lineata* and *Cocculus hirsutus*.

Turmeric has a long history of therapeutic uses as it is credited with a variety of important beneficial properties such as its antioxidant, antibacterial, anti-inflammatory, analgesic, and digestive properties, apart from its uses as a medicinal plant, the fresh juice, the aqueous extracts, and the essential oil of the plant are credited with interesting pesticidal properties against certain pests of agricultural importance as well as a noticeable repellent activity against noxious mosquito species (Dalamas 2011). The insecticide activity of *M. azedarach* has been thoroughly studied. Leaf and fruit of *M. Azedarach* extracts have been evaluated on

diverse pests with promising results (Padrón *et al.* 2003; Mazzonetto and Vendramim 2003). The fruits are rich in triterpenes, Azadirachtin and oil (Chiang and Chang 1973). Due to biologically active triterpenoids with an antialimentary effect, i.e., they inhibit the feeding of phytophage insects producing death and malformations of subsequent generations (Vergara *et al.* 1997; Carpinella *et al.* 2003). Its insecticidal character was studied on the larvae of certain mosquitoes such as *Culex* sp. (Thekkevlayil *et al.* 2004) and *Anopheles* species (Sengottayan *et al.* 2006a). The insecticidal properties of *E. communis* has been scarcely studied, this is the first report of *E. communis* tested against mosquito control.

Their medicinal values and known phytochemical substances are also listed in Table 6. Ethnobotanical uses of selected plants and their properties are:

1. *Acacia gageana* Craib

Family	: Mimosaceae
Mizo name	: Khanghu
Uses	: The young shoots are eaten as vegetable.

2. *Alstonia scholaris* L. R. Br

Family	: Apocynaceae
Local name	: Thuamriat
Common name	: Indian Devil tree
Uses	:The decoction of the bark was used to treat diarrhoea, malaria, skin disorders, malarial fever, urticaria, chronic dysentery, diarrhea, in snake bite, ulcers. Decoction of the leaves is taken orally in the treatment of dysentery, typhoid fever and hypertension. The gummy exudates, mixed with sesame oil, are used as ear drops in ear pain.

3. *Antidesma acidium* Retz.

Family	: Euphorbiaceae
Mizo name	: Thurte-an
Common name	: Amti
Uses	: The acid leaves are eaten as cooked vegetable. The fruits are eaten by man and birds.

4. *Blumea lanceolaria* Roxb.

Family	: Asteraceae
Local name	: Buarze
Common name	: Lance-leaved blumea
Uses	: The tender leaves are used as cooked as vegetable. The leaves are used for treatment of ulcer, indigestion, asthma, chronic dysentery. Juice of leaves is applied on the infected wounds of animals to kill maggots.

5. *Brugmansia suaveolens* (Humb. & Bonpl. ex Willd.) Bercht. & C. Presl

Family	: Solanaceae
Local name	: Tawtawrawt-par
Uses	: The dried leaf is smoked in case of asthmatic problem and respiratory failure. The leaf juice is mixed with milk is given orally in the treatment of venereal diseases. The crushed seed is mixed with common salt solution, and after 7 days, the mixture is given orally in the treatment of headache, pre-menstrual tension and pain. The juice of the root is used topically in dog bites.

6. *Centella asiatica* Brahmi.

Family	: Apiaceae
Local name	: Lambak
Common name	: Indian pennywort
Uses	: Taken as boiled vegetable in case of stomach problem; crushed leaf applied to skin problems, given to mother after childbirth.

7. *Clerodendrum colebrookianum* Walp.

Family	: Verbenaceae
Local name	: Phuihnam
Common name	: East Indian Glory Bower
Uses	: Cold infusion of leaves is drunk against hypertension and to decrease breast milk.

8. *Croton caudatus* Geisel

Family	: Euphorbiaceae
Local name	: Ranlungdamdawi
Common name	: Caudated croton
Uses	: The leaves are applied as a poultrice to spraints. The leaves are also given to kill the sore-worms for pigs and cattles.

9. *Curcuma longa* Linn.

Family : Zingiberaceae.
Local name : Aieng
Common name : Turmeric
Uses : The juice of the rhizome, mixed with the juice of *Mikania micrantha* is used for treatment of cancer. The rhizome along with crab is taken orally for treatment of asthma. Turmeric is used for treatment of ulcer, skin diseases and wounds.

10. *Cuscuta reflexa* Roxb

Family : Cuscutaceae
Local name : Japanhloral
Common name : Dodder plant
Uses : This plant is used for the diseases such as impotence, premature ejaculation, sperm leakage, frequent urination, ringing in the ears, lower back pain, sore knees, leucorrhea, dry eyes, blurred vision, and tired eyes. Juice of the pounded stem is also used for washing hair to eliminate dandruff and lice.

11. *Dysoxylum gobara* Buch.-Ham. Merr.

Family : Meliaceae
Local name : Thingthupui
Uses : Decoction of the leaves is used as a remedy for food poisoning, diarrhea and dysentery.

12. *Elaeagnus caudata* Schltdl. ex Momiy.

Family : Elaeagnaceae
Local name : Sarzuk-pui
Common name : Bastard Oleaster
Uses : The juice of the root is given orally in rheumatic pain.

13. *Elsholtzia communis* (Coll. and Hemsl.) Diels

Family : Labiatae
Local name : Lengser
Uses : It is an aromatic herb used for flavoring curry.

14. *Eryngium foetidum* Linn.

Family : Apiaceae.
Local name : Bahkhawr
Common name : Mexico coriander
Uses : The leaf juice is taken orally to stop convulsions during high fever. It is also used internally as stomachic.

15. *Eupatorium glandulosum* Linn.

Family : Asteraceae
Local name : Tlangsam suak
Common name : Sticky snakeroot
Uses : The crushed leaves are applied to wound as blood coagulant and antiseptic.

16. *Eupatorium odoratum* Linn.

Family : Asteraceae
Local name : Tlangsam
Common name : Christmas bush
Uses : The juice of crushed leaves is applied externally on fresh wounds as haemostatics and as an antiseptic. The juice is also applied externally to remove pinworm from the anus.

17. *Gmelina arborea*

Family : Verbanaceae
Local name : Thlanvawng
Common name : Beechwood
Uses : The leaf juice is given orally in coughs, gonorrhea and ulcers. It is also used topically in scorpion stings and snakebites. The roasted fruit is crushed and the juice is applied externally on the itching skin.

18. *Hedyotis scandens* Roxb.

Family : Rubiaceae
Local name : Kelhnamtur/ Laikingtuibur.
Medicinal uses : Infusion of the roots and leaves are taken as an effective remedy against malarial fever. Infusion of the leaves is common employed to cure jaundice, kidney trouble and removal of kidney/ gall bludder stones. The juice of crushed leaves is taken for dysuria.

19. *Hiptage benghalensis* L. Kurz.

Family : Maplighiaceae
Local name : Raisentur
Medicinal uses : Decoction of the root bark is consumed orally for stomachache, chewed in a raw for diarrhoea and the powdered root bark mixed with water for dysentery treatment.

20. *Homalomena aromatica* Schott.

Family : Araceae
Local name : Anchiri
Common name : Sugandhmantri

Uses : Rhizome is used as aromatic stimulant. Juice of whole is used as lotion in skin diseases. The burnt smoke of dried rhizome is used as mosquito repellent.

21. *Melia azedarach* Linn.

Family : Meliaceae
Local name : Nim suak
Common name : China berry tree
Uses : Decoction of leaves is taken orally against fever and hypertension.

22. *Mikania micrantha* H.B.K.

Family : Asteraceae
Local name : Japan-hlo
Common name : Chinese creeper
Uses : The juice of crushed leaves is used as an effective haemostatics in cuts and wounds. The leaves are boiled and the water is drunk against diarrhea and dysentery associated with fever. The leaves are locally used for pig's food.

23. *Millettia pachycarpa* Benth.

Family : Fabaceae
Local name : Rulei
Common name : Fish poison climber
Uses : The juice extract of the crushed root and seed are widely used as fish poison.

24. *Oroxylum indicum* Linn. (Benth.)

Family : Bignoniaceae
Local name : Archangkawm
Common name : Indian trumpet tree
Uses : The root bark is also used, administered as astringent, bitter tonic, stomachic and anodyne. The decoction of the bark is taken for curing gastric ulcer and a paste made of the bark powder is applied for mouth cancer, scabies and other skin diseases. The seed is ground with fire-soot and the paste applied to the neck for quick relief of tonsil pain. Also, a paste made of the bark is applied to the wounds of animals to kill maggots. Decoction of the bark is given to animals for de-worming.

25. *Polygonum plebeium* R. Brown

Family : Polygonaceae
Local name : Bakhate

Common name : Knotweed
Medicinal uses : Decoction of the plant is taken against cirrhosis of liver and gastric complain.

26. *Securinega virosa*

Family : Euphorbiaceae
Local name : Saisiak
Common name : White berry bush
Uses : Decoction of the leaves is used for bath in case of measles, chicken pox, scabies and skin itching.
Other uses : The bark is used for fish poisoning.

27. *Syzygium aromaticum* (L.) Merr. & Perry.

Family : Myrtaceae
Common name : Clove
Uses : Relieving toothaches, earaches, nausea, hypertension and pain from burns and wounds.

28. *Tagetes erecta* Linn.

Family : Asteraceae
Local name : Derhken
Common name : Marigold
Medicinal uses : The leaf juice is used as eardrops in otorrhoea. It is also applied on boils and carbuncles. The juice of the flower is used as eye drops in the treatment of ulcers of the eyes. It is given orally as a blood purifier and also in haemorrhoids.

29. *Thespesia lampas* Dalz and Gibs

Family : Malvaceae
Common name : Portia tree
Uses : Roots and fruits are used for treating gonorrhea, jaundices, syphilis. The stems of the plant are used for treatment of inflammation, acidity, bleeding nose, bronchitis, cough, dysentery, fever, sun stroke, urinary complaints, anthelmintic, carbuncle.

30. *Tithonia diversifolia* (Hemsl) A. Gray

Family : Asteraceae
Local name : Bawngpu-par
Common name : Mexican sunflower
Medicinal uses : An infusion of leaves is used as a medicine for constipation, stomach pains, indigestion, sore throat and liver pains.

RAPD profiling for Genetic studies

Organisms when continuously exposed to environmental stress may result in DNA damage. The explorations of random amplified polymorphic DNA (RAPD) as genetic markers have improved the detection of DNA alterations after the influence of many genotoxic agents (Atienzar *et al.* 2001). RAPD -PCR is one of the most reliably used techniques for detecting DNA damage as the amplification stops at the site of the damage. RAPD assay and related techniques like the arbitrarily primed polymerase chain reaction (AP-PCR) have been shown to detect genotoxin-induced DNA damage and mutations. The changes occurring in RAPD profiles following genotoxic treatments include variation in band intensity as well as gain or loss of bands. This can be done through the analysis of band intensities and/or band gain/loss variation between exposed and non-exposed individuals (Lalrotluanga *et al.* 2011a). Indeed, the gain/loss or intensity differences of RAPD bands may be related to DNA damage, mutations or structural rearrangements induced by genotoxic agents, affecting the primer sites and/or interpriming distances (Atienzar *et al.* 2002).

The RAPD method is a PCR-based technique that amplifies random DNA fragments with the use of single short primers of arbitrary nucleotide sequence under low annealing conditions. The technique has been extensively used for species classification and microorganism strain determination. Recently, the RAPD assay was also applied to detect genetic instability in tumors (Papadopoulos *et al.* 2002) and successfully detected genomic DNA alterations induced by several DNA damaging agents, such as benzopyrene (Atienzar and Jha 2004), heavy metals (Enan 2006) and UV radiation (Kumar *et al.* 2004). The final purpose of the present work was to identify the possible molecular site or gene-specific markers linked to biopesticides.

The aim of this study was to investigate the larvicidal, IGR, adulticidal, repellent, ovipositional deterrency of selected plant extracts on three mosquito vector species, namely *Aedes albopictus, Anopheles barbirostirs* and *Culex quinefasciatus*. In addition, the biochemical and molecular profiling was undertaken in the treated larvae.

Table 1. Efficacy of petroleum ether extracts of plants against mosquito vectors

Plant species	Family	Plant parts used	Target mosquito species	References
Artemisia vulgaris	Asteraceae	Leaf	*An. stephensi*	Sharma *et al.* (2006)
Acacia nilotica	Fabaceae	Leaf	*An. stephensi*	Saktivadivel and Daniel (2008)
Aloe barbadensi	Liliaceae	Leaf	*An. stephensi*	Maurya *et al.* (2007)
Eucalyptus globulus	Myrtaceae	Seed, leaf	*Culex pipiens*	Sheeren (2006)
Solanum xanthocarpum	Solanaceae	Root	*Cx. pipiens*	Mohan *et al.* (2006)
Thymus capitatus	Lamiaceae	Leaf	*Cx. pipiens*	Mansour *et al.* (2007)
Citrus aurantium	Rutaceae	Fruit peel	*Cx. quinquefasciatus*	Kassir (1989)
Myrtus communis	Myrtaceae	Flower and leaf	*Cx. pipiens molestus*	Traboulsi *et al.* (2002)
Jatropa curcas	Euphorbiaceae	Leaf	*Cx. quinquefasciatus*	Rahuman *et al.* (2007)
Pedilathus tithymaloides				
Phyllanthus amarus				
Argemone mexicana	Papaveraceae	Leaf	*Cx. quinquefasciatus*	Karmegan *et al.* (1997)
Jatropa curcus	Euphorbiaceae	Leaf		
Pergularia extensa	Aslepiadaceae	Leaf		
Withania somnifera	Solanaceae			
Piper nigrum	Piperaceae	Seed	*Cx. pipiens*	Shaalan *et al.* (2005)
Euphorbia hirta	Euphorbiaceae	Stem bark	*Cx. quinquefasciatus*	Rahuman *et al.* (2007)
E. tirucalli				
Ocimum basilicum	Lamiaceae	Leaf	*An. stephensi* and *Cx. quinquefasciatus*	Maurya *et al.* (2009)

Table 2. Efficacy of chloroform extracts of plants against mosquito vectors

Plant species	**Family**	**Plant parts used**	**Target mosquito species**	**References**
Plumbago zeylanica, P dawei and P. stenophylla	Plumbaginaceae	Root	*An. gambiae*	Maniafu *et al.* (2009)
Euphorbia tirucalli	Euphorbiaceae	Latex and stem bark	*Cx. pipiens pallens*	Yadav *et al.* (2002)
Nyctanthes arbortristis	Nycthantheceae	Flower	*Cx. quinquefasciatus*	Khatune *et al.* (2001)
Citrus sinensis	Rutaceae	Fruit peel	*An. subpictus*	Bagavan *et al.* (2009)
Aloe ngongensis	Asphodelaceae	Leaf	*An. gambie*	Matasyoh *et al.* (2008)
Millettia dura	Leguminaceae	seed	*Ae. aegypti*	Yenesew *et al.* (2003)
Cassia obtusifolia	Leguminaceae	Seed	*Ae. aegypti, Ae. togoi* and *Cx. pipiens pallens*	Yang *et al.* (2003)
Ocimum sanctum	Labiatae	Leaf	*Ae. aegypti* and *Cx. quinquefasciatus*	Anees (2008)

Table 3. Efficacy of acetone extracts of plants against mosquito vectors

Plant species	**Family**	**Plant parts used**	**Target mosquito species**	**References**
Tridax procumbens	Compositae	Leaf	*An. subpictus*	Kamaraj *et al.* (2011)
Ageratum conyzoides	Asteraceae	Leaf	*Cx. quinquefasciatus*	Saxena *et al.* (1992)
Cleome icosandra	Capparaceae	Leaf		
Tridax procumbens	Compositae	Leaf		
Ageratina adenophora	Asteraceae	Twigs	*Ae. aegypti* and *Cx. quinquefasciatus*	Raj Mohan and Ramaswamy (2007)
Feronia limonia	Rutaceae	Leaf	*Cx. quinquefasciatus, An. stephensi, Ae. aegypti*	Rahuman *et al.* (2000)
Millingtonia hortensis	Bignoniaceae	Leag	*Cx. quinquefasciatus, An. stephensi, Ae. aegypti*	Kaushik and Saini (2008)

Table 4. Efficacy of Methanol extracts of plants against mosquito vectors

Plant species	**Family**	**Plant parts used**	**Target mosquito species**	**References**
Atlantia monophylla	Rutaceae	Leaf	*An. stephensi*	Sivagnaname and Kalyanasundaram (2004)
Dysoxylum malabaricum /Melia azedarach	Meliaceae	Leaf	*An. stephensi*	Senthil Nathan *et al.* (2006a;b)
Moringa oleifera	Moringaceae	Bark	*Cx. gelidus*	Kamaraj and Rahuman (2010)
Ocimum gratissimum	Lamiaceae	Leaf	*Cx. gelidus*	Kamaraj and Rahuman (2010)
Solenostemma argel	Apocynaceae	Aerial parts	*Cx. pipiens*	Al-Doghairi *et al.* (2004)
Solanum xanthocarpum	Solanaceae	Root	*Cx. pipiens pallens*	Mohan *et al.* (2006)
Chrysanthemum indicum	Asteraceae	Leaf	*Cx. tritaenorrhynchus*	Kamaraj *et al.* (2010)
Azadirachta indica	Meliaceae	Leaf	*Cx. pipiens*	El Haq *et al.* (1999)
Rhazya stricta	Apocynaceae	Leaf		
Trichosanthes anguina / Momordica charantia	Cucurbirtaceae	Leaf	*Cx. quinquefasciatus*	Prabakar and Jebanesan (2004)
Vitex negundo, V. trifolia	Verbenaceae	Leaf	*Cx. quinquefasciatus*	Krishnan *et al.* (2007)
Centella asiatica	Umbelliferae	Leaf	*cx. quinquefasciatus*	Rajkumar and Jebanesan (2005)
Euphorbia tirucalli	Euphorbiaceae	Latex and stem bark	*Cx. pipiens pallens*	Yadav *et al.* (2002)
Eucalyptus globulus	Myrtaceae	Seed and leaf	*Cx. pipiens*	Sheeren (2006)
Atlantia monophylla	Rutaceae	Leaf	*Cx. quinquefasciatus*	Sivagnaname and Kalyasundaram (2004)
Pavonia zeylanica	Malvaceae	Leaf	*Cx. quinquefasciatus*	Vahitha *et al.* (2002)
Cassia tora	Caesalpinaceae	Seed	*Ae. aegypti* and *Cx. pipiens pallens*	Jang *et al.* (2002)
Coccinia indica	Cucurbitaceae	Leaf	*Ae. albopictus*	Rahuman and Venkatesan (2008)
Cucumus sativus				
Momordica charantia				
Annona squamosa	Annonaceae	Leaf		Das *et al.* (2007)
Gymnopetelum cochinchinensis,	Cucurbitaceae	Fruit/peri carp		
Chamaecytoparis obtuse	Cupressaceae	Leaf	*An. stephensi*	Jang *et al.* (2005)

Table 5. Efficacy of aqueous extracts of plants against mosquito vectors

Plant species	Family	Plant parts used	Target mosquito species	References
Carica papaya	Caricaceae	Seed	*Cx. quinquefasciatus* and *An. gambiae*	Rawani *et al.* (2009)
Murraya paniculata	Rutaceae	Fruit		
Cleistanthus collinus	Euphorbiaceae	Leaf		
Hemidesmus indicus	Asclepiadaceae	Root	*Cx. quinquefasciatus*	Khanna and Kannabiran (2007)
Gymnema sylvestre	Asclepiadaceae	Leaf		
Eclipta prostrata	Asteraceae	Leaf, root		
Artimisia cina	Compositeae	Leaf	*Cx. pipens*	Aly and Bardan (1986)
Cleome droserifolia	Capparidaceae	Leaf		
Piper retrofractum	Piperaceae	Un ripe and ripe fruit	*Cx. quinquefasciatus* and *Ae. aegypti*	Chansang *et al.* (2005)
Solanum villosum	Solanaceae	Leaf	*An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti*	Chowdhury et al. (2008)
Solanum nigrum	Solanaceae	Dried fruit	*An. Culicifacies, An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti*	Raghavendra et al. (2009)

Table 6. Ethnomedicinal plants tested for their insecticidal actions against mosquitoes and their properties

Plant	**Family**	**Parts used**	**Ethanomedicinal uses**	**Known phytochemical substances**	**Properties**	**Reference**
Alstonia scholaris (L.) R.Br	Apocynaceae	Leaf, bark, root	Toothache, rheumatism, snakebite, dysentery, bowl disorder, beri-beri, ulcer, dropsy, congestion of liver	Alkaloids, terpenoids, steroids, flavonoids, saponins, tannins	Antimicrobial, anticancer, antiplasmodial Anthelminthic, astringent, antiperiodic, antidiarrhia,	Mishra *et al.* (2011); Khan *et al.* (2003); Kaushik *et al.* (2011)
Antidesma acidium Retz.	Euphorbiaceae	leaf	Diabetic treatment,	-	-	Khan and Yadava (2010)
Blumea lanceolaria Roxb.	Asteraceae	leaf	wounds and chronic ulcers	methyl thymol	O p h t h a l m i a	Dung *et al.* (1991)
Brugmansia suaveolens	Solanaceae	Leaf, flower	dental and skin infections, toothache and alopecia	Tropane alkaloids, sapponins, tannins	Anti-inflammatory, respiratory decongestion	Devi *et al.* (2011) Banso and Adeyemo (2006)
Centella asiatica Brahmi.	Umbelliferae	Leaf	Skin diseases like chronic ulce ration, psoriasis and leprosy	Triterpene	collagen enhancement, antioxidant, anti-cellulite, psychoactive	Vishnurao (1996) Hasim *et al.* (2011)
Clerodendrum colebrookianum Walp.	Verbenaceae	Leaf	Diuresis, hypertension,	Steroids, terpenes	-	Yang *et al.* (2000) Joshi *et al.* (1979) Rai and Lalramng-hinglova (2010)
Croton caudatus Geisel.	Euphorbiaceae	stems and leaves	malaria, ardent fever, convulsions, rheumatic arthritis, and numbness	Flavonoids, dotriacontamol, bamyrin, b-sitosterol, Sesquiterpenes	Anti-oxidant	Anon. (1975) Deore *et al.* (2009) Wang and Zou (2008)

Curcuma longa	Zingiberaceae	rhizom e	Ulcer, wounds, facial tonic, ringworm, obstinate itching, eczema and other parasitic skin diseases, chicken pox, small pox, cold, cough, bronchitis, conjun-ctivitis and liver affections	curcuminoids, sesquiterpenes, Turmerin	Antiinflammatory, antiseptic, antiprotozoal, spasmolytic, CNS active,Antipa-rasitic, antispasmodic, antibacterial, antiarthritic, -	Joy *et al.* (1998); Khan *et al.* (2008)
Cuscuta reflexa Roxb.	Convolvulaceae	Whole plant	Jaundice, pains in muscles and joints, bilious disorders	Flavonoids, phenols	Astringent, anti-helminthic, anti-steroidogenic	Chopra *et al.* (1992); Yadav *et al.* (2000); Loffler *et al.* (1995)
Dysoxylum gobara Buch.-Ham. Merr.	Meliaceae	Leaf and Bud	diarrhoea and dysentery	-	-	Rai and Lalramnghinglova (2010)
Elaeagnus caudata Schltdl. ex Momiy.	Elaeagnaceae	Leaf	-	Flavonoids. Steroids	-	Rout *et al.* (2012) Dandge *et al.* (2011)
Elsholtzia communis (Coll. and Hemsl.) Diels	Lamiaceae	Leaf	-	Phenols, flavonois, tannins	-	Khomdaram and Singh (2011)
Eryngium foetidum	Umbelliferae	whole	Colds, fits, convulsions, fainting, ulcers	Phytosterols	anti-inflammatory , antihelminthic	Garcia *et al.* (1999); Mitchell and Ahmad (2006)
Eupatorium glandulosum	Asteraceae	Leaf	antimicrobial, antiseptic, blood coagulant, analgesic, antipyretic and enhan-cer of phenobarbitone induced sleep	Alkaloid, terpenoids, phenols, organic acid, flavonol glycosides,	Antibacteria, pesticide	Nair *et al.* (1995) Gurusubramanian *et al.* (2008); Ansari *et al.* (1983)
Eupatorium odoratum	Asteraceae	Christ	Wound dressing, colds,	Flavonoids,	Insecticidal,	Patel *et al.* (2010)

		mas bush	cough, bronchitis, dengue fever, arthritis, certain infectious diseases, migraine, intestinal worms, malaria, and diarrhea	steroids, triterpenes alkaloids, flavonoids, tannins, diterpenes, glycolsides, lactones, saponins	antihelminthic	Mitchell and Ahmad (2006) Prasad *et al.* (2005)
Gmelina arborea	Verbaneceae	Leaf, stem	stomachic, galactogogue, laxative and antihelminthic, abdominal pains, burning sensations, fevers and urinary discharge	Alkaloids, flavonoids, tannins, saponins	Cytotoxic, antimicrobial	Hartwell (1995); Anthony *et al.* (2012); El-Mahmood *et al.* (2010)
Hiptage benghalensis L. Kurz.	Malpighiaceae	Bark, leaf, flower, root bark	Stomachache, diarrhea, dysentry	Alkaloids, coumarin, flavonoids, phenols, tannins, terpenoids, hiptagin	Aromatic, refrigerant, expectorant	Murugan and Mohan (2011); Khare (2007); Lalnundanga (2000)
Homalomena aromatica (Roxb.)Schott	Araceae	Rhizome	Jaundice, influenza, anti-inflammatory agent, skin disease	Flavonoid, alkaloid, reducing sugar, saponin, tannin, steroids	Anti-microbial, anti-fungal, insecticidal	Singh and Maurya (2005); Das and Talukdar (2010)
Melia azedarach Linn.	Meliaceae	Leaf, fruit	Leprosy, scrofula, anthelmintic, antilithic, diuretic, deobstruent, resolvent, rheumatism	Triterpenoids, limonoid	insecticide	Carpinella *et al.* (2003)
Mikania micrantha H.B.K	Asteraceae	Leaf	fever, rheumatism, influenza and respiratory diseases	Linalool, α-pinene, terpenes, phenol, tannin, alkaloids, steroids, terpenoids	Antibacterial, antibacterial and anti-inflammatory, insect sting, skin irritation	Cabral *et al.* (2001); Wei *et al.* (2004); Smith (1991); Pérez-Pérez-Amador *et al.* 2010);Hajra *et al.* (2010)

Millettia pachycarpa Benth.	Fabaceae	Stem, leaf	Insecticide, blood tonic	Isoflavonoids rotenoids , prenylated chalcone	Anthelminthic insecticidal	Okamoto *et al.* (2006); Singhal *et al.* (1982); Su *et al.* (2012)
Oroxylum indicum L. Benth.	Bignoniaceae	Bark	Jaundice	Flavonoids. Alkaloids, phenols	Anti-inflammatory, anti-allergy	Khisha *et al.* (2012); Roy *et al.* (2007); Radhika *et al.* (2011)
Polygonum plebeium R. Brown	Polygonaceae	leaf	Pneumonia, bowel complaints, colic complaints	Polyphenol, flavonoid	-	Swapna *et al.* (2011); Katewa and Galav (2005)
Securinega virosa Roxb. ex Willd.	Euphorbiaceae	Leaf, bark	Inflammatory agent, analgesic, fever, body pain, stomach ache rheumatism, diar-rhoea,pneumonia, epilepsy	-	Antibacteria, antifungal antimalaria	Neuwinger (1996); Khan *et al.* (1980); Gbeassor *et al.*(1989)
Syzygium aromaticum (L.) Merr. & Perry.	Myrtaceae	Leaf, bud	Sexual disorders	Tannins, flavonoids, phenols, anthraquinone	Aphrodisiac	Tajuddin *et al.* (2003)
Thespesia lampas Dalz and Gibs	Malvaceae	Roots, fruits	Treating gonorrhea, jaundices, syphilis, diabetes	-	anti-microbial, hepatoprotective activity	Sangameswaran *et al.* (2008)
Tithonia diversifolia Hemsl.	Asteraceae	Leaf	antimalaria,anti-diarrhia, antiinflam-matory, antibacterial, antiproliferation	Sequiterpene	Insecticidal, antifungal	Rungeler *et al.* (1998); Akinbode (2010)
Tagetes erecta Linn.	Asteraceae	Leaf, flower	Eye diseases, conjunctivitis, coughs, ulcers	-	Antibacteria, antifungal, cytotoxic	Nikkon *et al.* (2007)

Hedyotis scandens Roxb.	Rubiaceae	Leaf	Gastro-intestinal problems	Alkaloids, anthraquinones, flavonoids, iridoids, triterpenoids, sterols, lignans	-	Gaikwad *et al.* (2008)

Table 7. Traditional plants used for mosquito repellent

Plant	**Family**	**Vernacular name**	**Parts used**
Cinnamomun glanduliferum	Lauraceae	Khiangzo/bulrimna	Root
Citrus macroptera	Rutaceae	Hatkora	Rind of fruit
Gmelina arborea	Lamiaceae	Vawngthla	Root, bark, leaf
Gmelina oblongifolia	Lamiaceae	Thlanvawng	Root, bark, leaf
Homalomena aromatica	Araceae	Anchiri	Rhizome

Fig. 1 Global distribution of human *Plasmodium* spp. and lymphatic filarial species; (A) malaria); and (B) lymphatic filariasis (Manguin *et al.* 2010)

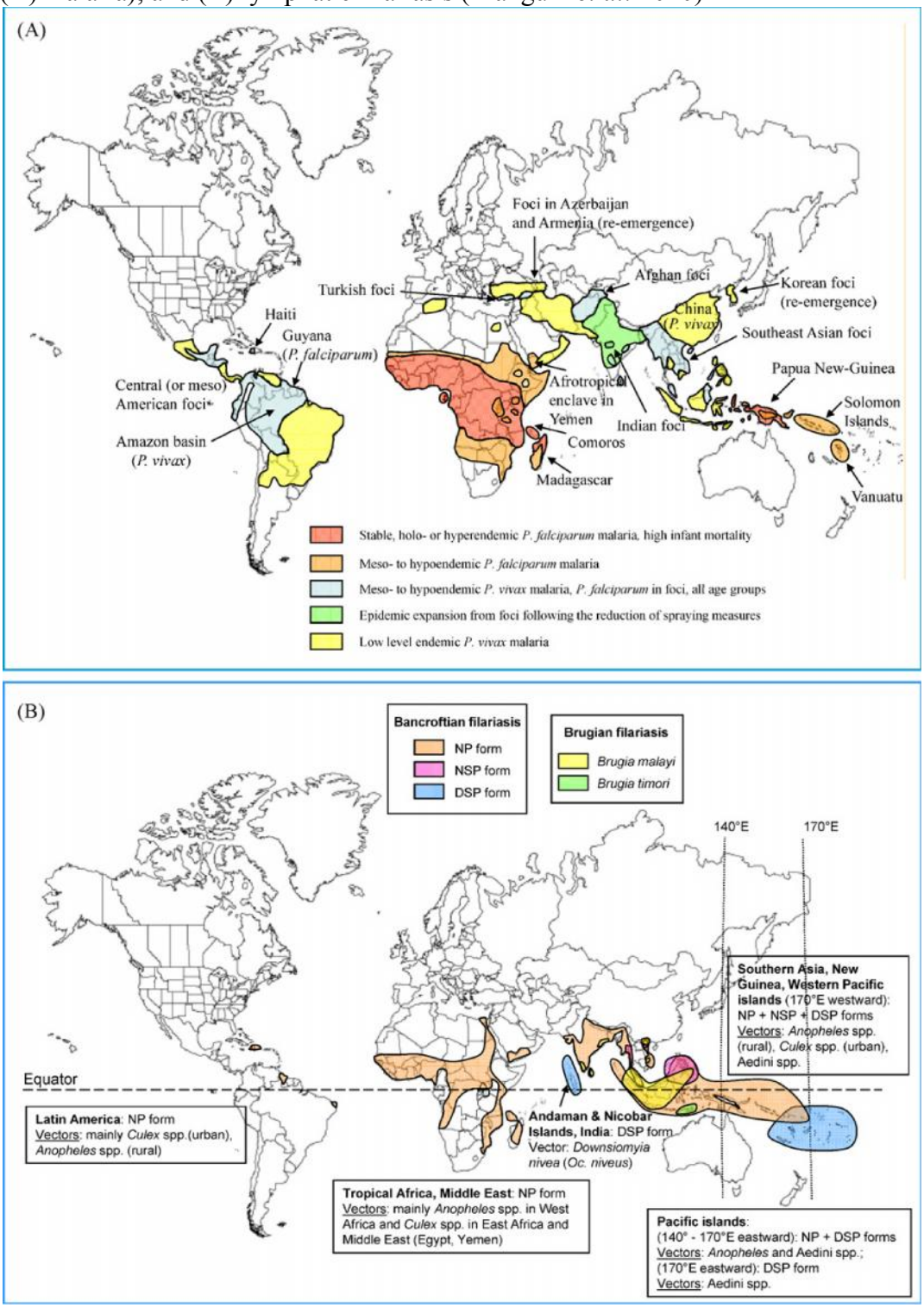
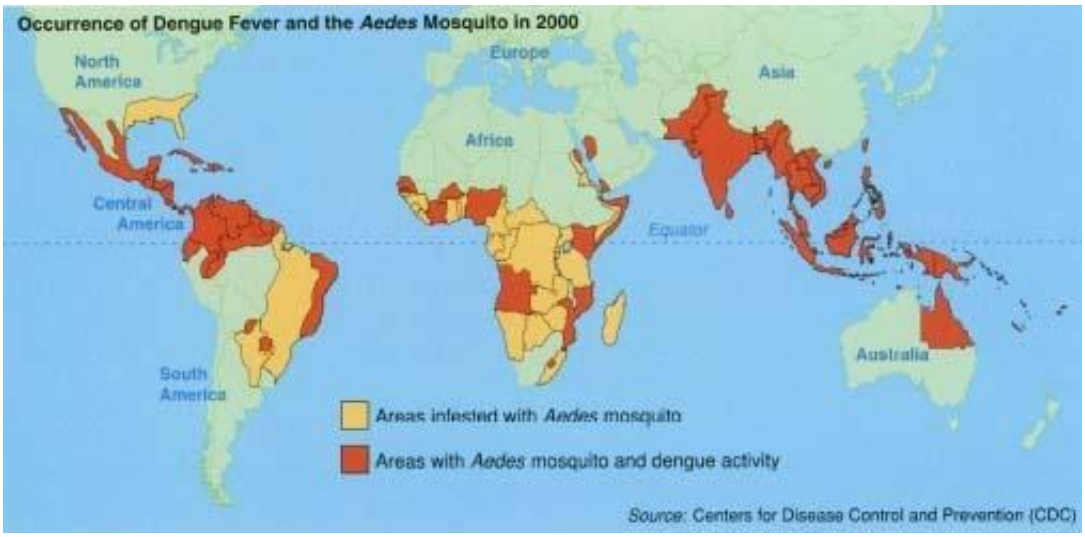


Fig. 2 Distribution of Dengue disease throughout the world.



2. OBJECTIVES OF THE STUDY

The present study approaches the following objectives:

1. Screening of mosquito larvicidal properties of thirty ethnomedicinal plants from Mizoram.
2. Selective bioassay - mortality, lethal concentration (LC_{50}) and lethal time (LT_{50}) of certain plant extracts.
3. Estimation of nutrient reserves in treated mosquito larvae
4. To study the effect of plant extracts as insect growth regulators
5. To study adulticidal effect, repellency and oviposition deterrency of plant extracts against mosquitoes
6. Assessment of DNA damage in the treated larvae by RAPD – PCR tools

3. MATERIALS AND METHODS

3.1. Mosquito culture

Mosquito eggs and larvae were reared in plastic and enamel trays containing tap water. They were maintained, and all the experiments will be carried out, at 27±2°C and 75 –85% relative humidity under 14:10 light and dark cycles (WHO, 2005). *Aedes albopictus, Anopheles barbirostris* and *Culex quinquefasciatus* larvae were collected from various places to start the colony and were reared in plastic and enamel trays containing tap water (Plate 2: A). The mosquito species were identified with the help of standard keys (Das *et al.* 1990; Reuben *et al.* 1994; Rueda 2004). The laboratory colonies were maintained at 25-30°C and 80-90% relative humidity under a photoperiod of 14:10 h (light/dark) in the insectary of the Department of Zoology, Mizoram University, Aizawl, Mizoram. Pupae were transferred from the trays to a cup containing tap water (Plate 2: B) and were maintained in our insectary (45×45×40 cm) where adults emerged (Plate 2: C). Larvae were fed on dog biscuit, soya flour, brewers yeast or algae collected from ponds. The adults were provided with 10% sucrose and it was periodically blood-fed on restrained rats or chicken. Beakers with 50 ml of tap water lined with filter paper were kept inside the cage for oviposition.

3.2. Preparation of plant extracts

The air dried plant materials (leaves, bark, flowers) were powdered mechanically using commercial electrical stainless steel blender and extracted with petroleum ether, acetone, chloroform, methanol and water. To determine the efficacy of these chemical extracts, the air dried leaf powders and the solvent were placed in

conical flasks at the ratio of 1 mg: 1 ml. The conical flasks were covered with air tight seals and the mixture was then left to stand for 2-7 day. The flasks were shaken everyday for about one to three hour. The mixture was then filtered through filter paper. The solvent was evaporated off with the help of a water bath (Kotze and Eloff 2002). The concentrated extract was then placed in vials. The filtrate was considered as pure material and redissolved in absolute ethanol to 10% (w/v) standard formulation. By further dilutions with required amount of water, different ppm concentrations were prepared.

3.3. Larvicidal bioassay

Control experiments were conducted in parallel with each replicant. All the experiments were performed according to World Health Organization standard protocols (WHO 1981) with suitable modifications. Mortality counts were made after 24. Dead larvae were identified when they failed to move after probing with dropper. Percentage mortality is recorded from the average for the three replicates taken. The percentage mortality was calculated by using the formula:

$$\text{Percentage of mortality} = \frac{\text{Number of dead insects}}{\text{Number of insects tested}} \times 100$$

Controls with more than 20% mortality were discarded. When the mortality ranged from 5-20%, the corrected mortality was calculated by Abbott's formula so as to remove the error, if any, on account of the mortality due to factors other than the toxic effect of the extract (Abbott, 1925). Initially, thirty III instar mosquito larvae in

three replicates were exposed to a particular concentration (500 ppm) of different plant extracts to find out the larvicidal activity.

3.3.1. Preliminary screening of larvicidal bioassay

In preliminary screening of larvicidal bioassay, third instar larvae of *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus* were treated in 500 ppm of plant extracts. A total of 30 third instar larvae were exposed in three replicates of 10 larvae each. Control experiments were conducted in parallel with each replicant. Experiments were conducted at room temperature (28±2°C).

3.3.2. Dose–response larvicidal bioassay (LC_{50})

After screening of different plant extracts against the third instar larvae of *Ae. albopictus, An. barbirostris* and *C. quinquefasciatus*, plant extracts showing high mortality rate were selected for dose – response bioassay. Required concentrations of different plant extracts (concentrations of 12.5, 25, 50, 100, 200, 400, and 500 ppm) were prepared through the mixing up of stock extract with variable amounts of sterilized distilled water. Each of the earlier prepared concentrations of different extracts was transferred into the sterile glass beakers (500 ml capacity). For bioassay test, third instar larvae of *Ae. albopictus, An. barbirostris,* and *Cx. quinquefasciatus* were divided into respective groups in four batches of 25 numbers in 249 ml of water and individually added with 1.0 ml of different concentration (12.5, 25, 50, 100, 200, 400, and 500 ppm) of plant extract. No food was provided during the treatment. Mortality was recorded after 24 and 48 h of post-exposure (WHO 1981). Dead larvae were identified when they failed to move after probing with a needle in the siphon or

cervical region. The experiments were observed four times and conducted at 27±2°C and 80–90 % relative humidity. The untreated control was set up with acetone solvent. The corrected mortality was calculated by Abbott's formula (Abbott 1925).

3.3.3 Time response Larvicidal Bioassay (LT_{50})

One milliliter of plant extract was added to 249 ml distilled water in a 500 ml plastic cup, which was shaken lightly to ensure a homogeneous test solution. Twenty five specimens each of third instar larval stages of *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus* were divided into respective groups and placed in cups. No food was provided during the treatment. Lethal time was observed at the concentrations of 12.5, 25, 50,100, 200 and 400 ppm. Mortality was recorded at regular intervals of 1, 3, 5, 8, 15, 20, 24 and 48 h of post-exposure in each concentration. Larvae were considered dead if they were incapable of rising to the surface or did not show the characteristic dicing reaction when the water was disturbed (WHO 1981). The mean mortality number was recorded. Each experiment was performed in four replicates with a simultaneous control (1 ml 70% ethanol in 249 ml water). LT_{50} values (lethal time for 50% mortality at a specific dose) were calculated using probit analysis. The mortality data was analyzed by Tukey's multiple range test (Snedecor and Cochran 1989).

3.4. Estimation of nutrient reserves from treated and untreated mosquito larvae

A sublethal concentration was selected on the basis of dose response bioassay to observe the effect on nutrient reserves of freshly emerged III instar larvae of *A. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus*. The bioassay experiment was

replicated thrice. Five mosquito larvae of the treated and untreated each mosquito species were placed separately in each microcentrifuge tube with 200 µl of sodium sulphate and crushed with a plastic pestle, and two x 0.8 ml volumes of chloroform–methanol (1:1) was used to wash the pestle. The tubes were vortexed and centrifuged at 3,000 rpm for 1 min. The supernatant was transferred to another microcentrifuge tube whereas pellet was retained for glycogen analysis. De-ionized water was mixed with the supernatant and centrifuged at 3,000 rpm for 1 min. Aqueous layer was separated for sugar analysis, and bottom portion was used for lipid analysis (van Handel 1985a).

3.4.1. Sugar analysis

The tubes were heated at 90°C until the solutions evaporated from the sample, and about 50–100 µl of solution is left in the sugar tubes. Anthrone (5 ml) was added to the the tubes, vortexed and heated for 20 min at 90°C. The absorbance was recorded at 625 nm.

3.4.2. Glycogen analysis

Anthrone (5 ml) was added to the precipitate of the tubes containing glycogen pellet, vortexed and heated for 20 min at 90°C. The absorbance was recorded at 625 nm.

3.4.3. Lipid analysis

Samples were heated at 90°C until all solutions were evaporated from the lipid. Sulphuric acid (200 µl) was added to the tubes containing lipid precipitate and

heated for 10 min at 90°C. 5 ml of vanillin–phosphoric acid reagent was added, vortexed and allowed to cool, and the absorbance was read at 525 nm (van Handel 1985b).

3.4.3. Protein analysis

Freshly emerged III instar larvae were collected from the experimental setup for the protein analysis. Five larvae were taken for each concentration and homogenized in 0.25 M sucrose solution in cold conditions. The homogenate was centrifuged at 12,000 rpm for 10–12 min, and the obtained supernatant was used to determine the total protein present in the sample (Lowry *et al.* 1951).

3.4.4. Standard curves

Known amounts of glucose and soybean oil (1 mg/ml) were prepared in deionized water and chloroform, respectively, as reported by van Handel (1985a,b). Glucose solution was prepared in amount of 25, 50, 100, 150 and 200 µg and brought to a volume of 5 ml with anthrone reagent, whereas soy bean oil was prepared in amount of 50, 100, 200 and 400 µg and brought to a volume of 5 ml with vanillin–phosphoric acid reagent. Three replicates were prepared for each concentration; absorbance was read at 625 nm for glucose and 525 nm for soybean oil, and nutrient amount was calculated from the resulting linear regression equations.

3.5. Insect Growth Regulators

The insect growth regulators bioassay followed Sagar and Sehgal (1997) and the World Health Organization standard protocols (WHO 2005) with slight

modifications. Insect growth regulatory (IGR) activity of different plant extracts were tested against *Ae. albopictus*. Ten first instar larvae were introduced into 500ml enamel bowls containing 249 ml of water. Three different test concentrations 5 ppm, 10 ppm and 20 ppm were tested against *Ae. albopictus* and each test concentration were replicated four times. 250 ml of distilled water and distilled water with petroleum ether and Tween-20 served as control. Tween-20 was used as emulsifier in all the experimental media. The control experiments were run parallel with each replicate. Mortality of the larvae, pupae, larval pupal intermediate and adult mortality was recorded at regular intervals. Observation was continued in both treated and control bowls until the last immature pupates. Morphological abnormalities were also noted. The dead larvae and pupae removed daily and counted. The percentage emergences at different concentrations were recorded. Growth index was assessed by the following formula:

$$\text{Developmental Period (DP)} = \frac{\text{No..of larvae moulted X Days taken}}{\text{Total no. of larvae moulted from that instar}}$$

$$\text{Growth Index (GI)} = \frac{\text{\% Survival in a particular stage}}{\text{Developmental period}}$$

Adult Emergence IE(%) = 100 – (T X 100/ C)

where T = percentage survival or emergence in treated batches

C = percentage survival or emergence in the control.

The moribund and dead larvae in four replicates were combined and expressed as a percentage of larval mortality of each concentration. Dead larvae were

identified when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within a reasonable period of time) or showing the characteristic diving reaction when the water was disturbed. All surviving larvae were separately reared and maintained at 25-30°C and 80-90% relative humidity in the insectary. Pupation and adult emergence of these mosquitoes were recorded. The assays were terminated 3 day after the last control mosquito emerged.

The various dispersed effects that interfere with ecdysis was studied based on the characteristic toxic effects on mosquito larvae and were recorded according to the following eight criteria of Lapcharoen *et al.* (2005):

3.5.1. L (death as larvae). This category represents death during the larval stage with no evident initiation of pupation.

3.5.2. L (P) (larval cuticle with pupa inside). Death in this category has occurred at an early stage of pupation. The pupal abdomen can be seen to be withdrawn from the terminal part of the abdomen and the pupal respiratory trumpets are visible.

3.5.3. L-P (larvae with pupae partly emerged). At this stage the larval skin has been ruptured and the pupal body has partly emerged from the thoracic split. The abdomen has retracted to at least halfway along the larval abdominal skin and has adopted the characteristic pupal shape.

3.5.4. WP (white pupae). The pupae have completely escaped from the larval cuticle but have remained completely unmelanized except for eye pigment. The abdomen is held in an abnormally straight position.

3.5.5. BP (brown pupae). The pupae show some melanization.

3.5.6. P (A) (pupae with adult visible inside). In this categories, most of the adult anatomy can be distinguished, but the pupal skin has not split. Unlike the previous categories, the dead insect normally floats, presumably because the internal air bubble is preserved.

3.5.7. P-A (pupae with adult beginning emergence). The adults have begun to escape from the pupal skin but are unable to free themselves completely. Sometimes the head and thorax are freed, but the abdomen remains enclosed. Occasionally, the whole body is nearly free except for the legs.

3.5.8. DA (death adult). This category is reserved for adults which have freed themselves completely from the pupal skin, but cannot escape from the water film.

When mortality in the control is over 20%, the tests would be discarded.

3.6. Adulticidal Bioassay

Three mosquito vectors, *A. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus* mosquitoes were selected for the testing of adulticidal activities (WHO 1996). Appropriate concentrations (10000, 25000 and 50000 ppm) were dissolved in 2.5 ml of acetone and applied on Whatman no. 1 filter papers (size 12 x15 cm2). Impregnated papers were left to dry at room temperature overnight prior to testing. Control papers were treated with acetone under similar conditions. Adulticidal activity was evaluated at three concentrations (10000, 25000 and 50000 ppm) with an untreated control.

Ten female mosquitoes (3-6 days old 10% glucose fed, blood starved) were collected and gently transferred into a plastic holding tube. The mosquitoes were allowed to acclimatize in the holding tube for 1 h and then exposed to test paper for 1

h. At the end of exposure period, the mosquitoes were transferred back to the holding tube and kept 24 h for recovery period. A pad of cotton soaked with 10 per cent glucose solution was placed on the mesh screen. Mortality of mosquitoes was determined at the end of 24 h recovery period. Per cent mortality was corrected by using of Abbott's formula (Abbott 1925). Three observations were made for each concentration.

3.7. Repellent Bioassay

The duration of protection provided by plant extract was tested by means of arm-in-cage studies, in which volunteers insert their repellent-treated arms into a cage with a fixed number of unfed mosquitoes, and the elapsed time to the first bite is recorded (Fradin and Day 2002). Testing of repellent was conducted in a laboratory to reduce potential confounding variables (wind, speed, temperature, humidity, density of the mosquito population, the level of the mosquitoes' hunger, and the species of the mosquitoes).

For each test, 10 disease-free, laboratory-reared *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus* female mosquitoes separately that were between 5-10 days old were placed into separate laboratory cages measuring 30 cm by 22 cm by 22 cm. A batch of 10 mosquitoes that had not been exposed to the repellent being tested was used for each arm insertion. Mosquitoes were provided with a constant supply of 5 percent sucrose solution. Cages were placed in a laboratory at 27 ± 2°C, 80–90% RH and 14:10 light and dark cycles. Fifteen volunteers (5 men and 10 women) were used in the study.

The plant extracts were sequentially diluted to 5000, 10000 and 20000 ppm in absolute alcohol and tested on each subject. The repellent action was being tested three times on each subject. Most subjects only completed one test per day. The average time to completion of all three tests was 10 days. Before each test, the readiness of the mosquitoes to bite was confirmed by having subjects insert their untreated forearm into the test cage. Once subjects observed five mosquito landings on the untreated arm, they removed their arm from the cage and applied the repellent being tested from the elbow to the fingertips (Fradin and Day 2002).

After the application of the repellent, subjects were instructed not to rub, touch, or wet the treated arm. Subjects were provided with a standardized log sheet to ensure accurate documentation of the duration of exposure and the time of the first bite. The elapsed time to the first bite was then calculated and recorded as the "complete-protection time" for that subject in that particular test (Fig. 3).

3.8. Oviposition Deterrent Test

The oviposition deterrent test was performed using the method of Xue *et al.* (2001). Fifteen gravid females of *Ae. albopictus* (10 days old, 4 days after blood feeding) were transferred to each mosquito cage (45 × 38 × 38 cm). 10% sucrose solution was given as food supplement. Serial dilutions of leaf extract were made in ethanol. Enamel bowls containing 100 ml of rainwater would be treated with leaf extract to obtain test solutions of 5, 10 and 20 ppm. Two enamel bowls holding 100 ml of rainwater were placed in opposite corners of each cage, one treated with the test material, and the other with a solvent control that contained 1% ethanol. The positions of the bowls were alternated between the different replicates so as to nullify

any effect of position on oviposition. Five replicates for each concentration were run, with cages placed side by side for each bioassay. After 24 h, the number of eggs laid in treated and control bowls would be recorded.

The percent effective repellency for each leaf extract concentration was calculated using the following formula

$$ER(\%) = \frac{NC - NT}{NC} X\ 100\ (\%)$$

Where ER = percent effective repellency; NC = number of eggs in control; and NT = number of eggs in treatment.

The oviposition experiments were expressed as mean number of eggs and oviposition activity index (OAI), which was calculated using the following formula.

$$OAI = \frac{NT-NS}{NT+NS}$$

Where NT = total number of eggs in the test solution and NS = total number of eggs in the control solution. Oviposition active index of +0.3 and above are considered as attractants, while those with – 0.3 and below are considered as repellents (Kramer and Mulla 1979). Positive values indicate that more eggs were deposited in the test cups than in the control cups and that the test solutions were attractive. Conversely, negative values indicate that more eggs were deposited in the control cups than in the test cups and that the test solutions were a deterrent.

3.9 RAPD-PCR profiling of treated mosquito larvae

3.9.1. DNA Extraction

DNA from mosquito larvae was extracted by the method of Ballinger-Crabtree *et al.* (1992) with slight modifications. Ethanol preserved specimens were ground in 200 µl lysis buffer (100 mM Tris–HCl, pH 8.0; 1% sodium dodecyl sulphate; 50 mM NaCl; 50 mM EDTA), and the mixture was treated with 5 µl of proteinase K (20 mg/ml) for 16 h at 37°C. The suspension was extracted twice with equal volume of phenol–chloroform, and DNA was extracted by the addition of 0.2 volumes of 5 M NaCl and 2.0 volumes of ethanol at room temperature. The mixture was incubated overnight at −20°C and spun at 12,000 rpm for 10 min to get pellet which was resuspended in 100 µl of sterilized distilled water and stored at 4°C. DNA concentrations were determined by spectrophotometeric analysis

3.9.2. RAPD-PCR amplification

RAPD amplification was done with a 15 μl PCR mix, containing 1x PCR buffer 1.5 µl, MgCl2 (1 mM) 0.6 µl, dNTP (0.2 mM) 0.3 µl, BSA (0.533µl/ml) 0.8 µl, primers (MA-09, MA-12 and MA-26 – Table 8) 0.3 µl, 1.5 unit of Taq (0.3µl) and filled up with sterile deionized water to the final volume. 1µl of extracted DNA was also added in each PCR tube. Three primers were randomly selected for RAPD analysis. The reaction mixture was given a short spin for thoroughly mixing of the cocktail components. PCR tubes were loaded on to a thermal cycler. The PCR programme included an initial denaturation step at 94°C for 4 mins followed by 45 cycles with 94°C for 1 min for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 2 minutes and final extension at 72°C for 10 minutes were carried out (Table 9).

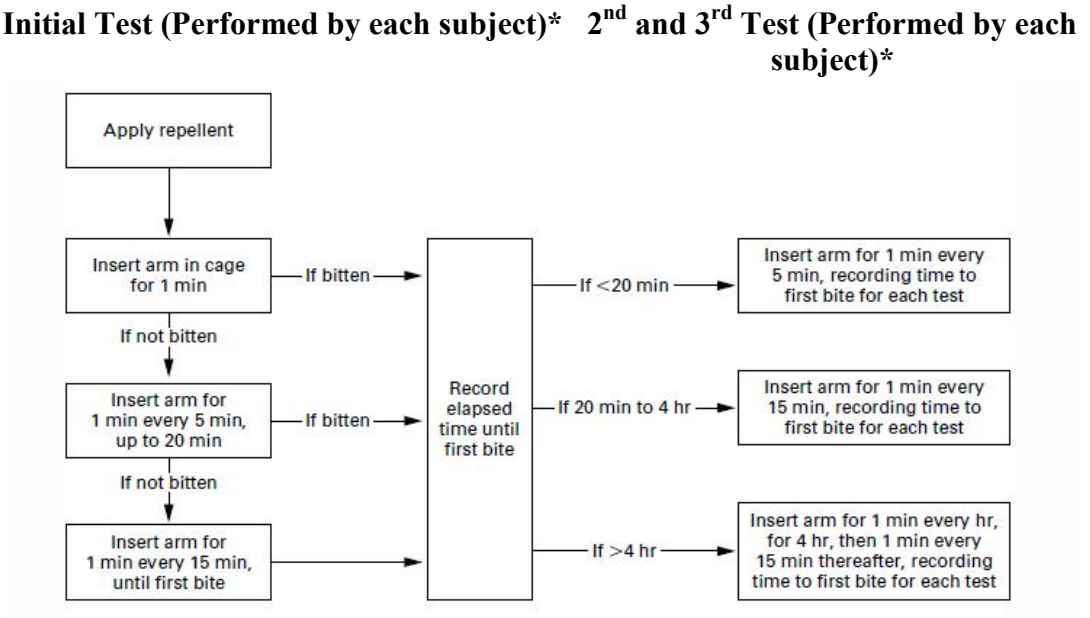
3.9.3. Agarose gel Electrophoresis

The amplification products were analyzed by electrophoresis (Sambrook *et al.* 1989). Along with the PCR amplified products, 100 bp DNA ladders as standard marker were subjected to electrophoresis in 1.5% agarose gel in TAE buffer and stained with ethidium bromide. Molecular size of the marker was 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. The amplified pattern was visualized on a UV transilluminator and documented. Polymorphism was evidenced as the presence and / or absence of DNA fragments between the samples. The RAPD profiles of the treated insects were evaluated on 1.5% agarose gel run for 30 min. at 120 volts.

3.10. Statistical analysis

Log probit for the larvicidal efficacy of plant extracts were calculated by following Finney (1971) method to generate regression equation, LC_{50}, and LT_{50} values (95% class intervals) with upper and lower fiducial limits, slope and chi square values. Results with $P<0.05$ were considered to be statistically significant. Completely randomized three-way factorial ANOVA and correlation coefficient were carried out using mosquito species, period of bioassay and different concentrations as variables to find the significance between the above parameters and mortality. Results of biochemical profile of nutrient reserves and primary metabolites studies were analysed statistically and Student's t test was used to analyse mean difference between control and treated groups. Data collected on adulticidal and repellent action were subjected to ANOVA and F value, critical difference (CD) and coefficient of variation (CV%) of mean ($P < 0.05$) and Tukey's multiple range test ($P < 0.05$) were used for taking statistical decisions (Snedecor and Cochran 1989).

Fig. 3 Study design for repellent test



*If at any time during testing, mosquitoes were seen to land on the skin but not bite (a sign of imminent failure of the repellent), then the interval between insertions was decreased to five minutes until the first bite was confirmed.

Table 8. List of random primers used for RAPD analysis

No	Primer	Sequence (5' to 3')	Annealing Tm °C/Sec
1	Primer MA-09	GACGGATCAG	32
2	Primer MA-12	ACCGCGAAGG	34
3	Primer MA-26	GACGTGGTGA	32

Table 9. RAPD-PCR reaction conditions

Profile	PCR programme	No of cycles
1	94°C 2min	Initial denaturation
2	94°C 1min	45cycles
3	36°C 1min	
4	72°C 30sec	
5	72°C 10min	Final extension
6	4°C	Hold

4. RESULTS

Preliminary larvicide screening results

The preliminary screening is a good means of evaluation of the potential larvicidal activity of plants popularly used for this purpose. Larvicidal activity of petroleum ether, chloroform, acetone, methanol and aqueous solvent crude extracts of thirty plants against III instar larvae of *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus* are presented in Table 10 ,11 and 12, respectively.

In preliminary screening test of different plant extracts against *Ae. albopictus*, petroleum ether extracts of *Blumea lanceolaria*, *Centella asiatica*, *Homalomena aromatic*a, *Oroxylum indicum*, *Tagetes erecta*, *Elsholtzia communis*, *Melia azedarach*, *Syzygium aromaticum*, *Curcuma longa*; Chloroform extract of *S. aromaticum*, *C. longa*; acetone extracts of *E. communis*, *Hiptage benghalensis*, *S. aromaticum* and *C. longa* have shown 100% mortality at 500 ppm. Petroleum ether extracts of *Antidesma acidum* (76%), *Croton caudatus* (73.3%), *Clerodendron colebrookanium* (83.3%); Chloroform extracts of *B. lanceolaria* (70%), *Oroxylum indicum* (76.7%), *E. communis* (96%); Acetone extracts of *Centella asiatica* (90%), *O. indicum* (73.3%), *T erecta* (75%), *C. caudatus* (73.3%) and methanol extract of *T. erecta* (90%) have shown larvicidal activities between 70 to 99% mortality. Petroleum ether extracts of *M. pachycarpa* (60%), *C. asiatica* (63.3%), *T. erecta* (60%), *Cuscuta reflexa* (50%), *C. caudatus* (60%); Acetone extract of *B. lanceolaria* (50%), *Eupatorium glandulosum* (63.2%), *C. reflexa* (53.3%), *A. acidum* (60%) and methanol extract of *C. asiatica* (56.7%) have shown 50-70% mortality (Table 10).

Petroleum ether extract of *E. glandulosum*, *C. asiatica*, *H. aromatica*, *T. erecta*, *E. communis*, *M. azedarach*, *S. aromaticum*, *C. longa*; chloroform extract of *O. indicum*, *E. communis*, *C. longa*; acetone extract of *E. glandulosum*, *T. erecta*, *E.communis*, *H. benghalensis* and *C. longa* gave 100% mortality in the third instar larvae of *An. barbirsotris*. 70-99% mortality were observed in petroleum ether extracts of *Mikania micrantha* (76.6%), *C. reflexa* (70%), *Brugmansia suaveolens* (73.3%); chloroform extracts of *E. glandulosum* (90%), *T. erecta* (80); acetone extract of *H. aromatica* (73.3%) and *S. aromaticum* (83.3%). 70% to 50% mortality were observed in chloroform extract of *E. odoratum* (60%), *Mikania micrantha* (63.3%), *H. aromatica* (60%), *C. reflexa* (53.3%), *S. aromaticum* (63.3%); acetone extract of *E. odoratum* (63.3%), *M. micrantha* (60%), *C. asiatica* (50%); methanol extract of *C. asiatica* (50%) and *T. erecta* (66.6%) (Table 11).

In screening test against *Cx. quinquefasciatus*, 100% mortality were observed in *M. micrantha*, *C. asiatica*, *O. indicum*, *T. erecta*, *M. pachycarpa*, *E. communis*, *Polygonum plebium*, *M. azedarach*, *S. aromaticum*, *C. longa*; chloroform extract of *C. longa*; acetone extract of *C. reflexa*, *E. communis*, *H. benghalensis*, *S. aromaticum* and *C. longa*. Mortality between 70% to 99% were observed in petroleum ether extract of *E. glandulosum* (90%), *E. foetidum* (80), *A. acidum* (76.3%), *D. gobara* (76.6%), *B. suaveolens* (80%); chloroform extract of *M. micrantha* (93.3%), *E. communis* (90%), *S. aromaticum* (80%); acetone extract of *E. glandulosum* (70%), *C. asiatica* (88.3%), *M. pachycarpa* (80%), *B. suaveolens* (73.3%), *H. benghalensis* (90%). The following extracts showed 50% to 70% mortality- *B. lanceolaria* (60%), *T. diversifolia* (56.6%), *H. aromatica* (66.6%), *C. colebrookanium* (63.3%); chloroform extracts of *E. odoratum* (60%), *C. asiatica* (56.3%), *E. foetidum* (50%);

acetone extracts of *E. foetidum* (66.7%), *A. acidum* (60%), *H. scandens* (66.7%) and methanol extract of *E. foetidum* (60%) (Table 12).

Based on the preliminary screening results, plant extracts that showed 100% larval mortality alone were selected and subjected to dose–response bioassay for larvicidal activity against *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasctiatus* larvae.

Table 10. Screening of larvicidal effecfs of ethnomedicinal plants against *Ae. Albopictus* at 500 ppm concentration in different solvents

Family	Plants	Common name	Part used	% Mortality ± SE				
				Pet. Ether	Chloroform	Acetone	Methanol	Aqueous
Asteraceae	*Blumea lanceolaria*	Lance-leaved blumea	Leaf	100	70±0	50±5.7	0	0
	Eupatorium glandulosum	Sticky snakeroot	Leaf	43.3±2.85	3.3±2.85	63.3±1.2	10±0	0
	Eupatorium odoratum	Christmas bush	Leaf	30±0	20±0	40±5	3.3± 2.85	0
	Mikania micrantha	Chinese creeper	Leaf	33.3±2.85	43.3±2.85	33.3±2.85	10±0	0
	Tithonia diversifolia	Mexican sunflower	Leaf	23.3±8.8	13.3±2.85	20±5	10±5	0
Apiaceae	*Centella asiatica*	Indian pennywort	Leaf	100	63.3±8.8	90± 5.7	56.7±7.6	0
	Eryngium foetidum	Mexico coriander	Stem	50±5	10±0	20± 0	0	0
Apocynaceae	*Alstonia scholaris*	Indian Devil tree	Bark	0	0	0	0	20±5
Araceae	*Homalomena aromatica*	Sugandhmantri	Rhizome	100	23±2.85	30±5	0	0
Bignoniaceae	*Oroxylum indicum*	Indian trumpet tree	Bark	100	76.7±8.8	73.3±8.8	0	0
Compositae	*Tagetes erecta*	Marigold	Leaf	100	60±0	75±7.7	90±5.7	0
Convolvulaceae	*Cuscuta reflexa*	Dodder plant	Stem	40±5.75	50±5.72	53.3±7.25	0	0
Euphorbiaceae	*Securinega virosa*	White berry bush	Leaf	0	0	3±2.85	0	0
	Antidesma acidum	Amti	Leaf	70±10	16.7±2.85	60±5	0	0
	Croton caudatus	Caudated croton	leaf	73.3±2.85	60±8.65	70±0	16.7±2.85	6.6±3.3
Elaeagnaceae	*Elaeagnus caudata*	Bastard Oleaster	Leaf	10±0	20±0	26.7±8.8	0	0
Fabaceae	*Millettia pachycarpa*	Fish poison climber	Root	60±5.8	46.7±8.8	43.3±8.8	23.3±5.7	0
Labiateae	*Elsholtzia communis*		Leaf	100	96±5.7	100	30±0	0
Polygonaceae	*Polygonum plebium*	Knotweed	Leaf	30±0	10±5.8	13.3±3.3	0	0
Malpighiaceae	*Hiptage benghalensis*	Hiptage	Root	0	0	100	0	0
Malvaceae	*Thespesia lampas*	Portia tree	Leaf	0	0	0	0	0
Meliaceae	*Dysoxylum gobara*		Leaf	0	13.3±5.7	0	23.3±8.8	0
	Melia azedarach	China berry tree	Fruit	100	26.6±7.6	33.3±5.7	0	0
Mimosaceae	*Acacia gageana*		Leaf	0	0	0	0	0
Myrtaceae	*Syzygium aromaticum*	Clove	Leaf	100	100	100	10±0	0
Rubiaceae	*Hedyotis scandens*	Bishma	Leaf	43.3±7.6	30±0	20±0	10±10	0
Solanaceae	*Brugmansia suaveolens*	Angel's trumpet	Leaf	36.7±5.8	0	3±0.6	0	0
Verbenaceae	*Gmelina arborea*	Beechwood	Leaf	10 ±0	0	20 ±0	0	0

	Clerodendrum colebrookianum	East Indian Glory Bower	Leaf	83.3±3.2	10±0	13.3±5.7	0	0
Zingiberaceae	*Curcuma longa*	Turmeric	Rhizome	100	100	100	46.7±7.6	3±5.7

Table 11. Screening of larvicidal effects of ethnomedicinal plants against *An. barbirostris* at 500 ppm concentration in different solvents

Family	Plants	Common name	Part used	% Mortality ± SE				
				Pet. Ether	Chloroform	Acetone	Methanol	Aqueous
Asteraceae	*Blumea lanceolaria*	Lance-leaved blumea	Leaf	43.3±2.8	20±0	60±0	0	0
	Eupatorium glandulosum	Sticky snakeroot	Leaf	100	90±5	100	16.67±2.8	30±0
	Eupatorium odoratum	Christmas bush	Leaf	20±0	60±10	63.3±7.6	23.3±4.4	10±5
	Mikania micrantha	Chinese creeper	Leaf	76.6±3.33	63.3±5.7	60±5	33.3±3.3	0
	Tithonia diversifolia	Mexican sunflower	Leaf	33.3±4.4	16.6±5.8	30±0	13.33±5.7	0
Apiaceae	*Centella asiatica*	Indian pennywort	Leaf	100	30±10	23.3±3.3	50±10	0
	Eryngium foetidum	Mexico coriander	Stem	40±8.6	30±5	30±5	33.3±2.8	0
Apocynaceae	*Alstonia scholaris*	Indian Devil tree	Bark	0	0	0	0	0
Araceae	*Homalomena aromatica*	Sugandhmantri	Rhizome	100	60± 5	73.3±2.8	0	0
Bignoniaceae	*Oroxylum indicum*	Indian trumpet tree	Bark	40±0	100	30±8.6	10±5	30±5
Compositae	*Tagetes erecta*	Marigold	Leaf	100	80±8.6	100	66.6±2.8	0
Convolvulaceae	*Cuscuta reflexa*	Dodder plant	Stem	70±5	53.3±2.8	30±7.6	10±0	0
Euphorbiaceae	*Securinega virosa*	White berry bush	Leaf	0	0	16.6±2.8	13.3±2.6	30±0
	Antidesma acidum	Amti	Leaf	0	23±2.6	10±0	0	0
	Croton caudatus	Caudated croton	leaf	26.7±2.6	33.3±2.6	10±0	10±	0
Elaeagnaceae	*Elaeagnus caudata*	Bastard Oleaster	Leaf	50±5	40±8.6	16.6±4.4	0	0
Fabaceae	*Millettia pachycarpa*	Fish poison climber	Root	53±5.7	33.3±5.7	43.3±5.7	10±2	0
Labiateae	*Elsholtzia communis*		Leaf	100	100	100	33.3±2.8	6.6±3.3
Polygonaceae	*Polygonum plebium*	Knotweed	Leaf	0	0	13.3±4.4	6.7±3.3	0
Malpighiaceae	*Hiptage benghalensis*	Hiptage	Root	0	0	100	26.6±4.4	0
Malvaceae	*Thespesia lampas*	Portia tree	Leaf	0	0	0	0	0
Meliaceae	*Dysoxylum gobara*		Leaf	0	0	0	0	0
	Melia azedarach	China berry tree	Fruit	100	20±0	30±4	16.6±4.4	30±0

Mimosaceae	*Acacia gageana*		Leaf	0	0	0	0	0
Myrtaceae	*Syzygium aromaticum*	Clove	Leaf	100	63.3±7.6	83.3±2.8	0	0
Rubiaceae	*Hedyotis scandens*	Bishma	Leaf	16.6±7.6		10±5	0	3.3±2.8
Solanaceae	*Brugmansia suaveolens*	Angel's trumpet	Leaf	73.3±2.8	16.6±2.8	20±5	20±5	0
Verbenaceae	*Gmelina arborea*	Beechwood	Leaf	16.6±2.8	10±5	20±0	0	0
	Clerodendrum colebrookianum	East Indian Glory Bower	Leaf	0	0	0	0	0
Zingiberaceae	*Curcuma longa*	Turmeric	Rhizome	100	100	100	20±5	13.3±2.8

Table 12. Screening of larvicidal effecfs of ethnomedicinal plants against *Cx. quinquefasciatus* at 500 ppm concentration in different solvents

Family	Plants	Common name	Part used	% Mortality of mosquitoes in different extracts				
				Pet. Ether	Chloroform	Acetone	Methanol	Aqueous
Asteraceae	*Blumea lanceolaria*	Lance-leaved blumea	Leaf	60±5	10±0	13.3±2.8	80±5	0
	Eupatorium glandulosum	Sticky snakeroot	Leaf	90±5	46.7±2.8	70±5	0	0
	Eupatorium odoratum	Christmas bush	Leaf	43.3±2.8	60±0	40±5	23.3±2.8	0
	Mikania micrantha	Chinese creeper	Leaf	100	93.3±2.8	20±5	0	0
	Tithonia diversifolia	Mexican sunflower	Leaf	56.6±5.7	13.3±1.8	40±5		10±5
Apiaceae	*Centella asiatica*	Indian pennywort	Leaf	100	56.3±2.8	88.3±5.7	43.3±5.7	
	Eryngium foetidum	Mexico coriander	Stem	80±5	50±5	66.7±2.6	60±5	0
Apocynaceae	*Alstonia scholaris*	Indian Devil tree	Bark	0	0	0	0	0
Araceae	*Homalomena aromatica*	Sugandhmantri	Rhizome	66.6±7.6	23.3±2.8	30±0	0	0
Bignoniaceae	*Oroxylum indicum*	Indian trumpet tree	Bark	100	30±0	43.3±7.6	0	0
Compositae	*Tagetes erecta*	Marigold	Leaf	100	30 ±5	50±5	0	0
Convolvulaceae	*Cuscuta reflexa*	Dodder plant	Stem	20±5	16.7±2.8	100	13.3±5.7	0
Euphorbiaceae	*Securinega virosa*	White berry bush	Leaf	0	13±2.8	0	0	0
	Antidesma acidum	Amti	Leaf	76.3±5.7	20±5	60±5	0	0

	Croton caudatus	Caudated croton	leaf	20±0	40±0	20±0	30±5	0
Elaeagnaceae	*Elaeagnus caudata*	Bastard Oleaster	Leaf	30±0	13.3±2.8	20±0	0±0	10±5
Fabaceae	*Millettia pachycarpa*	Fish poison climber	Root	100	40±5	80±5	36.7±2.8	0
Labiateae	*Elsholtzia communis*	-	Leaf	100	90±5	100	13.3±2.8	0
Polygonaceae	*Polygonum plebium*	Knotweed	Leaf	100	20±0	43.3±2.8	3.3±2.8	0
Malpighiaceae	*Hiptage benghalensis*	Hiptage	Root	0	0	100	90±5	0
Malvaceae	*Thespesia lampas*	Portia tree	Leaf	46.7±7.6	10±0	30±5	10±0	10±0
Meliaceae	*Dysoxylum gobara*	-	Leaf	76.6±2.8	43.6±2.8	30±0	40±5	0
	Melia azedarach	China berry tree	Fruit	100	30±5	46.6±2.8	0	0
Mimosaceae	*Acacia gageana*	-	Leaf	0	0	0	0	0
Myrtaceae	*Syzygium aromaticum*	Clove	Leaf	100	80±5	100	30±0	0
Rubiaceae	*Hedyotis scandens*	Bishma	Leaf	20±5	0	66.7±2.8	33.3±5.7	0
Solanaceae	*Brugmansia suaveolens*	Angel's trumpet	Leaf	80±5	43.3±2.8	73.3±5.6	40±8.6	0
Verbenaceae	*Gmelina arborea*	Beechwood	Leaf	30±5	46.7±2.8	10±0	0	0
	Clerodendrum colebrookianum	East Indian Glory Bower	Leaf	63.3±2.8	13.3±2.8	0	0	0
Zingiberaceae	*Curcuma longa*	Turmeric	Rhizome	100	100	100	40±5	0

Selective bioassay - mortality, lethal concentration (LC_{50}) and lethal time (LT_{50})

Acetone extract of *Hiptage benghalensis*

Five different solvent extracts (water, petroleum ether, acetone, chloroform and methanol) of *H. benghalensis* root bark were tested at 500 ppm concentration against third larval instars of *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefascitus* (Table 10, 11, 12). Among the solvent extracts, 100% larval mortality was found in acetone extract in all the three mosquito vectors whereas no mortality was observed in water, petroleum ether and chloroform. Methanol extract showed 0%, 26.6% and 90% larval mortality in *Ae. albopictus, An. barbirostris* and *Cx. quinquefasciatus.* Hence, acetone extract was selected for bioassays in relation to lethal concentration (LC_{50}), lethal time (LT_{50}) and effect on nutrient reserves

Acetone root bark extract of *H. benghalensis* showed efficacy against all the three mosquito species tested. Highest mortality of 100% was recorded in 100 – 500 ppm whereas 40-60% mortality was observed in 12.5 ppm during 24-48 h bioassay period. The mortality values were significantly greater than the values of control (Table 13). The LC_{50} of *H. benghalensis* ranged between 12.69 -16.71 ppm and 11.15 -15.08 ppm for 24 and 48 h, respectively. Minimum LC_{50} (LC_{50}-12.69 ppm 24 h and 11.15 ppm 48 h) and highest susceptibility to acetone root bark extract of *H. benghalensis* were found in *Ae. albopictus* followed by *An. barborostris* (LC_{50}-15.48 ppm 24 h and 14.83 ppm 48 h) and finally by *Cx. quinquefasciatus* (LC_{50}-16.71 ppm 24 h and 15.08 ppm 48 h). Chi-square value was significant at $P<0.05$ level (Table 13). Higher slope value (24 h - 3.91 ± 0.92; 48 h - 5.04±0.82) and lower fiducial limits at 95% of LC_{50} (6.67 - 16.58 ppm at 24 h; 9.34-12.48 ppm at 48 h) were

observed for *Ae. albopictus* than those of other mosquito species (Table 13). The susceptibility order of three mosquito species to the root bark extract was observed to be of *Ae. albopictus* > *An. barbirostris* > *Cx. quinquefasciatus*.

The lethal time (LT_{50}) decreased as the concentration of the acetone root bark extract of *H. benghalensis* was increased in all the three tested mosquito species. In terms of lethal time to kill 50% population of mosquito species, 200 and 400 ppm of acetone root bark extracts of *H. benghalensis* had minimum time i.e., 1.25 and 4.84h, followed by 100, 50 and 25 ppm (4.54 and 14.39 h, respectively). Root bark extract at 12.5 ppm had longest time (26.1 – 44.33 h, respectively) to cause 50% mortality (Table 15). Lethal times were also shorter in *Ae. albopictus*, particularly at higher dosages (400 and 200 ppm), which presented values ~1-2 times lower than those exhibited by *H. benghalensis* in *An. barbirostris* and *Cx. quinquefasciatus*. This could again be linked to the difference in toxicity noticed in the 1-15 hours of the experiment (Table 15).

There were significant effects of concentration (F = 174.68; df= 5, 143; P < 0.0001), mosquito species (F = 26727; df = 2, 143; P < 0.0001) and time after application (F = 27067.8; df = 1, 143; P < 0.0001) on mortality. There were also significant effects of all interactions including concentration×species (F = 5163.34; df= 10, 143; P < 0.0001), species×time (F = 12719.5; df= 2, 143; P < 0.0001), concentration×time (F = 5049.42; df= 5, 143; P < 0.0001) and concentration×species×time (F = 2631.24; df = 10, 143; P < 0.0001). Among the treatments, 50-500 ppm caused a significant mortality in all the three mosquito species. *Ae. albopictus* was significantly more susceptible than *An. barbirostris* and *Cx. quinquefasciatus*, ($P \leq 0.0001$, Tukey's test). Interactions among concentrations,

mosquito species and time revealed significant difference in larval mortality ($p<0.0001$) (Table 3). The results of regression analysis of crude acetone extract of *H. benghalensis* revealed that the mortality (Y) is positively correlated with the concentration of exposure (X) having a regression coefficient (R) between 0.53 and 0.58 (Table 14). The results of log probit analysis (95% confidence level) revealed that LC_{50} values gradually decreased with the exposure periods having the lowest value at 48 h of exposure to third instar larvae (Table 14).

Petroleum ether extract of *Curcuma longa*

The percent mortality for III instar larvae of *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus* treated with various concentrations (ranging from 6.3 to 400) of the rhizome extract of *C.longa,* and LC_{50} values with their 95% lower and upper limits for 24 and 48 h are given in Table 4.7. In the larvicidal screening test, 100% mortality was observed in *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus* when treated with petroleum ether extract of *C. longa* rhizome at 500 ppm. In dose response bioassay, 100% mortality was observed in 400 ppm, 200 ppm and 100 ppm concentration in *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus* (Table 16). After 24 h, the LC_{50} for *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus* were 15.46, 18.24 and 16.18 ppm. There is no significant increase in LC_{50} after 48 h (i.e. 15.20, 15.16 and 16.18 for *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus* respectively). From the results it is evident that the petroleum ether extract of *C.longa* rhizome showed efficacy against third instar larvae of all the three mosquito species tested.

Table 13. Mortality, log probit and regression analysis of third larval instars of *A. albopictus*, *A. barbirostris* and *C. quinquefascitus* in different concentrations of crude root extract of *H. benghalensis*[@]

Concn (ppm)	*Ae. albopictus*		*An. barbirostris*		*Cx. quinquefascitus*	
	Time (h)					
	24	48	24	48	24	48
	Per cent mortality ± Standard error					
500	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
400	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
200	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
100	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
50	96.0 ± 0.72	100 ± 0.0	96.0 ± 0.75	100 ± 0.0	100 ± 0.0	96.0 ± 0.75
25	72.0 ± 0.44	80.0 ± 0.76	72.0 ± 0.82	76.0 ± 0.64	72.0 ± 0.43	76.0 ± 0.67
12.5	44.0 ± 1.00	50.0 ± 0.40	40.0 ± 0.40	41.0 ± 0.75	40.0 ± 0.47	40.0 ± 0.40

Mosquito species	Period of bioassay (h)	LC_{50} (ppm)	95% confidence limits		Slope ± standard error	Chi square $\chi 2$	R value
			Lower limit	Upper limit			
Ae. albopictus	24	12.69	6.67	16.58	3.91±0.92	16.79	0.58
	48	11.15	9.34	12.48	5.04±0.82	0.05	0.53
An. barbirostris	24	15.48	13.39	17.43	0.92±0.49	1.82	0.57
	48	14.83	13.03	16.49	4.03±0.45	3.74	0.53
Cx. quinquefascitus	24	16.71	13.75	19.57	2.32±0.22	4.72	0.57
	48	15.08	13.28	16.75	4.05±0.45	4.03	0.55

[@] Mean of four observations; R – regression coefficient
Control—nil mortality. Significant at $P<0.05$ level. Degree of freedom -6.
LC_{50} - lethal concentration that kills 50% of the exposed larvae.

Table 14. Completely randomized three-way factorial ANOVA using mosquito species, period of bioassay and different concentrations as variables

Source of variation	SS	DF	MS	F value	P value
Total	160674800	143			
Cells	59784	5			
Factor A (Species)	3373490.7	2	1686745.4	26727	0.0001
Factor B (Time)	1708251	1	1708251	27067.8	0.0001
Factor C (Concentration)	55121.33	5	1102427	174.68	0.0001
A x B	1605456	2	802728	12719.5	0.0001
A x C	3258585	10	325858.5	5163.34	0.0001
B x C	1593346	5	318669.2	5049.42	0.0001
A x B x C	1660577	10	166057.7	2631.24	0.0001
Errors (within cells)	1136	63	18.00		

SS – sum of square; DF – degrees of freedom; MS – mean sum os square; CD – critical difference

Table 15. Lethal time (LT_{50}) of acetone extract of *H. benghalensis* (root) against the three mosquito vector species[@]

Conc. (ppm)	*Ae. albopictus*					*An. barbirostris*					*Cx. quinquefascitus*				
	LT_{50} (h)	95% CL		Slope ± SE	$\chi 2$	LT_{50} (h)	95% CL		Slope ± SE	$\chi 2$	LT_{50} (h)	95% CL		Slope ± SE	$\chi 2$
		Lower	Upper				Lower	Upper				Lower	Upper		
400	1.25	0.86	3.65	2.10 ± 0.71	17.91	1.27	0.75	4.87	2.07 ± 0.66	16.36	1.36	0.67	2.77	2.28 ± 0.52	9.05
200	2.83	0.19	6.05	1.31 ± 0.38	29.65	2.94	0.79	5.27	1.87 ± 0.46	32.95	4.84	1.13	14.54	1.67 ± 0.54	43.77
100	5.54	3.74	7.93	2.29 ± 0.37	26.02	4.54	2.19	8.04	1.92 ± 0.42	27.89	9.28	7.25	14.90	5.10 ± 1.08	21.01
50	7.75	4.88	12.97	2.32 ± 0.45	40.69	4.69	2.75	7.19	1.89 ± 0.33	28.79	9.24	7.49	12.87	4.34 ± 0.71	15.19
25	8.17	6.44	10.77	3.08 ± 0.43	18.73	12.32	7.75	23.45	1.83 ± 0.33	32.66	14.39	12.68	16.82	2.81 ± 0.24	1.95
12.5	26.1	17.08	52.53	1.34 ± 0.19	12.12	44.33	19.75	65.89	1.20 ± 0.31	31.67	28.92	23.06	40.37	2.32 ± 0.26	6.44

[@] LT_{50} was calculated by mean value of four observations

Conc. – concentration; SE – standard error; CL – confidence limits; $\chi 2$ – chi square

LT_{50} - time interval during which 50% of larval population may be expected to die following acute administration of root bark extract at a given concentration under a defined set of conditions.

Table 16. Mortality, log probit and regression analysis of third larval instars of *A. albopictus*, *A. barbirostris* and *C. quinquefascitus* in different concentrations of petroleum ether extract of *Curcuma longa* rhizome[@]

Concn (ppm)	*Ae. albopictus*		*An. barbirostris*		*Cx. quinquefascitus*	
	Time (h)					
	24	48	24	48	24	48
	Per cent mortality ± Standard error					
400	100 ± 00	100 ± 00	100 ± 00	100 ± 00	100 ± 00	100 ± 00
200	100 ± 00	100 ± 00	100 ± 00	100 ± 00	100 ± 00	100 ± 00
100	100 ± 00	100 ± 00	100 ± 00	100 ± 00	100 ± 00	100 ± 00
50	81.0 ± 2.1	81.0 ± 2.1	87.0 ± 2.1	81.0 ± 2.1	83.0 ± 0.42	83.0 ± 0.42
25	76.0 ± 1.9	79.0 ± 0.9	72.0 ± 1.9	79.0 ± 0.9	79.0 ± 0.60	79.0 ± 0.60
20	71.0 ± 2.5	73.0 ± 2.1	71.0 ± 2.5	73.0 ± 2.1	75.0 ± 0.86	75.0 ± 0.86
12.5	45.0 ± 2.6	45.0 ± 2.3	42.0 ± 2.6	45.0 ± 2.3	33.0 ± 0.26	33.0 ± 0.26
6.3	8.0 ± 1.8	7.0 ± 1.7	8.0 ± 1.8	7.0 ± 1.7	6.0 ± 0.35	6.0 ± 0.35

Mosquito species	Period of bioassay (h)	LC_{50} (ppm)	95% confidence limits		Slope ± standard error	Chi square $\chi 2$
			Lower limit	Upper limit		
Ae. albopictus	24	15.46	6.08	28.24	2.25 ± 0.60	20.75
	48	15.20	3.53	32.36	2.61 ± 0.70	27.10
An. barborostris	24	18.24	12.38	25.45	2.47 ± 0.51	27.72
	48	15.16	10.43	20.32	2.76 ± 0.47	31.42
Cx. quinquefascitus	24	16.18	5.53	33.32	2.94 ± 0.76	28.14
	48	16.18	5.53	33.32	2.94 ± 0.76	28.14

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 12.59

Petroleum ether, chloroform and acetone extracts of *Elsholzia communis*

Petroleum ether, chloroform and acetone extracts of *E. communis* leaf were tested against III instar larvae of *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus.* Percent mortality in different concentrations was calculated after 24 and 48 h. Among the petroleum ether extracts, highest mortality was observed against *Ae. albopictus* at 400 ppm (85 and 86% after 24 and 48 h respectively) (Table 17). The LC_{50} of petroleum ether extract against *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus* were 158.87, 117.62, 209.65 ppm and 138.05, 110.43 and 170.27 ppm after 24 h and 48 h, respectively.

Chloroform extract exhibited the highest mortality against *An. barbirostris* larvae (68 and 70% after 24 and 48 h, respectively). After 24 h and 48 h, the LC_{50} value of chloroform extract of *E. communis* leaf were 260.05, 249.34, 286.17 and 229.9, 229.55, 276.85 against *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus,* respectively (Table 18). At 400 ppm, the acetone extract of *E. communis* leaf showed highest mortality against *Cx. quinquefasciatus* larvae (55 and 56 % after 24 and 48 h). After 24 and 48 h, the LC50 were 353.34, 255.79, 363.20 and 298.20, 238.16, 345.42 ppm for *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus* respectively (Table 4.10).

Petroleum ether extract of *Homalomena aromatica*

Petroleum ether extract of *H. aromatica* rhizome showed efficacy against all the three mosquito species tested. Petroleum ether extract of *H. aromatica* have shown 49% to 67% mortality against third larval instars of *Ae. albopictus*, *An.*

barbirostris and *Cx. quinquefascitus* in different concentrations. The highest mortality was observed in *Ae. albopictus* at 400 ppm (58 and 67% mortality after 24 h and 48 h). No mortality was recorded at 25 and 12.5 ppm in all the treated mosquito larvae. Highest susceptibility to petroleum ether extract of *H.aromatica* were found in *A. albopictus* (LC50 - 283.40 ppm 24h and 274.93 ppm 48 h) followed by *A. barborostris* (LC_{50}-304.43 ppm 24 h and 260.89 ppm 48 h) and finally by *C. quinquefascitus* (LC_{50}-422.20 ppm 24 h and 374.31 ppm 48 h). Chi-square value was significant at $P<0.05$ level (Table 20).

Table 17. Log probit and regression analysis of larvicidal activity of petroleum ether extract of *Elsholtzia communis* (leaf) against different mosquito species[@]

Concn (ppm)	*A. albopictus*		*A. barbirostris*		*C. quinquefascitus*	
	Time (h)					
	24	48	24	48	24	48
	Per cent mortality ± Standard deviation					
400	85±3.82	86±5.16	85±3.82	82± 5.16	70 ± 9.52	79 ± 11.94
200	67±8.86	68±8.64	67±8.86	68±8.64	54 ± 10.58	61 ± 13.62
100	44±3.26	47±3.82	44±3.26	47±3.82	21 ± 7.56	27 ± 6.28
50	27±3.82	29±3.82	27±3.82	29±3.88	10 ± 5.16	13 ± 6.82
25	7±8.24	8±7.3	7±8.24	8±7.3	3 ± 3.32	8 ± 3.26
12.5	0±0	0±0	0±0	0±0	0 ± 0	0 ± 0

Mosquito species	Period of bioassay (h)	LC_{50} (ppm)	95% confidence limits		Slope ± standard error	Chi square $\chi 2$
			Lower limit	Upper limit		
Ae. albopictus	24	158.87	136.23	188.38	1.88±0.16	1.958
	48	138.05	119.05	161.87	1.93±0.16	1.57
An. barborostris	24	117.62	101.74	136.64	1.97±0.16	1.4
	48	110.43	95.35	128.29	1.94±0.16	1.51
Cx. quinquefascitus	24	209.65	180.30	249.70	2.08±0.18	2.46
	48	170.27	149.30	196.48	2.34±0.18	1.42

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 7.815

Control – Nil Mortality; [a]Mean value of four replicates

Table 18. Log probit and regression analysis of larvicidal activity of extract of *Elsholtzia communis* leaf (chloroform) against different mosquito species[@]

Concn (ppm)	*A. albopictus*		*A. barbirostris*		*C. quinquefascitus*	
	Time (h)					
	24	48	24	48	24	48
	Per cent mortality ± Standard deviation					
400	62±10.58	67±13.38	68±8.64	70±9.52	61±11.00	62±9.52
200	41±10.50	44±11.76	37±16.12	40±16.96	31±5.02	35±5.04
100	29±8.86	31±8.86	26±9.52	30±10.38	30±10.48	32±8.64
50	7±6.00	12±7.30	5±5.02	8±6.52	8±7.30	10±9.52
25	3±3.82	5±2.00	2±2.30	4±9.22	0±0.00	0±0.00
12.5	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

Mosquito species	Period of bioassay (h)	LC_{50} (ppm)	95% confidence limits		Slope ± standard error	Chi square χ^2
			Lower limit	Upper limit		
Ae. albopictus	24	260.05	214.35	332.05	1.71±0.17	3.38
	48	229.9	190.35	290.36	1.65±0.16	2.23
An. barborostris	24	249.34	212.56	302.32	2.09±0.19	3.88
	48	229.55	196.94	275.09	2.12±0.19	5.75
Cx. quinquefascitus	24	286.17	170.79	1012.96	1.86±0.35	10.97
	48	276.85	155.26	1435.30	1.7±0.38	13.3

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 7.815

Control – Nil Mortality; [a]Mean value of four replicates

Table 19. Log probit and regression analysis of larvicidal activity of acetone extract of *Elsholtzia communis* leaf (Acetone) against different mosquito species[@]

Concn (ppm)	*A. albopictus*		*A. barbirostris*		*C. quinquefascitus*	
	Time (h)					
	24	48	24	48	24	48
	Per cent mortality ± Standard deviation					
400	49±13.20	57±19.96	50±12.00	54±14.78	55±14.37	56±13.46
200	37±11.8	40±12.9	36±5.62	40±7.31	46±10.58	47±11.10
100	30±5.16	35±6.82	23±8.86	24±8.64	36±11.18	37±10.52
50	7±6.82	7±6.82	13±8.83	16±9.78	16±5.66	18±5.16
25	4±5.04	6±6.92	2±2.30	3±2.00	3±3.82	3±3.82
12.5	0±00	0±00	0±00	0±00	0±00	0±00

Mosquito species	Period of bioassay (h)	LC_{50} (ppm)	95% confidence limits		Slope ± standard error	Chi square $\chi 2$
			Lower limit	Upper limit		
Ae. albopictus	24	353.34	272.95	5.7.68	1.44±0.17	6.85
	48	298.80	239.32	401.39	1.56±0.16	4.96
An. barborostris	24	255.79	204.86	341.98	1.44±0.15	7.78
	48	238.16	146.01	602.26	1.62±0.28	15.33
Cx. quinquefascitus	24	363.20	279.994	524	1.45±0.17	2.69
	48	345.82	264.39	505.616	1.35±0.16	3.44

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 7.815

Control – Nil Mortality; [a]Mean value of four replicates

Table 20. Mortality, log probit and regression analysis of third larval instars of *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefascitus* in different concentrations of Petroleum ether extract of *Homalomena aromatica* rhizome[@]

Concn (ppm)	*Ae. albopictus*		*An. barbirostris*		*Cx. quinquefascitus*	
	Time (h)					
	24	48	24	48	24	48
	Per cent mortality ± Standard deviation					
400	58±16.8	67±10.00	55±12.38	64±11.76	49±8.86	51±11.00
200	30±6.84	32±6.02	39±17.08	40±16.50	28±6.32	33±7.56
100	16±5.68	16±5.68	21±3.82	21±3.82	14±2.30	14±2.30
50	9±3.82	9±3.82	7±6.82	7±6.82	11±10	11±10
25	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
12.5	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

Mosquito species	Period of bioassay (h)	LC_{50} (ppm)	95% confidence limits		Slope ± standard error	Chi square $\chi 2$
			Lower limit	Upper limit		
Ae. albopictus	24	283.40	240.56	347.28	2.16±0.20	7.16
	48	274.93	234.16	334.89	2.18±0.20	5.46
An. barborostris	24	304.43	252.47	387.66	1.91±0.19	3.65
	48	260.89	222.65	316.29	2.15±0.20	2.10
Cx. quinquefascitus	24	422.20	327.12	607.28	1.64±0.19	6.01
	48	374.31	297.54	513.23	1.72±0.19	5.74

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 7.815

Control – Nil Mortality; [a]Mean value of four replicates

Larvicidal action of *Syzygium aromaticum*

Larvicidal activity of petroleum ether, acetone, chloroform and methanol extracts of *S. aromaticum* leaf extracts against *Cx. quinquefasciatus* III instar lavae are presented in Table 21. It is evident that all extracts showed moderate and low larvicidal effects; after 24 hours, the highest larval mortality was found in chloroform, petroleum ether, acetone and methanol extracts of *S. aromaticum* (LC_{50}=127.78, 152.764, 161.903 and 9922.34 ppm) against the larvae of *C. quinquefasciatus*, respectively. After 48 hour, petroleum ether extract was found to be highly active, followed by chloroform, acetone and methanol extracts (121.544, 127.777, 136.038 and 333.722 ppm, respectively. Chi-square value was significant at $P<0.05$ level (Table 3). The mortality values were significantly greater than the values of control.

Biochemical profiling of treated mosquito larvae

Table 22 summarizes the alterations in the concentration of nutrient reserves in freshly emerged III instar larvae following treatment with sublethal concentration of acetone root bark extract of *H. benghalensis*. Sugar (7.91–18.33 µg), glycogen (5.13–5.59 µg), lipid (70.12–71.24 µg), and protein (153.18–168.23 µg) contents were found to decrease significantly ($P<0.05$) in the treated larvae of all the three mosquito species.

Table 21. Larvicidal action of *Syzygium aromaticum* leaves against 3rd instar larvae *of Culex quinquefaciatus* (Culicidae:Diptera)

Solvents	% Mortality at various concentration±SEa					
	After 24 hr			After 48 hr		
	400ppm	200ppm	100ppm	400ppm	200ppm	100ppm
Pet. Eth	100±0	57±1.45	33±1.88	100±0	86.6±0.7	32.83±1.9
Acetone	98±0.02	70±1.3	13±0.6	100±0	83.3±0.62	22.9±1.25
Chloroform	100±0	65±0.75	42±1.04	100±0	87.9±0.7	62.5±0.85
Methanol	21±0.6	15±0.75	19±0.85	50±0.5	54±1.77	32±1.08

Solvents	Period of Biossay (h)	LC_{50}	95% confidence limits		Slope±SE	$\chi 2$
			Lower limit	Upper limit		
Petroleum ether	24	152.76	133.08	175.36	3.79±1.54	14.90
	48	121.54	111.14	132.92	5.40±0.61	0.34
Acetone	24	161.90	149.58	175.24	5.35±0.51	0.085
	48	136.04	124.35	148.82	5.92±0.62	0.31
Chloroform	24	127.78	109.81	148.67	3.47±1.38	11.85
	48	127.78	109.81	148.68	3.47±0.53	1.77
Methanol	24	9922.34	237.60	414358.1	0.12±0.34	1.11
	48	333.72	111.95	994.77	1.02±0.29	2.79

Control – Nil Mortality; [a]Mean value of four replicates

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 7.815

Table 22. Biochemical profile of nutrient reserves and primary metabolites in freshly emerged III instar larvae treated with sublethal concentration (5 ppm) of acetone extract of *H. benghalensis* root [@]

Metabolite (µg)	*A. albopictus*		P value	*A. barbirostris*		P value	*C. quinquefascitus*		P value
	Control	Treated		Control	Treated		Control	Treated	
Sugar	21.74 ± 3.58	14.45 ± 2.12	0.0387	14.62 ± 1.80	7.91± 2.38	0.0178	21.16 ± 4.55	18.33 ± 2.70	0.0470
Glycogen	9.93 ± 1.58	5.27 ± 1.65	0.0245	7.73 ± 1.00	5.13 ± 0.93	0.0306	10.28 ± 1.43	5.59 ± 1.07	0.0105
Lipid	95.17 ± 2.57	70.12 ± 4.12	0.0010	94.01 ± 2.57	70.23 ± 4.54	0.0014	95.49 ± 1.19	71.24 ± 1.33	0.0001
Protein	221.09 ± 9.47	168.23 ± 2.44	0.0007	217.36 ± 10.29	153.18 ±11.06	0.0018	239.37 ± 9.59	161.83 ± 10.42	0.0007

[@]Values are represented as mean ± standard deviation in µg per five larvae

t – t–test; degrees of freedom = 4

Insect Growth Regulators

Table 23 has shown the insect growth regulatory effect of petroleum ether extracts of *M. azedarach* fruit against *Ae. albopictus* treated on the I instar larvae upto adult emergence. The per cent survival of *Ae. albopictus* upto their eclosion was 100, 70 and 75 with the concentration of 5 ppm, 10 ppm and 20 ppm respectively. No mortality was observed in 5 ppm concentration, while 10 ppm and 20 ppm showed 30 and 25 per cent mortality respectively. Treated tests at 10 ppm and 20 ppm resulted in the presence of dead larvae, malformed pupae and incomplete moulting adult (Table 23 and Plate 4). But there is no mortality and malformed individuals at 10 ppm concentration. However, the death of *Ae. albopictus* larvae and pupae may be due to failure of proper sclerotization (Zebitz 1986). These suggested that the petroleum ether extract of *M. azedarach* interfere with the hormonal control of moulting. The total development period to eclosion was prolonged by 0.25, 0.75 and 1.25 days at the concentrations of 5 ppm, 10 ppm and 20 ppm respectively from 13.25 days in the control.

Adulticidal Bioassay

Highest concentration (50,000 ppm) of *H. benghalensis* recorded 16.66–36.66 % mortality of mosquitoes after 24-h exposure. *C. quinquefascitus* adults were more susceptible followed by *A. albopictus* and least by *A. barbirostris*. A significantly main effect of concentration at 2 h ($F_{2,6025}$; $P<0.0012$ and $F_{2,6076}$; $P<0.0001$), 3 h ($F_{2,602.60}$; $P<0.1537$ and $F_{2,6020.94}$; $P<0.002$), and 24 h ($F_{2,609.0}$; $P<0.0156$ and $F_{2,6011.40}$; $P<0.009$) was also observed in C. quinquefasciatus and A. albopictus, respectively (Table 24).

Table 23. Consequence of different concentrations of petroleum ether extract of *Melia azedarach* fruit on the development of *Aedes albopictus* treated as the first instar larvae

Concn	Developmental stages	% survival	Cumulative developmental period	Growth index	% Total larval mortality	IE (%)
Control (Petroleum ether + Tween - 20)	L1-L2	100	1.75±0.25	57.14	0	100
	L1-L3	100	5.00±0.41	20.00		
	L1-L4	100	6.75±0.25	14.81		
	L1-LP	100	12.50±0.41	8.00		
	L1-A	100	13.25±0.47	7.54		
5ppm	L1-L2	100	2.75±0.25	36.36	0	100
	L1-L3	100	6.25±0.47	16.00		
	L1-L4	100	9.50±0.29	10.52		
	L1-LP	100	12.50±0.29	8.00		
	L1-A	100	13.50±0.29	7.40		
10ppm	L1-L2	92.50±4.78	2.75±0.25	33.63	30±4.08	70±4.08
	L1-L3	82.50±4.78	6.25±0.25	13.20		
	L1-L4	80.00±4.08	8.00±0.41	10.00		
	L1-LP	72.50±4.78	13.00±0.41	5.58		
	L1-A	70.00±4.08	14.00±0.41	5.00		
20ppm	L1-L2	90.00±4.08	2.25±0.25	40.00	25±2.88	75±2.88
	L1-L3	85.00±2.89	6.00±0.41	14.17		
	L1-L4	82.50±4.78	8.50±0.29	9.70		
	L1-LP	77.50±2.5	13.25±0.48	5.85		
	L1-A	75.00±2.88	14.50±0.28	5.17		

Table 24. Preliminary effect of acetone extract of *H. benghalensis* root on *Ae. albopictus*, *An.barbirostris*, and *Cx. quinquefasciatus* adults exposed continuously for 3 hours at different concentrations and mortality after 24 hour exposure[@]

Mosquito species	Conc. (ppm)	Time post treatment			
		1 h	2 h	3 h	24 h
	0	0	0^{a}	0^{a}	0^{a}
Ae. albopictus	10000	0	0^{a}	0^{a}	0^{a}
	25000	0	0^{a}	13.00 ± 1.52^{b}	13.00 ± 1.52^{b}
	50000	0	12.66 ± 1.45^{b}	15.33 ± 2.72^{b}	26.66 ± 6.67^{c}
P value		-	<0.0001	0.0020	0.0090
F value		-	76.00, df 2,6	20.943, df 2,6	11.404, df 2,6
An. barbirostris	0	0	0^{a}	0^{a}	0^{a}
	10000	0	0^{a}	0^{a}	6.66 ± 3.35^{a}
	25000	0	0^{a}	6.66 ± 3.33^{ac}	10.00 ± 5.77^{a}
	50000	0	6.67 ± 3.3^{a}	13.33 ± 3.33^{ac}	16.66 ± 3.33^{a}
P value		-	-	0.0370	-
F value		-	-	6.00, df 2,6	-
Cx. quinquefasciatus	0	0	0^{a}	0^{a}	0^{a}
	10000	0	0^{a}	10.00 ± 5.77a	16.66 ± 3.33^{a}
	25000	0	0^{a}	13.33 ± 3.33^{a}	26.66 ± 3.33^{ac}
	50000	0	16.66 ± 3.3^{b}	23.33 ± 3.33^{a}	36.66 ± 3.33^{bc}
P value			0.0012	0.1537	0.0156
F value			25.0, df 2,6	2.60, df 2,6	9.0, df 2,6

[@]Mean of three observations

Means ± SE followed by same letters within the same column are not significantly different (Tukey-test, $p < 0.05$).

Repellent Bioassay

The results from the skin repellent activity of *G. arborea* leaf in shown in Table 25. The acetone extract at 20000 ppm gave 50 minutes protection against *Ae. albopictus* female bite. The control provided only 2.2 minutes of protection. Other concentrations provided 48.75 to 6.25 minutes of protection. Petroleum ether extract of *C. longa* provided 125 minutes of protection at 20000 ppm (Table 26). Among the different extracts, *H. aromatica* petroleum ether extract at 20000 ppm provided 90 minutes protection (Table 27). The results clearly show that repellent activity was dose dependent. The repellency of *G. arborea* leaf extracts were also recorded against *An. barbirostris* (Table 28). Concentration of 20000 ppm has shown longest protection time of 32.5 minutes only by the petroleum ether of *G. arborea*. The control provided 3.25 minutes of protection.

Oviposition Deterrent Test

In the oviposition deterrency bioassay, gravid *Ae. albopictus* preferred to lay eggs in the control cups than in the cups treated with petroleum ether extracts of the tested plants (Table 29). There was also a marked difference in the number of eggs laid. The present results showed that 5 ppm; 20 ppm and 50 ppm treated cups of *C. longa* had a mean of 38±5.84, 72.25±4.07 and 122±3.8 eggs, respectively against the control cups of 171.25±7.82, 145±7.7 and 126.5±7.07 eggs with effective repellency of 28.75%, 50.17% and 69.96%, respectively. The petroleum ether extract of *E. communis* at 50 ppm, 20 ppm and 5 ppm concentration had reduced number of eggs against the control (122.75±6.22, 58.25±6.47 and 32.75±3.70 eggs against 161.5±9.13, 113.5±8.00 and 103.25±10.66, respectively) with effective repellency of

23.99%, 48.67% and 68.28%. *M. azedarach* extract also had oviposition deterrency of 80.5±4.69, 59.75±7.00 and 35.25±4.47 with eggs collected from the treated cups against 119±7.26, 127.25±8.46 and 116.5±10.41 eggs collected from the control cups at 5 ppm, 20 ppm and 50 ppm, respectively. The effective repellency of *M. azedarach* were 32.35%, 53.04% and 69.74 at 5 ppm, 20 ppm and 50 ppm. The OAI values of *C. longa* rhizome, *E. communis* leaf and *M. azedarach* fruit were -0.14, -0.13 and -0.19 at 5 ppm; -0.33, -0.32 and -0.36 at 20 ppm; -0.53, -0.52 and -0.25 at 50 ppm, respectively. The OAI values revealed that the solvent plant extracts have deterrent effect, and they caused a remarkable negative response resulting in oviposition of fewer eggs. The effective concentration of oviposition deterrency was also calculated as 17.58, 20.35 and 15.39 for *C. longa*, *E. communis* and *M. azedarach*.

Table 25. Repellent activity of *Gmelina arborea* leaf extracts against blood-starved female *Ae. albopictus*

Solvents	Concentration (ppm)	Complete protection time	
		Mean±SE	Range
Petroleum ether	5000	18.75±2.39	15-25
	10000	32.5±4.33	25-45
	20000	48.75±4.27	40-60
Acetone	5000	10±2.04	5-15
	10000	25±3.53	15-30
	20000	50±5.4	40-65
Chloroform	5000	6.25±2.39	1-10
	10000	15±2.04	10-20
	20000	20±3.53	10-25
Methanol	5000	6.25±3.75	1-15
	10000	20±4.08	10-30
	20000	30±4.08	20-40
Control		2±1.00	1-5

Each value (mean ± SE) represents mean of five values. Values with different letters are significantly different at $P < 0.05$ level (Tukey's test of multiple comparison).

Table 26. Repellent activity of *Curcuma longa* rhizome extracts against blood-starved female *Ae.albopictus*

Solvents	Concentration (ppm)	Complete protection time	
		Mean±SE	Range
Petroleum ether	5000	115±13.23	90-150
	10000	112.5±8.54	90-130
	20000	125±7.36	105-140
Acetone	5000	28.75±5.15	15-40
	10000	33.75±3.75	25-50
	20000	47.5±6.61	30-60
Chloroform	5000	3.75±2.39	0-10
	10000	13.75±2.39	10-20
	20000	16.25±4.73	10-30
Methanol	5000	5±2.04	0-10
	10000	7.5±1.44	5-0
	20000	12.5±3.22	5-20
Control		2±1.00	1-5

Each value (mean ± SE) represents mean of five values ± standard error . Values with different letters are significantly different at $P < 0.05$ level (Tukey's test of multiple comparison).

Table 27. Repellent activity of *Homalomena aromatica* rhizome extracts against blood-starved female *Ae.albopictus*

Solvents	Concentration (ppm)	Complete protection time	
		Mean±SE	Range
Petroleum ether	5000	35±2.88	30-40
	10000	70±9.13	50-90
	20000	90±10.80	70-120
Acetone	5000	8.75±4.27	0-20
	10000	10±4.56	0-20
	20000	13.7±2.3	10-20
Chloroform	5000	3±2.04	0-10
	10000	10±4.56	0-15
	20000	12.5±6.61	0-20
Methanol	5000	22.5±3.22	15-30
	10000	25±2.89	20-30
	20000	27.5±4.87	20-40
Control		2±1.00	1-5

Each value (mean ± SE) represents mean of five values. Values with different letters are significantly different at $P < 0.05$ level (Tukey's test of multiple comparison).

Table 28. Repellent activity of *Gmelina arborea* leaf extracts against blood-starved female *An. barbirostris*

Solvents	Concentration (ppm)	Complete protection time	
		Mean±SE	Range
Petroleum ether	5000	7.5±1.44	5-10
	10000	20±4.08	10-30
	20000	32.5±4.33	10-30
Acetone	5000	5±2.04	0-10
	10000	15±2.88	10-20
	20000	17.5±4.78	10-30
Chloroform	5000	2.5±1.44	0-5
	10000	20±4.08	10-30
	20000	22.5±6.29	10-40
Methanol	5000	2.5±1.44	0-5
	10000	3.75±1.25	0-5
	20000	5±2.04	0-10
Control		3.25±1.03	1-5

Each value (mean ± SE) represents mean of five values. Values with different letters are significantly different at $P < 0.05$ level (Tukey's test of multiple comparison).

Table 29. Oviposition deterrency of different plant extracts against *Ae. albopictus* female

Plants	Plant part	Concn (ppm)	Number of eggs ± SE		Effective repellency (%)	EC_{50} (ppm)	Oviposition active index
			Treated	Control			
Curcuma longa	Rhizome	5	122±3.8	171.25±7.82	28.75	17.58	-0.14
		20	72.25±4.07	145±7.7	50.17		-0.33
		50	38±5.84	126.5±7.07	69.96		-0.53
Elsholtzia communis	Leaf	5	122.75±6.22	161.5±9.13	23.99	20.35	-0.13
		20	58.25±6.47	113.5±8.00	48.67		-0.32
		50	32.75±3.70	103.25±10.66	68.28		-0.52
Melia azedarach	Fruit	5	80.5±4.69	119±7.26	32.35	15.39	-0.19
		20	59.75±7.00	127.25±8.46	53.04		-0.36
		50	35.25±4.47	116.5±10.41	69.74		-0.25

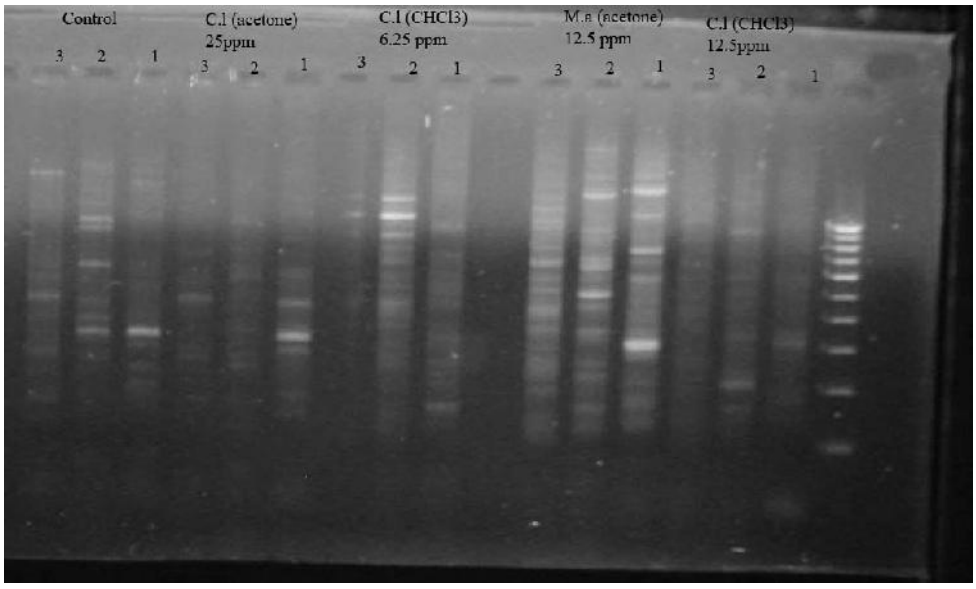
RAPD –PCR profiling of Cx. quinquefasciatus larvae treated with plant extracts

The extracted DNA samples of III instar *Cx. quinquefasciatus* larvae exposed to acetone and chloroform extracts of *C. longa* and *M. azedarach* at different concentration (6.25, 12.5 and 25 ppm) were further evaluated for their DNA changes in comparison with untreated control larvae. Different range of DNA modifications was observed in the treated larvae in comparison with untreated control. Number of bands increased in *M. azedarach* (acetone extracts) treatment whereas in *C. longa* acetone and chloroform treatments the number of bands were found to be decreased (Table 30 and Fig. 4). Further, it was observed that the number of DNA bands was more in the larvae of *Cx. quinquefasciatus* treated with plant extracts.

Table 30. Alteration in number of total bands in treated genomic DNA from larvae of *Cx. quinquefasciatus*

Primer	Plant extract	Solvent used	Concentration (ppm)	Total bands
MA-09	Control			7
	Curcuma longa	acetone	25	8
		chloroform	6.25	6
			12.5	2
	Melia azedarach	acetone	12.5	10
MA-12	Control			6
	Curcuma longa	acetone	25	5
		chloroform	6.25	8
			12.5	6
	Melia azedarach	acetone	12.5	10
MA-26	Control			6
	Curcuma longa	acetone	25	2
	Curcuma longa	chloroform	6.25	2
			12.5	smear
	Melia azedarach	acetone	12.5	11

Figure 4. Comparison of RAPD-PCR profiles of III instar larvae of *Culex quinquefasciatus* control regime and treatment regime with acetone and chloroform extracts of *Curcuma longa* and *Melia azedarach*. Lane on the right side is the DNA size marker (100 bp). 1- Primer MA-09; 2- Primer MA-12; 3- Primer MA-26; C.l - *Curcuma* longa; M.a-= *Melia azedarach*



5. DISCUSSION

Plant secondary compounds have been the subject of thorough investigation in an effort to discover new sources of botanical insecticides. The secondary metabolites of plants (such as steroids, alkaloids, terpenoids, saponins, phenolics, essential oil, etc.) are associated with a wide range of biological activities (Chowdhuri *et al.* 2007). Naturally occurring botanical compounds contain a broad range of chemical active ingredients can intervene in all biological processes of the mosquito, thus interrupt its life cycle and dispersal and reduce harms to humans and animals. Among the plant families studied, the Meliaceae, Rutaceae, Asteraceae, Labiateae, Piperaceae and Annonaceae are perhaps the most promising (Isman 1995). Naturally occurring botanical compounds contain a broad range of chemical active ingredients can intervene in all biological processes of the mosquito, thus interrupt its life cycle and dispersal and reduce harms to humans and animals. Other promising families are Lamiaceae, Annonaceae, Apiaceae and Asteraceae. The search for plant-derived chemicals that have potential use as crop protectants (insecticides, antifeedants, and growth inhibitors) often begins with the screening of plant extracts. Initially, the test insects are fed the extracts and effects on insect behaviour and development are monitored.

Once a promising extract has been discovered, the next step is to find out how it is affecting the insect. This kind of information is needed to ensure safety to non-target organisms (humans, beneficial insects). The phytochemicals derived from plant resources can act as larvicides, insect growth regulators, repellents and ovipositional attractants, having deterrent activities observed by different researchers

(Venkatachalam and Jebanesan 2001). Though many plants have been shown to possess insecticidal / larvicidal and growth inhibition activity against mosquitoes, most of these reports are based on laboratory observations only. However, some of the plant products have shown promise for mosquito control even under field conditions (Shaalan *et al.* 2005).

Larvicidal screening

Larvicidal test using fifteen plant extracts (at 500 ppm) showed 100% mortality against *Ae. albopictus*; sixteen plant extracts against *An. barbirostris* and sixteen plants against *Cx. quinquefasciatus*.

In this study, petroleum ether extracts of nine plants showed 100% mortality against *Ae. albopictus*, eight plant extracts against *An. barbirostris* and ten plant extracts against *Cx. quinquefasciatus*. Sharma *et al.* (2006) reported the larvicidal effect of the petroleum ether extract of *Artemisia annua* leaf possess larvicidal effects with LC_{50} values of 16.85 ppm against *An. stephensi*; leaves of *Argemone mexicana*, *Jatropha curcus* and *Pergularia extensa* causes 100% mortality at 250 ppm of each extracts against *Cx. quinquefasciatus* (Karmegan *et al.* 1997); leaves of *Acacia nilotica*, *Argemone mexicana*, *Jatropa curcas*, *Withania somnifera*, *Citrullus colocynthus* shown LC_{50} between 30.47 to 65.08 ppm (Saktivadivel and Daniel 2008); *Aloe barbadensi* and *Cannabis sativa* leaf have shown LC_{50} values of 29.06 and 376.58 ppm respectively, after 24 hours (Maurya *et al.* 2007); *Eucalyptus globulus* seed and leaf caused 100% mortality at 1000 ppm against *Cx. pipiens* (Sheeren 2006); Mohan *et al.* (2006) reported *Solanum xanthocarpum* root showed LC_{50} value of 41.28 ppm and 38.48 ppm after 24 and 48 hour respectively. Leaves of

Myrtus communis, *origanum syriacum*, *Mentha microcorphylla*, *Pistacia lentiscus* and *Lavandula stoechas* were tested against *Cx. pipiens molestus* with LC_{50} values of 16 mg/l, 36 mg/l, 39 mg/l, 70 mg/l and 89 mg/l respectively.

Acetone extracts of four plants showed 100% mortality against *Ae. albopictus*, five plants against *An. barbirostris* and five plants against *Cx. quinquefasciatus*. The acetone extract of *Tridax procumbens* leaf have shown LC_{50} value of 39.98 mg/l against *An. subpictus* (Kamaraj *et al.* 2011); *Ageratum adenophora* twigs have shown potent larvicidal activities against *Ae. aegypti* and *Cx. quinquefasciatus* larvae with LC_{50} values of 356.70 ppm and 227.20 ppm respectively (Raj Mohan and Ramswamy 2007). Rahuman *et al.* (2000) reported the LC_{50} values of 129.24, 7.58 and 57.23 ppm of *Feronia limonia* when tested against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* respectively. Leaf of *Millingtonia hortensis* showed LC_{50} values of 104.70, 138 and 83.18 ppm against *An. stephensi*, *Ae. eegypti* and *Cx. quinquefasciatus* larvae respectively after 24 hour (Kaushik and Saini 2008). The chloroform extract of *Plumbago zeylanica*, *P. dawei* and *P. stenophylla* roots have shown high larvical activities against *An. gambiea* (4.1, 6.4 and 6.7 mg/ml respectively) (Maniafu *et al.* 2009). Latex and stem bark of *Euphorbia tirucalli* were reported to have LC_{50} of 200.76 mg/l against *Cx. pipiens pallens* (Yadav *et al.* 2002). Fruit peel of *Citrus sinensis* also showed LC_{50} value of 58.25 mg/ml against *An. gambie* (Matasyoh *et al.* 2008). Yenesew *et al.* (2003) also reported rotenoids, deguelin and tephrosin isolated from the seeds of *Millettia dura* showed potent activities, with LC_{50} values of 1.6 and 1.4 µg/ml at 24 hour respectively. Tang *et al.* (2008) also reported seed of *Cassia obtusifolia* showed strong larvicidal of 100% mortality at 25 mg/l with the LC_{50} of the active component emodin were 1.4, 1.9 and 2.2 ppm against *Ae. eegypti*, *Ae. togoi*

and *Cx. pipiens pallens*. In this work, we observed that out of 30 plants, chloroform extracts of two plants showed 100% mortality against *Ae. albopictus*, three extracts against *An. barbirostris* and one extract against *Cx. quinquefasciatus.*

However, low mortality was observed from methanol and aqueous extracts at 500 ppm. Methanol extract of *Atlantia monophylla* leaf is reported to have LC_{50} value of 0.05 mg/l against *An. stephensi* larvae (Sivagnaname and Kalyanasundaram 2004). Senthil Nathan *et al.* (2006) observed that 4% concentration of *Dysoxylum malabaricum* leaf extract at 4% concentration killed more than 97% of first instars, 92% of fifth instars, 93% of pupae and 91% of adults of *An. stephensi*. Methanol extracts of *Momordica charantia*, *Trichosanthes anguina*, *Luffa acutangula*, *Benincasa cerifera* and *Citrullus vulgaris* leaves have LC_{50} values of 465.85, 567.81, 839.81, 1189.30 and 1636.04 against *Cx. quinquefasciatus* larvae respectively (Prabakar and Jebanesan 2004). When tested against *Cx. quinquefasciatus* larvae, *Vitex negundo*, *V. trifolia*, *V. peduncularis* and *V. altissima* leaves showed LC_{50} values of 212.57, 41.41, 76.28 and 128.04 ppm respectively (Krishnan *et al.* 2007). Das *et al.* (2007) observed root of *Aristolochia saccata*, *Annona squamosa* leaf, *Gymnopetelum cochinchinensis* fruit, *Caesalpinea* sp. bark and *Piper* sp. stem have shown good larvicidal properties against *Ae. albopictus* larvae with LC_{50} values of 14.52, 20.26, 50.67, 53.66 and 144.22 ppm respectively.

After subsequent extraction with petroleum ether, chloroform, acetone, methanol and water, our results showed that the petroleum ether extracts were a more effective than the other solvent extracts. Similar effects were obtained by other researchers (Satoto 1993; Komalamisra *et al.* 2005). The observation showed that variation of the larvicidal potential of the same plant changed with the solvents used

(Kishore *et al.* 2011). The bioactivity of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant part, age of plant part, solvent used in extraction and mosquito species (Table 1).

LC_{50} and LT_{50} of *Hiptage benghalensis*

Phytochemical analysis revealed the presence of alkaloids, coumarin, flavonoids, phenols, tannins, and terpenoids in *H. benghalensis* (Murugan and Mohan 2011). The stem and bark contain friedelin, epifriedelinol, octacosanol, α-amyrin, and β-sitosterol. The root bark contains a lead compound hiptagin, a nitrogenous glucoside, identical with endecaphyllin and mangiferin (glucosyl xanthone) (Khare 2007). *H. benghalensis* possesses cardiotonic, hepatoprotective, analgesic, antibacterial, anti-inflammatory, anthelmintic, and insecticidal properties (Jonville *et al.* 2011). Results of the present study suggest that the acetone root bark extract of *H. benghalensis* is a potential natural mosquito larvicide. The larvicidal efficacy of *H. benghalensis* leaf extracts is comparable to well established insecticidal plant species. Low LC_{50} and LT_{50} were observed in *Ae. albopictus* followed by *An. barbirostris* and *Cx. quinquefasciatus.* Significant interactions were observed among mosquito species, bioassay time and concentration in terms of mortality. The mortality was observed to be time- dose dependent. Toxicity of crude acetone root bark extract of *H. benghalensis* may be attributed to their major constituents, hiptagin, a nitrogenous glucoside, identical with endecaphyllin and mangiferin (glucosyl xanthone) (Khare 2007). The compound significantly reduced feeding by third instar larvae of *Costelytra zealandica* (Gnanasunderam and Sutherland 1986*)*.

Larvicidal action of *C. longa*

In larvicidal bioassay, *Ae. albopictus, An. barbirostris* and *Cx. quinquefasciatus* larvae are highly susceptible to petroleum ether extract of *C. longa* rhizome. Turmeric contains pungent, odoriferous oils and oleoresins; the rhizomes have been reported to possess many kinds of biological activities (Rath *et al.* 1998). Petroleum ether extract of *C. longa* rhizome has been reported as repellent to *Tribolium castaneum* (Jilani and Su 1983) and insecticidal to *Plutella xylostella* (Morallo-Rejesus *et al.* 1992). Sesquiterpenes and curcuminoids isolated from the rhizome are shown to have anti-inflammatory, antioxidant and antimicrobial activities (Singh *et al.* 2002). LC_{50} of *C. longa* essential oil against *Ae. aegypti* and *Cx. quinquefasciatus* were 12.5 and 9.2 mg/l respectively (Ranaweera 1996). The hydrolate of *C. longa* was having LC_{50} and LC_{90} of 24.7 and 43.8 (%v/v) against *Ae. albopictus* and 35.5 and 52.5 (%v/v) against *Cx quinquefasciatus* respectively. The ethanol extracts of *C. longa* had LC_{50} of 106.38 mg/l against the larvae of *Ae. aegypti* (Komalamisra *et al.* 2005). The larvicidal activity of hydrolates of *C. longa* was also found promising with LC_{50} of 24.7 (%v/v) against *Ae. albopictus* and 35.5 (%v/v) against *Cx. quinquefasciatus* (Rabha *et al.* 2012). The essential oil of *C. longa* was also reported to have high larvicidal activity (4.5 mg/l) against *An. culifacies* (Ranaweera 1996).

Larvicidal action of *H. aromatica, E. communis* and *S aromaticum*

H. aromatica rhizome is a rich source of essential oils, which have been attributed for various medicinal uses. The plant bears wide range economic and ethnobotanical uses (Das and Talukdar 2010). The acetone, petroleum ether, ethyl

acetate and methanol extracts show the presence of flavonoid, alkaloid, reducing sugar, saponin, tannin and steroids (Das and Talukdar 2010). Komalamisra *et al.* (2005) reported 75% ethanol extract of *H. aromatica* rhizome showed LC_{50} of 38.10 mg/l against *Ae. aegypti*. The findings of the present study showed that the petroleum ether extract of *H. aromatica* has moderate larvicidal properties against treated mosquito larvae. This suggested that rhizome of *H. aromatica* may be explored as potential natural mosquitocidal agent. The future potential use of extracts from *H. aromatica* will require their phytochemical analysis and examination of insecticidal activity of the individual biochemically characterized components.

S. aromaticum, the clove is an evergreen tree, yielding cloves, clove oil and oleoresin as major commercial products. Clove oil is found to have biological activities on wide range of organisms ranging from bacteria to human beings (Prashar *et al.* 2006). Ovicidal and adulticidal effect of clove oils against *Pediculus capitis* was reported (Yang *et al.* 2003). Their ovicidal and larvicidal activity against *Ae. albopictus* have been reported (Bhat and Kempraj 2009). In this study, we have evaluated the larvicidal nature of petroleum, acetone, chloroform and methanol leaf extracts of *S. aromaticum* against third instar larvae of *Cx. quinquefasciatus*. The results indicated that the mortality rates at 400 ppm concentration were highest amongst all concentrations of the crude extracts tested against all the larval instars at 24 and 48 h of exposure. Result of log probit analysis (at 95% confidence level) revealed that lethal concentration LC_{50} and LC_{95} values gradually decreased with the exposure periods in bioassay experiment with the crude plant extract. The results of regression analysis of crude extract of *S.aromaticum* revealed that the mortality rate is positively correlated with the concentration of the extracts.

This result is in consensus with earlier reports on larvicidal effect of *S. aromaticum* against *Cx. quinquefasciatus*, *An. dirus*, *Ae. aegypti*, and *Ae. albopictus* (Trongtokit *et al.* 2005; Makhaik *et al.* 2005; Kishore *et al.* 2011; Bagavan *et al.* 2009). Mosquitocidal efficacy of eugenol and β- carryophyllene analyzed in comparison with bark and leaf oils of *S. aromaticum* against adult mosquitoes of *An. tessellates*, *Cx. quinquefasciatus* and *Ae. aegypti* has been reported (Sutthanont *et al.* 2010). In the light of this finding, the larvicidal effects of clove leaf extracts against *Cx. quinquefasciatus* observed during the current investigation can be attributed to eugenol and β- carryophyllene contained in the extracts. The current study has ascertained the potential of clove leaf extract as a source of mosquitocidal product. However, more trials on 'test formulation' prepared using eugenol/ β- carryophyllene are warranted for further standardization/optimization of the current technology for practical utility. Among the crude extracts, chloroform extract of *S. aromaticum* may be considered as a potent source of a mosquito larvicidal agent. The study suggests that the active ingredients of the chloroform extract should be identified and utilized, if possible, in preparing commercial product formulation as a mosquito larvicide (Lalrotluanga *et al.* 2011b).

The petroleum ether extract of *M. azedarach* fruit could kill the mosquito as well as it resulted in the formation of malformed individuals. Exposure of mosquito larvae to sub lethal concentrations of active botanical derivatives can often result in an extension of the duration of development. But there is no significant increase in the development period in the treated mosquitoes. But simple prolongation of various developmental stages is of little use for practical vector control since the recruitment rate from larval to adult populations is of fundamental importance to the maintenance

of disease transmission cycles (Shaalan *et al.* 2005). Insect growth regulatory activity of *Citrullus vulgaris* against *An. stephensi* was studied (Mullai *et al.* 2008). The total mortality with different concentrations ranged from 13.15 to 92.82%, 9.16 to 81.81%, 11.49 to 98.50% and 8.82 to 88.15% with benzene, ethyl acetate, petroleum ether and methanolic extracts and the plant extracts affect the length of the larval developmental period. But there is no significant increase in extension of larval and pupal development period.

The IGR activity of the methanolic extract of *Atlantia monophylla* against *Ae. aegypti* and *Cx. quinquefasciatus* were 325 times and 162.5 times more sensitive than *An. stephensi* respectively (Sivagnaname and Kalyanasundaram 2004). Sivagnaname and Kalyanasundaram (2004) also reported dead larval intermediates, prepupal stage larvae, dead adults with curved tarsi and malformed wings and albino pupae in the treated mosquitoes. The active fraction of *Moschosma polystachyum* showed IGR activity against 3rd instar larvae of *Cx. quinquefasciatus* with the EI_{50} value of 13.19 ppm (Rajkumar and Jebanesan 2004).

Several plants have been reported to have mimics of insect ecdysones and juvenile hormone activity. As with toxicity, growth inhibition from phytochemicals may also be species-specific (Novak 1985). Sujatha *et al.* (1988) observed that *Acorus calamus* extract induced malformations to a greater extent in *An. stephensi*, and to a lesser extent in *Cx. quinquefasciatus* and *Ae. aegypti*. The active fractions of *Solarium suratense* leaf and *Abrus precatorius* seed coat induced malformation, extended the larval furation and inhibited adult emergence; the active fractions, at concentrations less than 37.64 ppm inhibited emergence of 50% of the larvae (Muthukrishnan *et al.* 1997). Saxena *et al.* (1993) also evaluated alkaloids isolated

from *Annona squamosa*, which have shown larvicidal, growth-regulating and chemosterilant activities against *An. stephensi* at concentrations of 50 to 200 ppm Sagar and Sehgal (1997). The biological activity of the plant extract might be due to a variety of compounds in this plant. These compounds may jointly or independently contribute to cause the mosquitocidal activities against *Ae. albopictus.* The present studies have confirmed that *M. azedarach* fruit extract can be used as a potential agent for the control of mosquito population as insect growth regulator.

Over one thousand plant species contain bioactive substance with many of these containing phytoecdysones, phytojuvenoids and anti-juvenile hormones, which act as IGRs (Varma and Dubey, 1998). Identification of phytochemicals with growth inhibition properties combined with a considerable capacity to reduce adult emergence is the desired endpoint of botanical insecticide research. Another desirable quality would be that a control agent induced IGR effects at less than the lethal dose so that recruitment could be reduced over time. The lower dose treatments inhibited growth and caused mortality in a dose-dependent manner and also growth inhibiting effects on the various developmental stages of different mosquito species. A range of pre-emergent effects occur such as delays in larval development and extended pupal durations, moulting inhibition, morphological abnormalities, and mortality especially during moulting and melanization processes (Shaalan *et al.* 2005).

` Furthermore, in the present study, larval progress was affected showing several deformities, including dechitinized body wall, and body length of matured larvae was reduced as compared to controls. Deformities that developed in the body wall of larvae may be attributed to the dechitinizing effect of extract as reported by Saxena and Sumithra (1985). Tabassum *et al.* (1993) observed that phytoextracts

affect larval morphology, resulting in pigmentation and alterations in head and abdomen shape. Adults emerging from physically deformed pupae remained trapped in the pupal eclusion. Likewise, Murty *et al.* (1997) have reported significant inhibition in adult emergence in *Culex quinquefasciatus* when treated with *Polyalthia longifolia* leaf extract.

Many plant extracts tested to date have shown potential larvicidal activity against insect pests including mosquitoes. However, many have failed to demonstrate adulticidal effects. A study by Lee and Chiang (1994) showed a good larvicidal property of *Stemona tuberosa*, however no adulticide was detected. Choochote *et al.* (1999) also tried to demonstrate the adulticidal property of *Kaempferia galanga*, however it only caused a knockdown effect at the initial stage of exposure but after transferring to the holding tube, the mosquito recovered from the knockdown effect. Therefore they concluded perhaps *K. galanga* might be useful as a repellent instead.

Extracts from the various parts of *T. minuta* revealed extracts of floral parts to have the greatest biocidal effect on *Ae. aegypti* and *An. stephensi* adults with impressive LC_{90}s of 0.4% and 0.45%, respectively (Perich *et al.* 1994). Choochote *et al.* (2006) demonstrated the ethanolic extracts of *Piper longum*, *Piper ribesoides* and *Piper sarmentosum* showed adulticidal activity when tested against female mosquitoes by topical application. The highest adulticidal effect was established from *P. sarmentosum*, followed by *P. ribesoides* and *P. longum*, with LD_{50} values of 0.14, 0.15 and 0.26 µg/female, respectively. By using the aerosol spray test, the essential oil of *Piper aduncum* leaf induced significant mortality in *Ae. aegypti* (80%) and *Ae. albopictus* (71.6%) (Misni *et al.* 2011). By exposing adult *Ae. aegypti* females to filter paper treated with ethanolic plant extracts, *Annona squamosa* and *Artemisia*

vulgaris shown 63% and 79% mortality after 24 hours (Sharma *et al.* 2011). In the present study, *H. bengalesis* also showed good larvicidal activity but less adulticidal activity.

Yang and co-workers (2005) have evaluated adulticidal activity of five essential oils against *Cx. quinquefasciatus*. Ethanol extract of *Apium graveolence* exhibited adulticidal activity against *Ae. aegypti* with LD_{50} and LD_{90} values of 6.6 mg/cm_2 and 66.4 mg/cm2 (Choochote *et al.* 2004). Essential oil of *L. camara* leaves showed more adulticidal activity against mosquitoes compared to earlier reports and almost all mosquitoes showed signs of paralysis at exposure to 0.208 mg/cm_2 impregnated paper within 10 to 15 min, and at the end of 1 h exposure all mosquitoes become inactive. At 24 h holding period per cent mortality ranged from 93 to 100 per cent against all test mosquitoes. The symptoms observed in adult mosquitoes were similar to those caused by nerve poisons i.e., excitation, convulsion, paralysis and death (Choochote *et al.* 2004). Dua *et al.* (2010) observed that the LD_{50} values of the essential oil of *Lantana camara* treated against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. culicifacies*, *An. fluvialitis* and *An. stephensi* were 0.06, 0.05, 0.05, 0.05 and 0.06 mg/cm_2 respectively. For mean mortality value after 24 hours, the leaf essential oil of *Cymbopogon nardus*, wood of *Eurycoma longifolia* and wood of *Fernandoa adenophylla* gave mortality of 15.7% ± 0.3, 10.9% ± 0.2 and 10.6% ± 0.2, respectively after 24 hours. Kamaraj and Rahuman (2010) also reported that above 90% mortality was found in the ethyl acetate and methanol extract of all experimental plants viz. *Momordica charantia*, *Moringa oleifera*, *Ocimum gratissimum*, *Ocimum tenuiflorum*, *Punica granatum* and *Tribulus terrestris* against *Cx. gelidus* and *Cx. quinquefasciatus* at the concentrations of 500 µg/mL.

Although crude acetone root bark extract of *H. benghalensis* was less toxic against adults, it is encouraging that *H. benghalensis* was lethal against larvae. Larvicides target larvae in the breeding habitat before they can mature into adult mosquitoes and disperse, thus they can reduce overall pesticide use in control programs by reducing or eliminating the need for the ground or aerial application of adulticiding chemicals. The results suggest that, in addition to their medicinal properties, the crude acetone root bark extract of *H. benghalensis* also have potential to develop natural insecticides against mosquitoes.

G. arborea leaf, rhizomes of *C. longa* and *H. aromatica* were tested for repellency against mosquito. On comparing with the other extract, petroleum ether extract of *C. longa* rhizome was found to give better protection time against the bites of *Ae. albopictus*. Thousands of plants have been tested as potential botanical sources of insect repellent (Quarles 1996). Rajkumar and Jebanesan (2005b) reported that concentrations of 0.001, 0.005, 0.01, 0.015, and 0.02 % of *Solanum trilobatum* provided 70 to 120 minutes protection against mosquito bites. The Pine oil was reported to have strong repellent action against mosquitoes as it provided 100% protection against *An. culicifacies* for 11 h and 97% protection against *Cx. quinquefasciatus* for nine hours espectively (Ansari *et al.* 2005). Das *et al.* (2000) reported 7.4, 6.5 and 6.4 h protection against the bites of mosquitoes with 60% (0.57 mg/cm2) concentration of essential oil of *Z. armatum* (fruits), *Curcuma aromatica* (rhizomes) and oil of *A. indica* respectively in mustard oil base in field conditions. It is stated that petroleum ether extract of *Vicoa indica*, *Buddleja asiatica*, *Chenopodium ambrosoides*, *Clerodendrum inerme* and methanol extract of *Solanum erinthum* gave 3 h protection against mosquitoes at 9% concentration (Venkatachalam

and Jebanesan 2001). It is also reported that 1% of garlic extract gave 8 h protection against *Cx. fatigans* (Bhuyan *et al.* 1974). Sharma *et al.* (1993b) reported that two percent neem oil mixed in coconut oil, when applied to the exposed body parts of human volunteers, provided complete protection for 12 h from the bites of all anopheline species. But botanical repellents that have been tested in initial studies, regardless of their active ingredients and formulations, gave very short-lived protection (Fradin and Day 2002). The insect repellent components in turmeric are turmerones and arturmerone (Su *et al.* 1982). Further work is needed to isolate, identify and exploit the lead bioactive constituent(s) as promising mosquito repellent. This repellent activity is comparable to previously screened plants using different species of mosquitoes (Rajkumar and Jebanesan 2004; Venkatachalam and Jebanesan 2001).

The present results showed that petroleum ether extracts of *C. longa*, *E. communis* and *M. azedarach* have shown oviposition repellent activity against *Ae. albopictus* gravid females. Xue *et al.* (2006) pointed out the oviposition-deterrent effectiveness (76–100% repellency) against *Ae. albopictus* of 21 commercial insect-repellent products (at 0.1% concentration), including 12 botanical, six deet-based and three synthetic organics. Rajkumar and Jebanesan (2009) have reported that the oviposition-deterrence effects of ethanolic leaf extract of *Cassia obtusifolia* at higher concentration (400 mg/l) showed 92.5% effective repellency against oviposition, followed by 300, 200, and 100 mg/l that showed 87.2%, 83.0%, and 75.5%, respectively. The leaf extract of *Solanum trilobatum* reduced egg laying by gravid females of *An. stephensi* from 18% to 99% compared with ethanol-treated controls at 0.01%, 0.025%, 0.05%, 0.075% and 0.1% (Rajkumar and Jebanesan 2005b). Mehra

and Hiradhar (2002) revealed that the crude acetone extract of *Cuscuta hyaline* was an effective oviposition deterrent against *Cx. quinquefasciatus* at a concentration of 80 ppm. Coria *et al.* (2008) have reported that the full oviposition deterrency was obtained with *Melia azedarach* leaf extract at 1 g/L against *Ae. aegypti*. Autran *et al.* (2009) have reported that the essential oil from leaves and stems of *Piper marginatum* exhibited an oviposition deterrent effect against *Ae aegypti* at 50 and 100 ppm in that significantly lower numbers of eggs (<50%) were laid in glass vessels containing the test solutions compared with the control solution. Elango *et al.* (2009) reported that the OAI value of acetone, ethyl acetate and methanol extracts of *Aegle marmelos*, *Andrographis lineata* and *Cocculus hirsutus* at 500 ppm were −0.86, −0.87, −0.90, −0.78, −0.87, −0.86, −0.91, −0.94 and −0.86, respectively. There were no significant differences in the numbers of eggs laid on distilled water or on distilled water containing Tween 20 and acetone for day 3 (p=0.6412) or day 5 (p=0.1344) and the numbers of eggs laid on terpineol or its control on day 3 (p=0.939) and day 5 (p=0.857) against *Ae. aegypti* (Waliwitiya *et al.* 2009). Elango *et al.* (2009) also showed that the 500-ppm-treated cups received a mean number of 26±1.51 and 32±1.42 eggs that tested the leaf ethyl acetate and methanol extracts of *Aegle marmelos* respectively against *Cx*. *quinquefasciatus.*

Jeyabalan *et al.* (2003) observed the oviposition-deterrent properties against *An. stephensi* for various plant extracts including the methanol extract of *Pelargonium citrosa*, which exhibited 56% and 92% inhibition of oviposition at 1 and 4 ppm, respectively. Tawatsin *et al.* (2006) have reported that the relatively high oviposition deterrencies were obtained from essential oils of *Curcuma longa* (94.7%), *Schefflera leucantha* (91.6%), *Zingiber officinale* (90.1%), *Vitex trifolia* (89.1%),

Melaleuca cajuputi (87.9%), *Hedychium coronarium* (87.5%), *Psidium guajava* (87.1%), *Manglietia garrettii* (86.1%), and *Houttuynia cordata* (85%); and moderate degrees of deterrency were obtained from *Piper nigrum* (82%), *Litsea cubeba* (80.6%) and *Eleutherococcus trifoliatus* (80.2%) against *Aedes aegypti*.

A significant reduction of protein, sugar, glycogen, and lipid was observed when the larvae were treated with a sublethal concentration (5 ppm) of crude acetone root bark extract of *H. benghalensis*. It is well documented that during larval growth, extensive biosynthesis of reserves such as protein, carbohydrate, and lipids occurs in mosquitoes which serves as precursors for the metamorphosis of larvae into pupae and adults (Timmermann and Briegel 1999). Our results are in conformity with the work of Senthilkumar *et al.* (2009) and Preet and Sneha (2011) who reported a drastic fall in the concentrations of carbohydrates, glycogen, proteins, and lipids. Maximum reduction in glycogen, protein, and lipid concentrations followed by sugar was observed in *Ae. albopictus* and *Cx. quinquefascitus*, whereas in *An. barbirostris*, the order of decline of biochemical reserve was sugar, protein, lipid, and glycogen. An immediate explanation to maximum reduction in sugars and glycogen could be that they were utilized in maximum and depleted to combat this chemical stress. Hence, treatment with crude acetone root bark extract of *H. benghalensis* might have interrupted this process and resulted in larval mortality.

Modifications of the RAPD patterns can be due to changes in primer binding sites, structural changes due to DNA damage. The interpretation of the molecular events responsible for differences in the RAPD patterns is not an easy task since different DNA alterations can induce similar type of changes. RAPD analysis allows a qualitative assessment of the DNA effects and the nature of the changes in profiles

can only be speculated unless amplicons are analyzed with sequencing, probing etc. RAPD assays have been shown to detect DNA damage caused by selected plant extracts at different doses. The changes occurring in RAPD profiles treatments include variation in band intensity as well as gain or loss of bands. Exposure of an organism to a these plant extracts may result in the formation of covalently bound adducts between the chemical or its metabolites and the DNA; faulty repair of these adducts often results in mutations and, sometimes, cytogenetic changes. In addition, Jones and Kortenkamp (2000) demonstrated that genomic alterations can only be picked up by the RAPD assay if they affect at least 2% of the cells. It was found that RAPD polymorphism due to mosquito larvae DNA exposed to mosquitocidal plant extracts can be distinguished from the untreated mosquito larvae.

In conclusion, the present plant extracts have potential for the development of new and safe control products for mosquitoes. As naturally occurring insecticides, these plant-derived materials could be useful as an alternative for synthetic insecticides. In the present study, plants are easily available, accessible and affordable; therefore, the usage of traditional plants should be promoted among the local residents in order to reduce the man–vector contact as well as vector-borne diseases. The screening results suggest that the petroleum extract of *C. longa*, *E.communis* and *M. azedarach* are promising in mosquito control. Further studies on isolation of bioactive fraction/constituent may provide futuristic lead products for field application of mosquito control.

6. SUMMARY

Plant-based insecticides for vector control are urgently needed for *Aedes albopictus*, *Anopheles barbirostris* and *Culex quinquefasciatus* which are the primary vectors of malaria, lymphatic filariasis, and dengue, respectively, in India and other South East Asian countries.

In the present study, larvicidal, adulticidal, oviposition deterrency, insect growth regulator and repellent activities of petroleum ether, acetone, chloroform, methanol and water extracts of thirty ethnomedicinal plants from Mizoram were tested against the larvae and adults of the three mosquito vectors.

Larvicidal assay

- Preliminary screening of larvicidal activitiy of 30 medicinal plants in five different solvents (petroleum ether, chloroform, acetone, methanol and aqueous extracts) were perfomred at 500ppm against three mosquito vectors - *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus*

- About fifteen plant extracts, petroleum ether extracts of *Blumea lanceolaria*, *Centella asiatica*, *Homalomena aromatic*a, *Oroxylum indicum*, *Tagetes erecta*, *Elsholtzia communis*, *Melia azedarach*, *Syzygium aromaticum*, *Curcuma longa*; Chloroform extract of *S. aromaticum*, *C. longa*; acetone extracts of *E. communis*, *Hiptage benghalensis*, *S. aromaticum* and *C. longa* showed 100% mortality at 500 ppm against *Ae. Albopictus*.

- Petroleum ether extract of *E. glandulosum*, *C. asiatica*, *H. aromatica*, *T. erecta*, *E. communis*, *M. azedarach*, *S. aromaticum*, *C. longa*; chloroform extract of *O. indicum*, *E. communis*, *C. longa*; acetone extract of *E. glandulosum*, *T. erecta*, *E.communis*, *H. benghalensis* and *C. longa*] showed 100% mortality at 500 ppm against *An. Barbirostris*

- In screening test against *Cx. quinquefasciatus*, 100% mortality were observed in *M. micrantha*, *C. asiatica*, *O. indicum*, *T. erecta*, *M. pachycarpa*, *E. communis*, *Polygonum plebium*, *M. azedarach*, *S. aromaticum*, *C. longa*; chloroform extract of *C. longa*; acetone extract of *C. reflexa*, *E. communis*, *H. benghalensis*, *S. aromaticum* and *C. longa*..

Dose response bioassay

- Dose response larvicidal bioassay was performed using *H. benghalensis*, *E. communis*, *H. aromatica*, *C. longa* and *S. aromaticum* extracts against *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasctiatus* larvae.

- Larvicidal effect was found to be dose-dependent. *A. albopictus, A. barbirostris* and *C. quinquefasciatus* larvae were highly susceptible to petroleum ether extract of *C. longa* and *H. benghalensis*.

- Results of log probit analysis (at 95 % confidence level) and regression analysis of crude extracts of *H. benghalensis*, *E. communis*, *H. aromatica*, *C. longa* and *S. aromaticum* against *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasctiatus* larvae revealed that lethal concentration (LC50) values gradually decreased with the exposure periods; lethal time (LT50) decreased with the concentration, and the mortality is positively correlated with the concentration.

- The order of susceptibility of the three mosquito species was as follows: *A. albopictus*>*A. barbirostris*>*C. quinquefascitus*.

Disruption of nutrient profiles in larvae and plant extract

- Biochemical changes were also evidenced in third instar larvae of three mosquito species [*Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasctiatus*] following a sublethal exposure (10ppm) of *H. benghalensis* for 24 h. The level of sugar, glycogen, lipids, and proteins was significantly ($P<0.05$) reduced in larvae treated with *H. benghalensis*.
- A significant reduction of sugar, glycogen and lipid was noticed when the three mosquito larvae were treated with sub-lethal concentration suggesting disruption of nutrient profiles in the larvae leading to eventual reduction in growth and development.

Insect growth regulators

- Insect growth regulatory effect of petroleum ether extracts of *M. azedarach* fruit was observed against *Ae. albopictus*.
- Treated larvae at 10 ppm and 20 ppm resulted in, malformed pupae and incomplete moulting adults.
- The total development period to eclosion was prolonged by 0.25, 0.75 and 1.25 days at the concentrations of 5 ppm, 10 ppm and 20 ppm respectively from 13.25 days in the control.

Adulticide bioassay

- The acetone root bark extracts of *H. benghalensis* is less toxic to adults and repelled laboratory-reared female *An. barbirostris, Ae. albopictus*, and *Cx. quinquefascitus* with the short median protection times of 57.66–135, 72.41–134.16, and 47.66–93 min, respectively.

Repellant bioassay

- Skin repellent activity of *G. arborea* leaf, *C. longa* rhizome and *H. aromatica* rhizome was performed against females of *An. barbirostris, Ae. albopictus*, and *Cx. quinquefascitus*. The results clearly show that repellent activity was dose dependent.

- The acetone extract of *G. arborea* leaf at 20000 ppm gave 50 minutes protection against *Ae. albopictus* female bite. The control provided only 2.2 minutes of protection.

- Petroleum ether extract of *C. longa* and *H. aromatica* provided 125 min and 90 min of protection at 20000 ppm against *Ae. albopictus* female bite.

- The repellency of *G. arborea* leaf extracts were also recorded against *An. barbirostris*. Concentration of 20000 ppm has shown longest protection time of 32.5 minutes only by the petroleum ether of *G. arborea*. The control provided 3.25 minutes of protection.

Oviposition deterrent

- Oviposition deterrency was observed against gravid *Ae.albopictus* preferred to lay eggs in the control cups than in the cups treated with petroleum ether extracts of *C. longa, E. communis* and *M. azedarach*.

- Significant reduction in the number of eggs laid was observed in all the test substances. The effective repellency ranged between 68.28 and 69.96%.

- The EC50 of the three plant extracts was between 15.39% and 20.35% and the oviposition active index was observed least in *C. longa* (-0.53) followed by *E. communis* (-0.52) and *M. azedarach*(-0.25).

RAPD profile and DNA damage

- The random amplified polymorphic DNA (RAPD) assay was used to assess the level of DNA damage in various exposed and unexposed *Cx quinquefasciatus* larvae to acetone and chloroform extracts of *C. longa* and *M. azedarach* at different concentrations (6.25, 12.5 and 25 ppm).

- This is the first report of an analysis of genomic alterations in plant extracts-treated mosquito larvae using RAPD-PCR fingerprinting.

- In comparison to the control larvae, larvae treated with the plant extracts caused greater changes in the RAPD patterns.

- DNA strand breakage was more in the treated larvae of *Cx. quinquefasciatus*.

The present investigation proves it as the medicinal plants used in this study are the potent larvicide, repellent, IGR and deterrents against *Ae. albopictus, An. barbirostris*, and *Cx. quinquefascitus*, which can be recommended to control these mosquito species on its breeding site as well as in their habitats. However, further investigations are needed to confirm the lethal effects of these potent plant insecticides in field conditions and its impact on the nontarget organisms.

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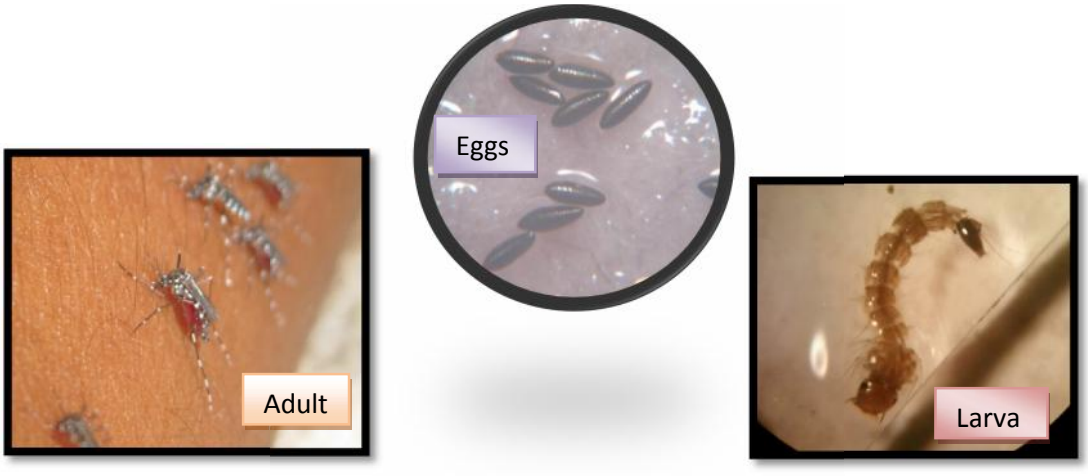
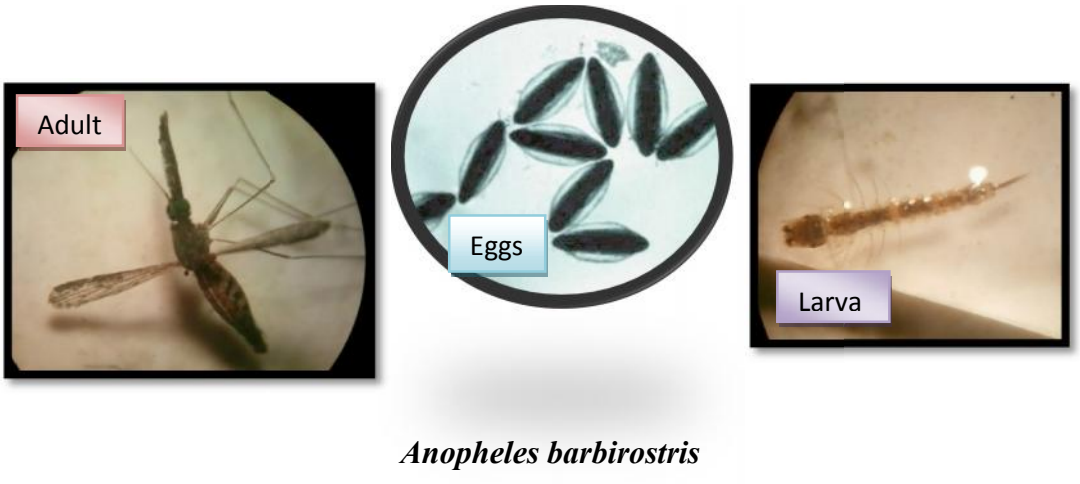
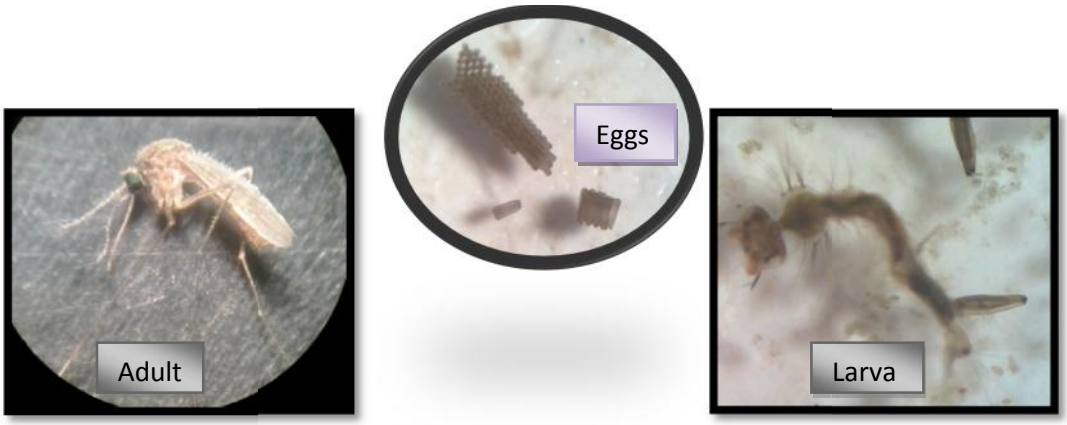
PLATE 1: Mosquito Vectors Used In The Present Study



Aedes albopictus



Anopheles barbirostris



Culex quinquefasciatus

PLATE 2: MOSQUITO CULTURE

A. Mosquito larvae in a tray

B. Mosquito pupae about to emerge in a culture plastic container

C. Adult culture cage

PLATE 3 Plants tested for mosquitocidal properties

Acacia gagaena

Alstonia scholaris

Antidesma acidum

Blumea lanceolaria

Brugmansia suaveolens

Centella asiatica

Clerodendron colebrookanium

Croton caudatus

Curcuma longa

Cuscuta reflexa

Dysoxylum gobara

Elaeagnus caudata

Elsholtzia communis

Eryngium foetidum

Eupatorium glandulosom

Eupatorium odoratum

Gmelina arborea

Hedyotis scandens

Hiptage benghalensis

Homalonema aromatic

Melia azedarach

Mikania micrantha

Millettia pachycarpa

Oroxylum indicum

Polygonum plebeium

Securinega virosa

Syzygium aromaticum

Tagetes erecta

Thespesia lampas

Tithonia diversifolia

PLATE 4: Effect of petroleum extract of *Melia azedarach* on the development of *Cx. quinquefasciatus* larvae

A. Incomplete moulting from larvae to pupa

B. Dead Larva

C. Incomplete moulting from pupa to adult

D. Incomplete moulting

Published papers:

i) **Lalrotluanga**, Senthil Kumar N, Gurusubramanian G (2011) Mosquito larvicidal activities of *Syzygium aromaticum* leaf extract against *Culex quinquefasciatus*. In Proc: Advances in Environmental Chemistry: 269-271

ii) **Lalrotluanga**, Senthil Kumar N, Gurusubramanian G (2011) Evaluation of the RAPD assay for the detection of DNA damage. Sci Vis 11(3): 155-158

iii) **Lalrotluanga**, Ngente L, Senthil Kumar N, Gurusubramanian G (2012) Insecticidal and repellent activity of *Hiptage benghalensis* L. Kruz (Malpighiaceae) against mosquito vectors. Parasitol Res 111:1007-1017

iv) **Lalrotluanga**, Laldikbera I, Vanlalruati C, Zothansanga, Brinda S, Senthil Kumar N, Gurusubramanian G (2008) Butterfly faunal diversity in Aizawl, Mizoram, India. Sci Vis 8(3):65-75.

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vii) Saipari Sailo, Lalremsanga HT, Hooroo RNK, **Lalrotluanga** and Ohler A (2009). *Ingerana borealis* (Annandale, 1912): a new record from Mizoram (India), with notes on its systematic position and natural history. Alytes 27 (1): 1-12.

viii) Lalrinchhana C, Lalnunsanga, Laltanpuia TC, Kumari A, Renthlei V, Lalrintluangi S**, Lalrotluanga**, Ramherliana J and Lalremsanga HT (2009). Collections on the Saurian (Reptilia: Squamata) fauna around Aizawl City area with notes on their ecology. Sci Vis 9(2): 57-72.

ix) Lalremsanga HT, Lalmalsawma Khawlhring and **Lalrotluanga** (2010) Three additional lizard (Squamata: Sauria) records for Mizoram, India. Journal of Threatened Taxa 2(2): 718-720.

x) Britz R, Lalremsanga HT, **Lalrotluanga**, Lalramliana (2011) *Monopterus ichthyophoides*, a new species of scaled swamp eel (Teleostei: Synbranchiformes: Synbranchidae) from Mizoram, India. Zootaxa 2936: 51-58

Papers Presentation at Symposia / Seminars /Workshop / Conferences:

i) Presented paper entitled **'Pesticidal action of plant extracts against mosquito vectors. *Aedes albopictus* and *Culex quinquefasciatu* (Culicidae: Diptera)'** in National seminar cum training program on "Green and Environmental Chemistry" (May 30, 2011) organized by Department of Chemistry, Mizoram University, Aizawl.

ii) Presented paper entitled **'Mosquito larvicidal activities of *Syzygium aromaticum* leaf extract against *Culex quinquefasciatus* Say'** (AEC 2011) in the International Conference on Advances in Environmental Chemistry (16-18 November 2011) organized by Department of Chemistry, Mizoram University, Aizawl, Mizoram.

iii) Presented paper entitled **'Insecticidal properties of *Diospyros variegata* Kruz.(Ebenaceae) extracts against *Aedes albopictus*, *Anopheles barbirostris* and *Culex quinquefasciatus* (Diptera: Culicidae)"** in "National seminar on emerging trends in Biosciences and future prospects" organized by Department of Zoology, Pachhunga University College, Aizawl, Mizoram on 29-30 November 2011.

Symposia / Seminars / Training / Workshop / Conferences Participated:

i) Training course on **'Bioinformatics – General Concepts and applications'** organized by the Bioinformatics infrastructure Facility, Department of Biotechnology, Mizoram University, sponsored by Department of Biotechnology, Ministry of Science and Technology, Government of India (March 26-27, 2009).

ii) Hands-on training in **'Winter School on Training in Insect Taxonomy'** (DST-FIST programme) (25th January – 6th February 2010) organized by Department of Zoology, Tripura University, Suryamaninagar, Tripura.

iii) One day Awareness Programme on **'UGC-infonet Digital Library Consortium'** (November 16 2010) organized by Department of Library & Information Science, Mizoram University, Aizawl, Mizoram in collaboration with INFLIBNET centre, Ahmedabad.

iv) Workshop on **'Molecular Phylogenetics and Evolution'** (November 22-24, 2010) organized by the Bioinformatics Infrastructure Facility, Department of Biotechnology, Mizoram University, Aizawl, Mizoram, sponsored by Department of Biotechnology, Ministry of Science and Technology, Government of India.

v) Training on **'Bioinformatics Proteins and Their Structure Prediction'** (November 23-23, 2011) organized by the Bioinformatics Infrastructure Facility, Department of Biotechnology, Mizoram University, Aizawl, Mizoram, sponsored by Department of Biotechnology, Ministry of Science and Technology, Government of India.

vi) State level workshop on **'Status and Conservation of Forest Resources in Mizoram'** (April 7-8, 2011) organized by Department of Environmental Science, Mizoram University, Aizawl, Mizoram.

vii) DBT sponsored **1st Summer School cum Workshop Training** (May 23-27, 2011) organized at the Institutional Biotech Hub, Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences (RIPANS), Aizawl, Mizoram.

viii) National seminar on **'Environment, Biodiversity, Veda and Traditional Systems'** (April 1-12, 2012) jointly organized by Department of Zoology, Mizoram University, Aizawl, Mizoram, MANU – International Council for Sustainable, and Efficacious Development and Awareness (ASEA), Rikhisesh, Uttarakhand.

ix) Short term course on **'One Week Workshop on Applied Statistics'** (July 23 – 28, 2012) organized by UGC-sponsored Academic Staff College, Mizoram University.

CHAPTER – 1

INTRODUCTION AND REVIEW OF LITERATURE

CHAPTER – 2

OBJECTIVES OF THE STUDY

CHAPTER – 3

MATERIALS AND METHODS

CHAPTER – 4

RESULTS

CHAPTER – 5

DISCUSSION

CHAPTER – 6

SUMMARY

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