

VALUATION OF SOME INDIGENOUS PLANTS EXTRACT
FOR POSSIBLE INSECTICIDAL ACTIVITY AGAINST VECTOR
OF FILARIASIS

THESIS SUBMITTED
UNIVERSITY OF MUMBAI

FOR THE DEGREE OF
MASTER OF SCIENCE
IN
APPLIED BIOLOGY

SUBMITTED BY
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UNDER THE GUIDANCE OF
DR. (MRS.) C.H. PANDIT

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**EVALUATION OF SOME INDIGENOUS PLANT EXTRACTS
FOR POSSIBLE INSECTICIDAL ACTIVITY AGAINST
VECTOR OF FILARIASIS**

**A Thesis Submitted
to the
University Of Mumbai
for the
M.Sc. (By Research) Degree
in
Applied Biology**

**Submitted By
Mr. Suryaji T. Jadhav**

**Under The Guidance Of
Dr. (Mrs.) C. H. Pandit**

**Department of Clinical Pathology,
Haffkine Institute for Training, Research and
Testing.**

OCTOBER 2006.

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DEDICATED

TO

MY FATHER

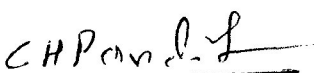
STATEMENT BY THE CANDIDATE

As, required by the University regulation No. 2190, I wish to state that the work embodied in this thesis titled **“Evaluation of some indigenous plant extracts for possible insecticidal activity against vector of filariasis”** from my own contribution to the research work carried out under the guidance of Dr. (Mrs.) C. H. Pandit, M.D., Head, Clinical Pathology Department, Haffkine Institute for Training, Research and Testing Parel, Mumbai. This work has not been submitted for any other degree of this or any other university. Whenever reference have been made to previous works of others, it has been clearly indicated as such and indicated in the bibliography.


Signature of the candidate.

(Mr. Suryaji T Jadhav)

Certified by


Signature of the Guide

(Dr. Mrs. C. H. Pandit)



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INTRODUCTION

INTRODUCTION:

Control of insect borne diseases poses a serious challenge to public health sector. There can be no doubt that these insect borne diseases or vector borne diseases are of global importance. Major among these diseases are mosquito borne infections like malaria, filariasis, yellow fever. Apart from malaria, control of diseases like filariasis continues to remain a priority even today all over the world. No wonder the World Health organization (WHO) has included it among the six tropical diseases requiring special attention in their Tropical Disease Research programme.

Filariasis is the name for a group of diseases caused by certain nematodes (filariasis worms). It affects people of rural areas as well as those living in urban areas with poor sanitation.

As per WHO out of a total population of 3287 million persons in South East Asian countries, where the disease is endemic, about 751 million live in areas where transmission is known to occur, of these 72.8 million persons are infected with *Wuchereria bancrofti* and 5.8 million with *Brugia malayi*. These two are the commonest filarial infections of humans in the tropical countries.¹²¹

In India 374 million persons living in endemic areas and 45 million persons are infected, an increase over the 18 million estimated in 1983, due mostly to an overall increase in population living in endemic areas.

In India the disease filariasis is caused by two species of nematodes viz. *Wuchereria bancrofti* and *Brugia malayi*. Filariasis due to *W. bancrofti* occurs mostly in urban situations and *B. malayi* in rural situations. Though bancroftian filariasis is the most prevalent in the most parts of India, brugian filariasis is also found mostly in Kerala, with scattered foci of low prevalence in Orissa, Assam, Madhya Pradesh, Andhra Pradesh and Tamil Nadu. Endemic states for *W. bancrofti* are Andhra Pradesh, Assam, Bihar, Gujrat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu, Uttar Pradesh, West Bengal, Andaman and Nicobar islands, Goa, Lakshadweep and Pondicherry.

Filariasis commonly known as elephantiasis was recorded in India far back as the sixth century B.C. by the famous Hindu physician Susruta. Another physician Madhvakara described the signs and symptoms of filariasis in the seventh century A.D. which hold good even today. Though the disease is not fatal it is responsible for considerable morbidity and social stigma especially among young adults and unmarried girls. Filariasis constitutes a major health problem in India, because of the increase in the trend of transmission, spread of the infection to newer areas, the huge population exposed to the risk of this disease and the large number of estimated persons with irreversible manifestations.

In recent years the incidence of bancroftian filariasis has been registering increasing trend. This is mainly due to unplanned urbanization, rapid industrialization, creating more mosquitogenic

conditions and frequent movement of people between endemic and nonendemic areas. In India lymphatic filariasis yet to be systematically and completely surveyed in Maharashtra, it has been observed, particularly in the city of Bombay, microfilaria rate is above 6% i.e. the problem that quite serious. (MCGB 1990-91)

In the city of Mumbai (Bombay), Basu et. al. shown had there was high infection rate of *W. bancrofti* in the mosquitoes (infection rate of 2.3% and infectivity rate of 0.7%). Recent data by Municipal Corporation of Greater Bombay (MCGB) likewise reveal high infection rate and infectivity rate in the mosquitoes prevalent in the city. (Annual Report, MCGB 1988) These reports indicate that the problem of bancroftian filariasis is very much there with us and needs serious attention. At the data collected by MCGB in the year 1991 shows the present situation of filarial infection in mosquitoes and intensity of mosquito population in the city of Mumbai (Bombay).

The worms responsible for causing filariasis in man are minute nematode worms that live in the blood and lymph. Although seldom fatal the disease is known to produce enlargement and deformities in legs, arms, genital organs and some other parts of the body, hence the name 'Elephantiasis'. The embryos or the Larval forms of this parasite are found in the deeper blood vessels of man (definitive host) during day time and when the patient is resting, especially in the night time the worms swarm into the superficial blood vessels and mosquitoes feeding at this time may draw in some of them with the Diseased blood. These worms then undergo transformation in the muscles of the

mosquito and after 2-3 weeks, when about 1/16 inch long, enter through the wound caused by the labium while the mosquito feeds on a healthy person, then migrate to the lymphatics where they grow to the length of about 3 inches and a diameter of about 1/100 inch, they mate and the female produces young ovoviviparously. Several species of genus *Culex* are suspected vectors in the tropics.

VECTOR OF FILARIASIS IN INDIA:

The filarial parasites found in the lymphatics of man in India are nocturnally periodic *W. bancrofti* and *B. malayi*, of these *W. bancrofti* is widely prevalent and *B. malayi* has localized distribution in the rural areas. So far no animal reservoir of *W. bancrofti* and *B. malayi* has been recorded. Mosquitoes are known to be the only intermediate hosts or vectors of *W. bancrofti* and *B. malayi*. *Culex quinquefasciatus* is the proved vector of *W. bancrofti* in India. Though some Anophelines and some other Culicines are found to support development of microfilariae of *W. bancrofti* up to infective stage in the laboratory e.g. *Anopheles subpictus*, *An. stephensi*, *Armigeres subaltus*, they have not been found suitable to act as vectors of the disease in nature.

In general these mosquitoes can practically breed in all the forms of water. Any place where water accumulates for a long time can be their breeding place. Hence it is common to observe breeding of

mosquitoes in all types conditions how ever adverse. However they predominantly breed in polluted water.

Cx. quinquefasciatus is the only known vector of *W. bancrofti* in Mumbai. It breeds in association with human habitations, preferring polluted water such as sewage and sullage water including pools, cell pits, septic tanks, drains, etc. In addition, it has been observed to breed in comparatively clean waters also. This is also the dominant species found more or less throughout the year. Periodical increase or decrease in natural population is linked with season.

CONTROL OF FILARIASIS:

There are two main approaches for the suppression of filariasis.⁴² First: To destroy the worms inside the patient's body so that there are no microfilariae left for transmission to other healthy individuals, this is the 'chemotherapeutic approach'. Second: Suppress the means of transmission, i.e. 'Vector control', so that they cannot carry over the infection to healthy people. This is the entomological approach. In this approach, it is necessary to maintain the suppression of mosquitoes continuously for several years. Unfortunately before achieving good control soon they become resistant to insecticides and chemical control becomes difficult.

MOSQUITO CONTROL:

This approach involves extensive use of synthetic insecticides. Generally speaking mosquito abatement is carried out on a community wide or large area basis under the direction of experts who determine the exact species involved, their breeding habits, and the most effective and economical means of suppression. Satisfactory control depends on intimate knowledge of mosquito biology, especially such factors as life cycle, larval development, breeding habits, adult flight range, resting places, adult feeding preferences, insecticide resistance etc.

ENVIRONMENTAL MANAGEMENT:

Mosquitoes depend on land, as well as aquatic habitats for growth and development. Because of the need for aquatic habitat for development the most fundamental means of control lies in the elimination of breeding places by drainage, filling, pumping, or temporary dewatering. Larvivorous fish such as *Gambusia* can occasionally be employed to keep ornamental pools, small ponds and lakes mosquito free, but due to the preference of this species over highly polluted water, survival of these fishes becomes difficult. In spite of their usefulness, however, such natural approaches rarely achieve complete mosquito control and must be reinforced by other measures such as house screening and the application of insecticides i.e. chemical control.

The use of synthetic or petroleum based insecticides for the control of insect pests and vectors poses problem of resistance environmental pollution and serious health hazard to mankind. Their hazardous nature has promoted research to find out the least disruptive newer option in the field of pest control technologies. Several attempts have been made to search for new classes of insecticides derived from plants, owing to low toxicity to non-target organisms and less persistence in nature. as a part of a continuing series of screening of new Phytolarvicide, an attempt has, therefore been made in the present study to assess the larvicidal activity of *Jatropha curcus*, *Ricinus communis*, *Strychnos nuxvomica*, *Pongamia pinnata* and *Echornia crassipes* against the larvae of *Culex quinquefasciatus*. (Diptera: Culicidae).

**REVIEW
OF
LITERATURE**

REVIEW OF LITRATURE:

Mosquitoes are highly specialized members of the class Insecta. Order Diptera is divided into three suborders, viz., Nematocera, Brachycera and Cyclorhapha¹⁰⁹. Members of Nematocera differ from that of the other two by having long, many segmented filamentous antennae. Smith (1973) divides it into eight families of which Culicidae forms one of the largest, embracing all mosquitoes. They are differentiated from the others (families) in having the second longitudinal vein forked once.⁹¹ Some authors include dixid and chaborid midges in this family as well, but they are more commonly placed separately.⁵¹ The family is divided into three subfamilies viz., Anophelinae, Culicinae and Toxorhynchitinae with numerous genera.^{49,50}

The subfamily Culicinae includes many genera such as Culex, Aedes, Armigeres, Mansonia, Psorophora, Haemagogus and Sabethes of great economic importance.⁵¹ Mosquitoes of the genus Culex are vectors of filaria and encephalitis, Aedes of dengue and yellow fever, Mansonia of Malayan filariasis; Psorophora of venezuelan equine encephalitis virus; and Haemagogus and Sabethes of Sylvan yellow fever virus of the above Psorophora and Haemagogus are not represented in India being confined mainly to the New World (Harwood and James, 1979).

About 3200 species of mosquitoes have been described throughout the world some with several subspecies (Service 1986).

The genus *Culex* comprises 466 species.¹⁷ As many as 250 species are reported from India.²⁷ Several species of the subgenus *Culex* act as vectors of a number of communicable diseases, of which *Culex pipiens* Linno complex and *Culex* 'vishnui' group Theobald are very important.

Cx. pipiens mosquitoes are highly domestic and are widely distributed throughout the world.⁹³ The females are night biters and usually highly anthropophilic. The larvae of the species breed usually in open ditches, sewage pools and artificial containers of polluted water. They occur in a complex form, and are grouped under several geographical or physiological subspecies, among which the following are of practical importance. These are:

- a] *Cx. p. pipiens* Linnaeus,
- b] *Cx. quinquefasciatus* Say,
- c] *Cx. p. pallens* Coquillette,
- d] *Cx. p. molestus* Forskal,

In India, *Cx. quinquefasciatus* is the main vector of bancroftian filariasis.⁹³

CULEX MOSQUITO:

About 550 sp. of *Culex* have been described most of them from tropical and subtropical region some species are important as vector of bancroftian filariasis and arboviruses such as Japanese encephalitis. In some areas, they are a considerable nuisance.²⁰

LIFE CYCLE:

Egg rafts of 100 or more eggs are laid on the water surface. The rafts remain afloat until hatching occurs 2-3 days later. *Culex* species breed in a large variety of still waters, ranging from artificial containers and catchments basins of drainage systems to large bodies of permanent water. But *Culex* breeds more in polluted water with organic material, such as refuse and excreta or rotting plants. Examples of such breeding sites are soak away pits, septic tanks pit latrines, blocked drains, canals and abandoned wells. In many developing countries *Culex quinquefasciatus* is common in rapidly expanding urban areas where drainage and sanitation are inadequate.

BEHAVIOUR:

Culex quinquefasciatus is a markedly domestic species. The adult female bite people and animals throughout the night, indoors and outdoors. During the day they are inactive and are often found resting in dark corners or in holes in trees in forested areas.²⁰

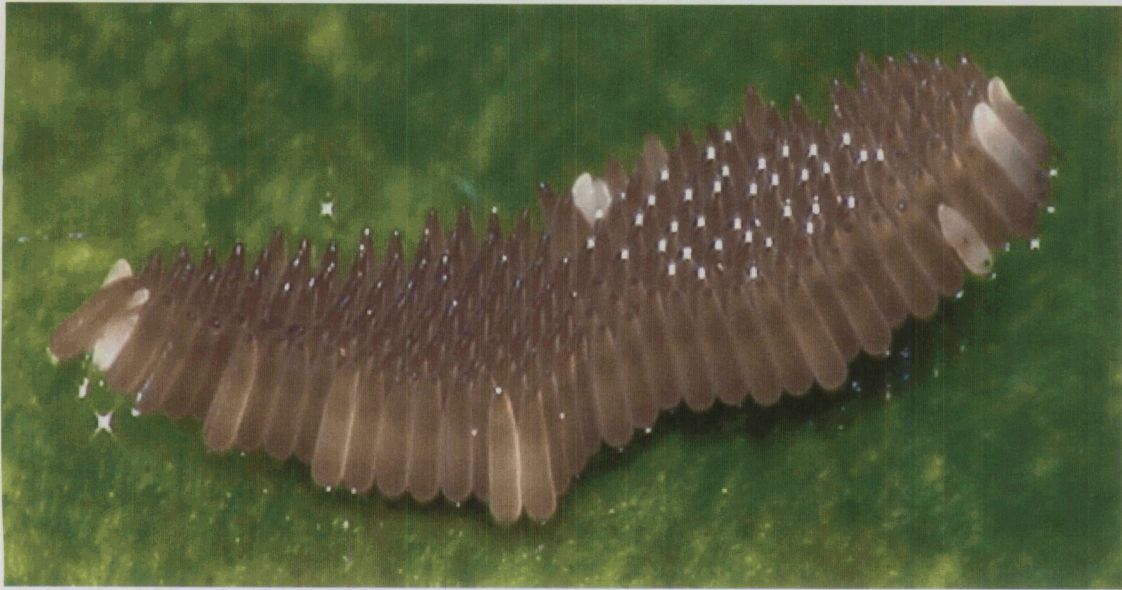
CULEX QUINQUE FASCIATUS FEMALE MOSQUITO :



CULEX QUINQUE FASCIATUS MALE HEAD :



CULEX QUINQUE FASCIATUS EGGRAFT :



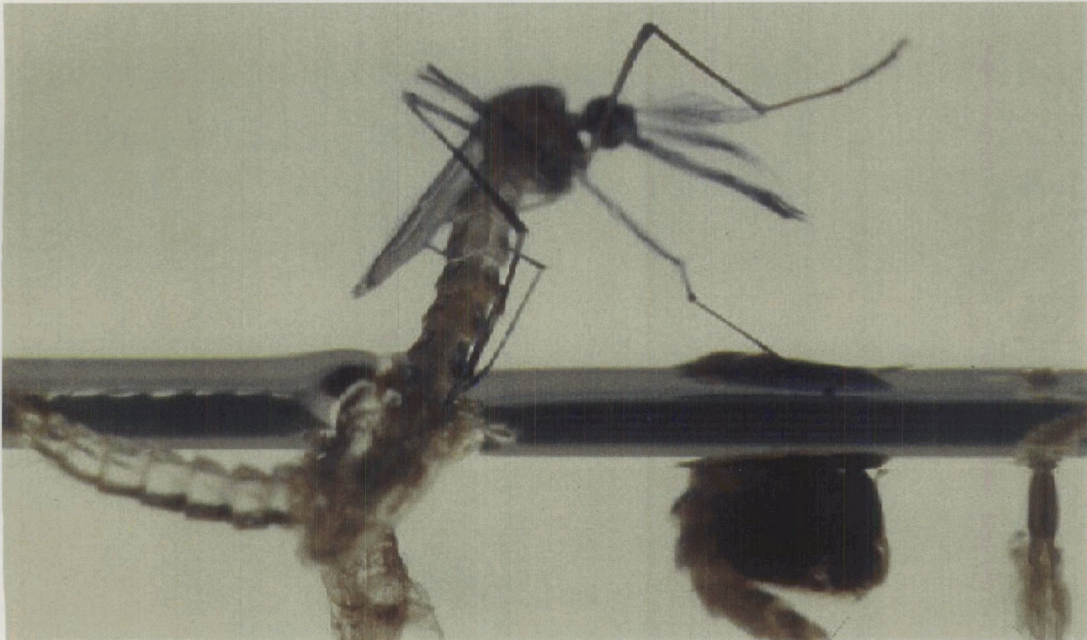
CULEX QUINQUE FASCIATUS LARVAE :



CULEX QUINQUE FASCIATUS PUPA :



CULEX QUINQUE FASCIATUS EMERGING ADULT :



LYMPHATIC FILARIASIS:

Lymphatic filariasis is ranked as the second most common cause of physical disability and is next to malaria among the debilitating vector borne tropical diseases¹²⁰. Estimated to infect over 120 million people globally, this disease is the commonest cause of lymphoedema and hydrocele in India, where the nocturnally periodic forms of *Wuchereria bancrofti* transmitted by *Culex quinquefasciatus* is responsible for 95 % of the disease burden and *Brugia Malayi* transmitted by *Mansonia* mosquito for the remaining 5 % together accounting for 40 % of the infected people in the world⁵⁷. This disease is geographically limited to economically backward countries of the world hampering their socio-economic progress considerably⁷⁴. It is identified as one of the six potentially eradicable diseases by the international task force for disease eradication. Lymphatic filariasis is now neglected for elimination globally as a public health problem based on the World Health Assembly resolution in the year 1997.^{18,75}

In India, 15 state and 5 union territories are endemic for Bancroftian filariasis, while Brugian filariasis is prevalent only in certain regions in Kerala and in some isolated foci in six other states.^{73,121} while and estimated 450 million population are 'at risk' of infection in this country, there are 31.26 million population with microfilaremia, 7.44 million with lymphoedema, and 12.88 million with hydrocele.⁵⁷ Even though Lymphatic filariasis usually presents with chronic manifestations, acute attacks of adenolyphangitis (ADL) are common

during the course of this illness. Thus, it is reported that a total of 40.6 million ADL episodes occur annually in the affected population. While these acute attacks by themselves cause considerable short-term disability, or they result in significant long-term incapacity by worsening the underlying lymphoedema.^{83,77} Estimates of the health burden suggested that 2.06 million disability adjusted life years (DALY'S) are lost in India due to this disease and the annual wage loss is estimated to be US \$ 811 million.⁸⁴

The recent advances in the understanding of pathogenesis of Lymphatic filariasis. The biology of the parasite: development of new diagnostic tools and chemo therapeutic agents that are safe and effective, have all contributed to new hope that this disease can be eradicated globally. This programme elimination of Lymphatic filariasis (PELF) is based on a dual approach consisting of (a) alleviation of the disease and (b) interruption of transmission to prevent the disease in future generations.⁹⁸ addressing the issue of disability prevention is extremely important in the context of the national control programme to ensure that there is a 'visible impact' on those who already suffer from this malady.¹⁰⁵

Many recent studies have illustrated the devastating social psychological, sexual and economic issue due to the deformities caused by this disease, which were not assessed so far. These massive swelling of the limbs in elephantiasis interfere with day-to-day activity of these patients, resulting in severe functional impairment and disability³⁹. They work for fewer hours, discontinue their activity or

change to lighter and less remunerative jobs, impairing their earning capacity⁸⁵. Genito-urinary disease, especially hydrocele, results in strange feelings of shame, fear and embarrassment associated with sexual disability and dysfunction²⁸. This disease hampers the marriage prospects of the young, mainly females. Other social problems among young patients include feeling of shame, embarrassment and frequent absence from or even discontinuation of studies⁸⁴.

Another significant development is the awareness that Lymphatic filariasis is first acquired mostly in childhood. Recent studies have shown that children residing in endemic areas suffer from lymphoedema of limbs, hydrocele and ADL attacks¹⁶. It was also found that in children microfilariae rates were 30 % of four-year-old children by ICT card test⁵². Existence of live adult filarial worms was shown on ultrasound examination of scrotal lymphatics in eight boys aged 14-16 years, which also revealed diffuse dilation of the affected lymphatic vessels²⁹. It has been found that, once established, this lymphatic dilation is irreversible and promotes progression of the disease.³⁴

BANCROFTIAN FILARIASIS:

Bancroftian filariasis is caused by the mosquito-borne nematode, *Wuchereria bancrofti*. The adult worms, males and females live in lymphatic vessels and nodes of human host, where the females produce first stage larvae called microfilariae that circulate in the blood. The infection is most often clinically unapparent, but it is now clear that

even these asymptomatic individuals almost always have underlying lymphatic damage caused by local mechanical damage by the highly motile adult worms with progressive infection, inflammatory immune damage to lymphatics results in a wide spectrum of clinical manifestation that depend on the part affected. Humans are the only natural host for *Wuchereria bancrofti*. The lack of an experimental laboratory animal model has severely hampered research in to the clinical, biochemical, and genetic determination of the disease process.^{3,13}

HISTORY:

Descriptions of elephantiasis are found in early India, Egyptian and Persian writings and drew the attention of medical observers in African and the South Pacific in the 17th and 18th centuries. The worldwide distribution of elephantiasis in tropical and subtropical areas and the epidemiological association of the elephantiasis with hydrocele, chylocele, and chyluria were established by the middle of the 19th century. Their common etiology, however, remained a mystery until discoveries were made of microfilariae in hydrocele fluid (Demarquay, 1863), Chylous urine (Wucherer, 1868), and blood (Lewis, 1872) and of the adult worm in a lymphatic abscess (Bancroft, 1877), Manson's great contribution was his description, while working in Amoy, China between 1875 and 1879, of the uptake of microfilariae by *Culex* mosquitoes and their maturation to infective forms. This is the first description of the mosquito as a vector of any of the parasitic

disease. Manson also made the association of endemic microfilarimex with elephantiasis and other lymphatic disease. Effective drug therapy awaited the description of the microfilaricidal effect of the Piperazine derivative diethylcarbamazine (Hewitt,1947) and more recently ivermectin. The adulticidal effect of DEC has been appreciated in the last decade.^{63,76}

ETIOLOGY:

Wuchereria bancrofti (Cobold, 1877) reaches sexual maturity in lymphatic vessels and nodes of humans. The adults are white, thread-like worms are convoluted in lymphnodes but have been shown by ultrasound to be almost completely extended in lymph vessels. The female, which is about twice the size of the male, measured between 80 and 100 mm in length and 0.2 to 0.3 mm in width. Thousands of developing embryos may be found within the gravid paired uteri of the female, each enclosed within translucent hyaline membranes that elongated to become the sheaths of the microfilariae once they are released. When examined in stained blood films a microfilaria has a length of 240 to 300 μ m and a width of 7 to 9 μ m and is covered by a translucent sheath. It usually assumes a graceful curved position. Differential features are shown in fig. In Asia the Pacific, *W. kalmanni*, a filariid infecting animals, has infective stage larvae and microfilariae that are morphologically indistinguishable from those of *Wuchereria bancrofti*.

The human *Wuchereria bancrofti* parasites have diversified in to a large number of strains, as is clear from the different periodicities of the microfilaremiæ that they cause. Each strain has established particular transmission equilibrium with the natural vector. The nocturnally periodic microfilariæ adapted to culicine and anopheline vectors, reach peak densities between 9 pm. and 2 am. And disappear completely from the circulation soon thereafter. The rapidity with which the levels taper off depends on peak intensity. Less commonly and predominantly in the South Pacific, *Aedes* adapted microfilariæ have a sub-periodic cycle.³³

EPIDEMIOLOGY AND NATURAL HISTORY:

Wuchereria bancrofti is the most abundant and wide spread of the human filarial infections, occurring throughout much of the tropical and subtropical regions of the world. It infects an estimated 115 million individuals worldwide. However, the use of assays that detect infection by measuring adult worm derived antigens in the circulation has indicated that infection rates in endemic areas may be twice that estimated from prevalence of microfilaria in blood samples. Most infection occurs in South Asia and tropical sub-Saharan Africa. In Asia, the parasite is endemic in both urban and rural areas in the Indian subcontinent. The Island of Srilanka and Burma. Infection predominate

in scattered rural foci in Thailand and Indochina extensively in the south and eastern alluvial plains of China.⁵⁸

Among above regional and proto-Malay people in the hill forest of the Mlay peninsula and northern Borneo and in many forested and agricultural regions of the Philippines. Most of the cities in these regions are spared infections. In Indonesia the parasite occur focally

Global Burden of Disease (GBD) Estimates of the Number of cases and prevalence of Filariasis (Infection and Chronic Disease Combined) By Endemic Regions:

Region	Population (millions)	Total No. of case (millions) <i>Wuchereria bancrofti</i>	Prevalence (%)
Sub-Saharan Africa	564	50.6	9.0
Asia	2770	62.3	2.3
Pacific Island	6	1.8	29.1
Latin America	441	0.4	0.1
World	3781	115.1	3.0

with low prevalence in low lying hill forests of Sumatra, Kaliountan and Sulawesi and more intensely along coastal fringes of small islands east of Lombok. Bancroftian filariasis is transmitted in urban areas along the northern coast of Java, particularly in the cities of Jakarta and Semarang. Over all the South pacific countries to be highly endemic for filariasis, particularly in coastal and low-lying hills in Papua New

Guinea and Iran, Java and many Islands of Melanesia, Micronesia, and Polynesia. The parasite is diurnally subperiodic throughout Polynesia, New Caledonia and the Loyalty Islands.

In sub-saharan Africa the nocturnally periodic, *Anopheles* rural strains of *Wuchereria bancrofti* are found in patchy distribution throughout a wide belt from 25 degrees north to 20 degrees south. In East Africa, *Culex* species as well as *Anopheles* transmit *Wuchereria bancrofti* all along the coastal regions and offshore islands of the Indian Ocean, particularly in Kenya, Tanzania, and Madagascar, where transmission occurs in both urban and rural areas. The other areas of high prevalence of filariasis extend from Senegal to Southern Sudan and low lying areas of Western Ethiopia, with populous Nigeria being one of the more intensely affected African countries. In Egypt, the parasite occurs mostly in Nile delta and is transmitted mainly by *Culex* mosquitoes. Within the past two decades the prevalence of Bancroftian filariasis has increased markedly in these foci and spread to urban areas in Egypt. Filariasis is no longer found in the Mediterranean or in the Middle East.⁵⁸

Bancroftian filariasis was introduced to the western hemisphere from Africa with the slave trade. The disease has been greatly reduced in the Americas, with only Haiti and the Dominican Republic remaining important foci in the Caribbean. In South America filariasis occurs only on the eastern tropical coast extending from Guyana to Salvador, Brazil primarily around urban and semi urban areas, particularly Recife.

FILARIASIS LIFE CYCLE:

The mosquito is infected when it ingested microfilariae from an individual with the disease. Passage of microfilariae in to the mosquito at the time of the human skin bite is apparently a passive mechanism. An extremely variable number of embryonic forms (microfilariae) may be ingested by the mosquito at any given time.

Yamada divides the development of microfilariae within the mosquito in to four-stages.⁵⁴

FIRST STAGE:

Up on entering the stomach of the mosquito the microfilaria lengthens, and in approximately 6 hours it loses it's cuticle, thus becoming a young larva. Those embryos, which do not undergo this transformation probably, disintegrate and are digested by the mosquito.

SECOND STAGE:

The larval form presents a pointed tail *and* migrates through the gastric wall until it lodges in the thoracic muscles. In this location the larva loses motility and becomes shorter. Toward the 5th day, it becomes sausage-shaped and on the 9th day undergoes an initial moult

THIRD STAGE:

During the next 4 days the larva lengthens up to 1 mm. and shows marked motility

FOURTH STAGE:

Toward the 15th day, it undergoes a second moult and becomes an infectious larva measuring 1.75 mm. in length. It then travels to the proboscis of the mosquito, where it is in position to be transmitted to man through the bite of the mosquito fig. The microfilaria penetrates the skin of man through the mosquito bite and spreads through the lymphatic system. For a period of time estimated to be approximately 3 months: the microfilaria undergoes two additional moults and then develops into the adult form of the nematode.

Man is the only known definitive host for *Wuchereria bancrofti*.

INSECTICIDE:

Insecticides have played an important role in reducing malaria and other insect borne diseases in past decades. At a stage public health authorities were hopeful of eradicating vector borne diseases like malaria. However, the widespread optimism based on the control or eradication of vectors with insecticides did not take into account the total energy of such vectors and problem of insecticide resistance that may arise. In view of these difficulties, which have been experienced by public health personnel, the necessity for studies on vector ecology

has now been reemphasized. New approaches have now been developed to analyze population dynamics such as, the number of animals, their survival, their reproductive capacity and an attempt has been made to apply these approaches to mosquito population, WHO (1988) has emphasized the need for a detailed study of the biology and ecology of the vector population in order to assess the role in disease transmission. It is felt that such kind of study would help in discovering the vulnerable points in the vector's life processes at which they can be attacked by some appropriate means. Seasonal population trends density parameters, vector competence, biting behavior developmental rates are some of these variables that are particularly required to be estimated in the endemic areas.

One of the major problems in the control of diseases transmitted by the vectors is resistance of the vector population to major groups of insecticides that are currently employed in that area. Studies carried out by various workers have demonstrated that resistant strains of Arthropods often have larger mean development time than their susceptible counterparts.^{4,35} To understand the relationship between the resistance phenomenon and the biology of mosquitoes it is very important to know about the major groups of insecticides, their mode of action and their role in the development of insecticide resistance.

CLASSIFICATION OF INSECTICIDES:

There are six major classes of insecticides used in vector control today; organophosphates (OPs), organochlorines (OCs), carbamates, pyrethroids, growth regulators and microbial insecticides. The first four of these insecticides act by affecting the passage of impulses in the insect nervous system, whereas the last two interfere with development and membrane permeability respectively. These insecticides can enter the insect body in one of three ways.

i) Contact poisons: - These penetrate the insect cuticle to reach the target site e.g. OPs (malathion), OCs (DDT), pyrethroids (permethrin) or carbamates (propoxur) or juvenile hormone analogues (methoprene) and the chitin growth inhibitor (diflubenzuron)

ii) Stomach poisons: - These are ingested and absorbed through the gut e.g. bacterial insecticides such as *Bacillus thuringiensis israelensis*. These insecticides act by releasing an endotoxin, which disintegrates the midgut cell wall.

iii) Fumigants: - These enter the insect body through the spiracles of the respiratory system. A number of contact poisons also have fumigant action e.g. dichloroovos.

iv) Chemosterilants: - In addition to insecticides efforts are being made to use chemicals for achieving insect control by sterilization.

The chemosterilization of mosquitoes such as *Aedes aegypti*, *Ae. togoi*, *Anopheles melas*, *An. gambiae*, *An. quadrimaculatus*, *An.*

labranchiae, *Culex pipiens var molestus* and *Cx. fatigans* with different alkylating agents gave encouraging results^{24,118}. Studies on sterilization tests carried out with the alkylating agent apholate on insectary reared *Cx. fatigans* showed that apholate induces sterility in both sexes²⁵. Females were affected much more when they were treated in the adult stage. The average number of eggs laid by a treated female was found to be reduced no matter whether the treatment was during the larva or adult phase. Male mosquitoes chemosterilized by dusting were more competitive than control males. Later it was shown that phosphoramides like hempa and ENT-51007 were quite promising for larval treatment and resulted in 80% and 83% control of reproduction respectively at nontoxic doses. Hempa at a toxic dose induced complete sterility. Triazines (hempa) were also shown to have similar effects³⁸. It was found that hempa and hemel were more effective in females of *Cx. p. fatigans* than in the males. They showed considerable reduction in oviposition. Reduction in oviposition is a common effect of alkylating and nonalkylating chemosterilants.

It was further observed that the lethal effects of chemosterilants appear in the F_1 generation⁸¹. It was found later that the phosphoramides and S-triazines also caused significant mortality in the F_1 progeny of *Cx. p. fatigans* and that they also induce dominant lethal mutations that are expressed in the F_1 progeny. The production of mortality in the F_1 generation of *Musca domestica* was characteristic of all S-triazines¹⁵.

The findings of the specificity of hempa and ENT-51007 and hemel against *Cx. p. fatigans* clearly suggest that, further trials on similar compounds should be continued because of the specific properties of the phosphoramides and S-triazines.

The ability of chemosterilants to induce mortality in the F generation an additive effect and would be advantageous in sterile male release programmes. Genetic control of insect populations in the past has generally involved the rearing of numbers of insects in insectaries for sterilization and release. But this has several disadvantages such as specialized staff and local use by a community. Ikeshoji et. al. have discussed for work aimed at genetic control utilizing insects trapped from wild populations which can then be chemosterilized and released circumventing these and other objections.⁴⁸ Thus more studies these lines may help scientists public and on workers to develop more and more sophisticated and quicker solutions to the problems due to vectors and vector borne diseases.

MODE OF ACTION OF INSECTICIDES ACTING ON THE NERVOUS SYSTEM:

NORMAL ACTION OF THE NERVOUS SYSTEM:

In an insect nerve there is a potential difference between the intra and extraneuronal cytoplasm. The potential is maintained because the nerve membrane is impermeable to sodium ions, but permeable to potassium and chloride ions. Sodium ions are actively

pumped out of the nerve, so that there is a net negative charge inside the nerve. An electrical impulse passes down the nerve by altering the permeability of the nerve membrane to sodium ions. Sodium channels or 'gates' are opened and sodium floods into the nerve causing a net positive change in the charge. The sodium channels shut almost immediately, but the resting potential cannot be renewed until the sodium is pumped back into the extra cellular fluid. Once this is achieved the nerve is ready to transmit another impulse¹¹⁵.

When a nerve impulse arrives at the end of a Cholinergic neuron, synaptic vesicles fuse with the presynaptic membrane and release a neurotransmitter, acetylcholine (ACh) which diffuses across the synapse and binds with a receptor on the post-synaptic membrane. Fusion of the synaptic vesicles to the membrane is thought to happen because the fluid inside the neuron decreases in viscosity when an electrical impulse alters its charge. The binding of ACh to the receptor causes a change in conformation, which alters the ionic permeability of the membrane, so that the impulse is passed on. Once impulse has passed, excess ACh has to be removed to prevent repetitive firing and to allow the transmission of succeeding message. The removal is effected by the enzyme acetyl-holinesterase (EC 3.1.1.7.) which catalyses hydrolysis of the ACh ester bonds, breaking it down to choline and acetic acid.

The active site of acetylcholinesterase (AChE) has two subsites, an anionic site and an esteratic site, the latter containing a serine hydroxyl residue, which is involved in cleavage of the ester

group. Hydrolysis of ACh involves the formation of a reversible enzyme substrate complex, followed by the acetylation of the esteratic subsite and release of choline into solution. The acetylated cholinesterase is very unstable and reacts with water to give free enzyme and acetic acid^{47,21}.

MODE OF ACTION OF ORGANOPHOSPHATES AND CARBAMATES:

Organophosphates And Carbamates are potent inhibitors of esterases, inhibiting these enzymes by phosphorylating or carbamylating them at or near the enzyme active site. Their prime action is to inhibit AChE in the insect nerve synapse, by acting as analogues of ACh. However, OP's and carbamates are poor substrates of AChE as they form, in case of OP- inhibition, a stable covalently linked phosphorylated enzyme, or with carbamate inhibition, a carbamylated enzyme. The release of free enzyme from these complexes still occurs, but the reaction is very much slower than with ACh. Consequently, AChE cannot deal efficiently with bursts of ACh release, the transmitter builds up and nerve function is disrupted⁹⁰.

Evidence for this form of inhibition in insects was documented in structure activity studies, which demonstrated that in order to be effective, organophosphates and carbamates need to possess similar binding characteristics to ACh²¹. More direct evidence was shown by the application of organophosphates and carbamates to cockroach

nerve cords. Changes in the normal excitatory post-synaptic potential were determined using electrophysiological methods. Application of the insecticides to the nerve prolonged this potential, presumably due to the persistence of ACh in the synapse (Narahashi 1971). More convincing data was presented by Uchida et. al. who reported that the ability of a series of carbamates to inhibit AChE correlated with their ability to excite nerve cords, which in turn was directly related to the insecticide's efficiency.¹¹⁴

MODE OF ACTION OF PYRETHROID ON THE NERVE MEMBRANE:

The precise mode of action of pyrethroids at the molecular level is not known. It is known that they act at the nerve membrane interfering with protein conformational changes at the lipid-protein interface, thus a delay in the closing of the sodium channels after an impulse has passed (Zerba 1988). Thus, at the end of an action potential, sodium ions still move into the axon, so that the membrane potential difference is positive. This causes the negative after potential to increase often to such a level that the after potential exceeds the membrane threshold and another action potential is passed. This results in repetitive firing of the nerve with subsequent paralysis.

Neurophysiological studies have indicated that pyrethroids fall into two groups, Type I compounds which lack an α -cyano group which have mainly central nervous system action. The toxicological effects c

the two compounds are different with Type I compounds the least potent of the two. However, the net result of poisoning with either Type I or II repetitive nerve charging⁵⁹.

MODE OF ACTION OF DDT:

DDT prolongs the inward sodium current of a nerve axon and suppresses the inward potassium permeability, increasing the negative after potential. This effect causes repetitive of the nerve, resembling the effect of pyrethroids. Narahashi has proposed that these two classes of compounds have same type of action but that the kinetics of the closing of insecticide modified channels different^{72,21}

INSECTICIDE RESISTANCE:

Crow found that years of insecticide pressure in the laboratory did not produce resistance in isogenic strains *Drosophila* that initially lacked resistance.²² Similarly, 150 generations of *Drosophila* were reared in a DDT contaminated media without any increase in resistance¹⁶. Based on this type of evidence, it has been argued that insecticide resistance is the result of a pre-adaptation, where the mutants which confer resistance do not arise as a consequence of insecticide usage but occur independently, at a frequency dependent on the natural mutation rate. The mutations disappear or remain at a very low frequency depending on the extent to which deleterious to the individual. If application of insecticide gives the mutant insects a

selective advantage, increasing their chance of survival, the frequency of individuals within a population capable of resisting the insecticide increases.

BIOCONTROL:

Biocontrol has two practices one is a naturally occurring process and another is a purposeful use of one organism to control the other.

Biocontrol can be achieved by Classical Biocontrol, Inundated release

The Biopesticide approach, Conservation Biocontrol.^{124,125}

HERBAL INSECTICIDES:

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. Some herbal products such as nicotine obtained from tobacco leaves, *Nicotiana tabacum*, anabasine and lupinine, the alkaloids extracted from Russian weed *Anabasis aphylla*¹⁸, rotenone from *Derri: elliptica* and pyrethrums from *Chrysanthemum cinerifolium* flowers⁴ have been used as natural insecticides even before the discovery of synthetic organic insecticides⁴⁴. However, the discovery, development and use of synthetic organic chemicals with persistent residual action not only overshadowed the use of herbal products against mosquitoes but also became the major weapon for mosquito control. Since the discovery of DDT, mosquito control approach has been almost

completely based on synthetic organic insecticides. But the extensive uses of synthetic organic insecticides during the last five decades have resulted in environmental hazards and also in the development of physiological resistance in major vector species. This has necessitated the need for search and development of environmentally safe biodegradable, low cost, indigenous methods for vector control, which can be used with minimum care by individual and communities in specific situations.

Phytochemicals obtained from plants with proven mosquito control potential can be used as an alternative to synthetic insecticides or along with other insecticides under the integrated vector control. Plant products can be used, either as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites depending on the type of activity they possess. A large number of plant extracts have been reported to have mosquitocidal or repellent activity against mosquito vectors¹¹⁰, but very few plant products have shown practical utility for mosquito control. Plant products can be obtained either from the whole plant or from a specific part by extraction with different types of solvents such as aqueous, methanol, chloroform, hexane, etc., depending on the polarity of the phytochemicals. Studies carried out so far have shown that some phytochemicals act as general toxicant (insecticide/larvicide) both against adult as well as larval stages of mosquitoes, while others interfere with growth and development (growth inhibitors) or with reproduction (chemosterilant) or produce olfactory stimuli thus acting as repellent or attractant. Ar

attempt has been made in the present write-up to review the reports on the mosquitoicidal and repellent activity of plant based products published during the past one decade with an emphasis on neem (*Azadirachta indica*) based products which have shown their practical utility under field conditions.

INSECTICIDES AND GROWTH INHIBITORS:

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. Products of some plants are effective at very high concentrations and thus may not be of much practical importance. However, some of the plant products have shown promise for mosquito control even under field conditions. One of the most commonly studied plants for control of mosquitoes is *Azadirachta indica*, (Meliaceae) commonly known as neem in India.

NEEM:

Neem contains at least 35 biologically active principles,⁶⁵ of which azadirachtin (AZA), a triterpenoid is the predominant insecticide active ingredient in the seeds, leaves, and other parts of the tree. Neem products containing azadirachtin and other ingredients have antifeedant, ovipositional deterrence, repellency, growth disruption, sterility and larvicidal action against insects⁹⁷. Neem based pesticides are now extensively used in agricultural practices all over the world.

Neem oil and other commercial preparations of neem have been found as potential mosquito larvicide⁶¹. Dhar et. al.²⁶ demonstrated the effect of neem oil volatiles on gonotrophic cycle and inhibition of oviposition in *An.stephensi* and *An. culicifacies*. Control of mosquito breeding has also been demonstrated in the field in some confined habitats using indigenous methods of application of neem oil in water and neem oil coated on wooden scraps^{11,69}. Wood scrap balls soaked in 5 to 20% neem oil in acetone were tested in overhead tanks of 0.50 cubic meter against *An. stephensi* breeding. Though it did not prohibit egg laying, it arrested pupal formation and eventually the adult emergence for about 45 days⁶⁹. Neem oil emulsion in water was also found to control breeding of *Cx. quinquefasciatus*, *An.stephensi* and *Ae. aegypti* in pools, basement tanks and desert coolers, and the effective control lasted for 2 to 3 weeks¹¹. Neem cake powder and urea coated with neem cake powder were evaluated for the control of mosquito breeding in rice fields⁸⁸. Application of neem cake powder alone or coated on urea resulted in drastic reduction in the late instar larvae and pupae of culicine mosquito for several weeks. Aqueous extract from deoiled neem seed kernels exhibited toxic and growth regulating activities against *Cx. quinquefasciatus* larvae with a 100% larval mortality especially during the first and second instars at all the tested concentrations⁹². Though neem products show high larvicidal activity, they do not show adulticidal action. Zebitz¹²³ suggested that azadirachtin acts as an antiecdysteroid and thus kills the larvae by growth inhibition effect. This, along with other delayed effects of neem

products²⁶ provides an alternative approach to chemical larvicides in mosquito control.

OTHER HERBAL PRODUCTS:

Several other plants have demonstrated toxic effects on mosquitoes mostly under laboratory conditions. *Tagetes* sp., commonly known as marigold has shown both larvicidal as well as adulticidal activity against mosquitoes^{79,37,53}. Active components have been isolated from different parts of this plant. Green et. al.³⁷ reported mosquito larvicidal activity in the extract of *Tagetes minuta* flowers. Perich et. al.⁸⁰ compared biocidal activities of the whole-plant extracts of three *Tagetes* species and showed that *T. minuta* had the greatest biocidal effect on the larvae and adults of *Ae. aegypti* (L.) and *An. stephensi* (L). Bioassays of simultaneous steam distilled extracts of *T. minuta* flowers showed larval mortality at LC₉₀'s of 4 and 8 ppm and against the adult at 0.4 and 0.45% against *Ae. aegypti* and *An. stephensi*, respectively⁸⁰. The extract from *T. minuta* was found to be most active among 83 plant species belonging to the compositae family, with a LC₅₀ of 1 mg/l against *Ae. fluviatilis*. Active components of *T. minuta* have also been identified as thiophene derivatives, a class of compounds present in many plants of family asteraceae⁵³. Pathak et. al.⁷⁹ reported 100% mortality with steam distilled oil extract from the whole plant of *T. erecta*, against larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* at doses lower than 100 ppm. Ethanol extract of another plant *Eclipta paniculata* belonging to family

compositae, has also shown significant insecticidal activity, with LC₉₀ of 17.2 mg/l and LC₅₀ of 3.3 mg/l⁴⁵. Leaf extract of *Polyalthia longifolia* exhibits larvicidal and growth inhibition effect against larvae of *Cx. Quinquefasciatus*⁶⁶. Application of the extract at the dose of 250 to 350 ppm produced 64- 96% inhibition of adult emergence of *Cx. Quinquefasciatus* in tanks and U-drains. Another plant, *Murraya koenigii* has also showed mosquito larvicidal activity^{79,87}, due to the presence of carbazole alkaloids, mahanimbine, murrayanol, and mahanine. Volatile oil from the peel of citrus fruits has also shown toxic effects on mosquito larvae as well as adults^{23,32,68,67}. Susceptibility tests carried out against *Cx. quinquefasciatus* larvae and adults using peel oil extracts of bitter orange (*Citrus aurantium*), orange (*C. sinensis*) and lemon (*C. limon*) indicated that the extracts may contain potentially useful insecticides. Volatile extracts of *C. sinensis* showed greater insecticidal potency³². The larvicidal action of three ethanol extracts of peels of lemon, grapefruit and navel orange, against *Cx. pipiens* revealed LC₅₀ values as 18.5, 20.3 and 26.5 ppm, respectively²³. The peel oil fulfilled other required specifications like suitable specific gravity, spreading pressure and viscosity. It is toxic at a wide pH range, stable to heat and light in terms of chemical change, which could alter larvicidal action. However, it is volatile and did not form a permanent film on water surfaces for long periods. This affected its larvicidal action⁶⁸. Jaiprakash et. al.⁴⁵ isolated three limonoids, namely limonin, nomilin and obacunone, from the seeds of *C. reticulata* which showed growth inhibition effect on 4th instar larvae of *Cx. quinquefasciatus* and

the EC₅₀ for inhibition of adult emergence was 6.31, 26.61 and 59.57 ppm for obacunone, nomilin and limonin, respectively. The pattern of mortality at around the EC₅₀ levels was indicative of moult inhibiting activity. Crude extract of leaves of *Solanum nigrum* in water showed larvicidal activity against *An.culicifacies*, *Cx. quinquefasciatus* and *Ae. aegypti* at a dose equivalent to LC₉₀ ranging between 0.18 and 0.21%³⁸. Toxicological studies on the three ethanolic extract preparations of *S. nigrum* leaves showed larvicidal activity against larvae of *Ae. caspius* and *Cx. pipiens*, (LC₅₀ 51.29 and 125.89 mg/l within 24 h, and 21.38 and 38.11 mg/l within 48 h, respectively). Sunlight, pH, and turbidity did not affect the activity of this extract. The concentrated extract (1000 mg/l) can be stored at room temperature for six months without any change in its activity, but diluted solutions of this extract lost their activity after four weeks¹.

Alcoholic extracts of leaves and stems of *Vanilla fragrans* fractionated with ethyl acetate and aqueous butanol possess mosquito larvicidal activity¹¹¹ and 4-butoxymethylphenol was found to be the most effective compound against mosquito larvae. Butenolides 1 and 2, isolated from the endemic plants *Hortonia floribunda*, *H. angustifolia*, and *H. ovalifolia*, also exhibited potent mosquito larvicidal activity against the second instar larvae of *Ae. Aegypt*³¹. Saxena et al⁹⁵ discovered growth inhibitory and juvenile hormone mimicing activity in the larvae of *Cx. quinquefasciatus* treated with acetone extracts of *Ageratum conyzoides*, *Cleome icosandra*, and *Tridax procumbens* resulting in larval pupal intermediates, demelanised pupae, defective

egg rafts and adult with deformed flight muscles. Loss of fecundity was also observed in the treated mosquitoes but no sterilitant effects could be seen. However, alkaloids from *Annona squamosa* exhibited chemosterilant effect in addition to larvicidal and growth inhibition in *An. stephensi*⁵. *Annona squamosa* and *Lansium domesticum* showed highest larvicidal potential against *Ae. aegypti* and *Cx. Quinquefasciatus* amongst the five plant species, viz. *A. squamosa*, *Eucalyptus globulus*, *Lansium domesticum*, *Azadirachta indica* and *Codiaeum variegatum* with extracts showing maximum insecticidal activity after 48 hours of exposure. *L. domesticum* and *A. squamosa* were most effective against larvae of *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. While, *Ae. aegypti* was more susceptible than *Cx. quinquefasciatus* to neem but *Cx. quinquefasciatus* was more susceptible than *Ae. Aegypti* to *Eucalyptus globulus*⁶². Petroleum ether extract of thyme plant, *Thymus capitatus* was found to be toxic against the larvae and adults of *Cx. pipiens* (L). Among different fractions isolated from this extract, the volatile oil, thymol, and the unsaponifiable portion showed high larvicidal potency (LC_{50} =49.0, 58.0, and 100.0 ppm, respectively). Non-lethal concentrations of these substances synergized the toxicity of malathion, while unsaponifiable portion and volatile oil showed the highest adulticidal potency. Thymol as well as volatile oil affected egg hatchability⁵⁵.

MOSQUITO REPELLENTS:

Though various plants have been reported to possess repellent activity against mosquitoes¹¹⁰, *Azadirachta indica*, *Eucalyptus* sp (Myrtaceae), *Lantana camara* (Verbanaceae), *Cymbopogon* spp (Gramineae), *Mentha piperita* (Labiatae), *Tagetes minuta* (Compositae) and some other plants products have been studied more extensively during the past one decade. Smoke produced by burning of dried leaves of *Azadirachta indica* has been used for the protection against mosquitoes since ancient times.

Neem oil:

Though neem oil has been used in various insecticidal and medicinal preparations, its mosquito repellent activity was not known. Recent studies carried out at the Malaria Research Centre (MRC) Delhi and elsewhere have shown repellent action of neem oil^{100,64,103,101}. Topical application of 2% neem oil mixed in coconut oil produced varying degree of protection against different vector species and the repellent effect was more pronounced against *Anopheles* spp than against *Cx. Quinquefasciatus*^{82,102,64}. A complete protection for 12 h from the bites of all the anopheline mosquitoes species was reported by using 2% neem oil in coconut oil on the exposed part of the body¹⁰⁰. However, Rajnikant and Bhatt²¹ reported only 89 and 98% protection against *An. fluviatilis* and *An. culicifacies* respectively and only 68% protection against all anopheline species by using 2% neem oil. The

protection from *Culex* and *Aedes* mosquitoes ranged between 76-86%. In another study 81-91% protection against *An. culicifacies* was reported during 12 h by using 1-4% neem oil in coconut oil⁶⁰. But Moore et al⁶⁴, did not find any significant protection from *An. darlingi* by using 2% neem oil, while a eucalyptus based repellent provided 96% protection for 12 h. Sharma et al¹⁰², reported only a week repellent effect of neem oil against *Ae. aegypti*. Vanishing cream with 5% neem oil also provided 67 to 100% protection against malaria mosquitoes in different ecological terrains in India^{30,70,103}. Application of the neem cream for protection against mosquitoes was more acceptable than neem oil because of its easy application, pleasant odour and more effective repellency up to 4 h after the application. Moreover, the application of neem oil and cream has been found safe¹¹⁶ and hence can be used as a personal protection measure against mosquito bites, particularly against malaria vectors.

Neem oil mats and lamps as mosquito repellent devices:

In addition to the topical application, other methods of using neem oil were also developed and evaluated at MRC, Delhi^{100,5}. Cardboard mats soaked in 5 and 10% neem oil were tested as mosquito repellent. Results revealed that mean catch per night per person in case of *Cx. quinquefasciatus* was 129.7 and 124.9 with mats containing 5 and 10% neem oil respectively and 187.6 with commercially available mat (containing allethrin) as compared to 729 mosquitoes in the control (no mat)²³. When indoor resting density of

mosquito was compared, 78 *Cx. quinquefasciatus* and 2 *An. culicifacies* mosquitoes were collected in rooms with 5% neem oil mats as against 142 *Culex* and 8 *An. culicifacies* in room with commercial mat and 212 *Culex* and 95 *An. culicifacies* in the control room without mat. Smoke produced by burning of neem oil mixed in the kerosene oil in lamps provided protection against mosquito bites³⁰. Use of kerosene lamps with 1% neem oil mixed in kerosene, produced 100% protection from all *Anopheles* mosquito species for 10 nights, but against *Culex* spp only 79% protection was observed. The feasibility of malaria control by burning neem oil in kerosene lamps was also demonstrated in a village scale trial³¹. Results revealed that burning of neem oil in kerosene lamps resulted in the displacement of *An. culicifacies* from living rooms to cattle sheds. This was also reflected when malaria incidence was compared in experimental and control villages. Human cases per 1000 persons and *Plasmodium falciparum* rates per 1000 person were 1.03 and zero respectively in experimental village as against 9.6 and 4.3 in the control village. Discontinuing the burning of neem oil in kerosene lamps resulted in the recurrence of *An. culicifacies* in living rooms and an increase in malaria incidence in experimental village.

OTHER HERBAL PRODUCTS:

In addition to neem some other plant-based products have also been found as effective mosquito repellents and have been evaluated against different vector mosquitoes. Flowers of *Lantana camara*

extracted in methanol and mixed with coconut oil provided 94.5% protection against *Ae. albopictus* for two hours.⁴⁰ Four fractions viz MRC - HR1, HR2, HR3 and HR4 were isolated from Lantana flowers using solvent extraction and chromatographic methods. Of these MRC-HR2 showed maximum repellency against *Aedes* mosquitoes with a mean protection time of 2.43 h. Repellent action of MRC-HR2 gave 85% protection for up to 6 h against *Aedes* sp. in field conditions³⁰. Oils of *Cymbopogon martini*, *Cymbopogon citratus* and *Cymbopogon nardus* provided more than 95% protection against *Cx. quinquefasciatus* and *An. culicifacies* in whole night landing collection on human baits⁸. Essential oil extracted by steam distillation of *Mentha piperita* and *Dalbergia sisoo* provided 84.5 to 100% protection against *Cx. quinquefasciatus* and *An. culicifacies* during the whole night landing collection^{7,8}. A high degree of repellency (>90% protection for 2 h. and >50% up to 4 hr) was observed in the essential oil extract of *Tagetes minuta* against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes in the laboratory studies¹¹³. Govere et. al.³⁶ studied the repellency effect of three plants viz fever tea (*Lippia javanica*), rose geranium (*Pelargonium reniforme*) and lemon grass (*Cymbopogon excavatus*) against laboratory reared *An. arabiensis* mosquitoes. The alcoholic extracts of these plants provided significant protection ($p = 0.012$). *L. javanica* provided better and longer (76.7% for 4 h) protection against *An. Arabiensis* compared to *C. excavatus* and *P. reniforme*, (66.7 and 63.3% protection for 3 h, respectively). At five hours post application only *L. javanica* alcoholic extract provided

appreciable protection (59.3%) against *An. arabiensis*. Methanol extract of *Ferronia elephantum* leaves provided 100% protection against *Ae. aegypti* at 1.0 and 2.5 mg/ cm² up to 2.14 and 4.00 h respectively. The total protection of *Ferronia elephantum* was 45.8% at 1.0 mg/cm² and 59.0% at 2.5 mg/cm² for 10 h¹¹⁷.

Eucalyptus-based products have been found effective as mosquito repellents in various studies^{64,112,96}. Schreck and Leonhardt⁹¹ evaluated Quwenling-an insect repellent product of China derived from extracts of the lemon eucalyptus plant (*Eucalyptus maculata*) and compared its repellency with deet against *An. albimanus*, *An. quadrimaculatus*, *Ae. aegypti* and, *Ae. albopictus* in laboratory tests and with *Ae. taeniorhynchus* in field tests. Cloth treated with Quwenling at 2x the dosage of deet was effective against 2 of the 4 species tested (*Ae. albopictus* 29 days, *An. quadrimaculatus* 28 days). On the skin of volunteers at 2x the dosage of deet, the duration of protection for Quwenling was significantly less compared to deet for *Ae. aegypti* and *Ae. taeniorhynchus*, but was not significantly different for *Ae. albopictus*. Both repellents were ineffective against the anopheline species. As a topically applied mosquito repellent, Quwenling, had a shorter duration of effectiveness than deet. Moore et al²⁵ reported up to 96.8% protection against *An. darlingi* using a eucalyptus based repellent as against 84.8% protection by deet. Eucalyptus and deet provided higher protection than 2% neem oil. Trigg¹¹² reported complete protection for 6-7 h against malaria vectors *An. gambiae* and

An. funestus using a eucalyptus based insect repellent with active ingredient p-methane 3,8 diol.

Palsson and Jaenson⁷⁸ collected data on plant species and plant derived products or methods used by people to reduce mosquito biting activity in Guinea Bissau and identified the potential plants for mosquito repellent activity. Fresh or smouldering *Hyptis suaveolens* Poit and smoke produced by the bark of *Daniellia oliveri* Rolfe, *Elaeis guineensis* Jacq, seed capsules of *Parkia biglobosa* Benth, leaves of *Azadirachta indica* and Eucalyptus sp. and fresh *Ocimum canum* Sims and *Senna occidentalis* L. were identified as showing mosquito repellent activity. The repellent activity of these plants was compared with that of two commercially available mosquito repellents. In the first experiment, smouldering *H. suaveolens*, fresh *H. suaveolens* burning of the bark of *D. oliveri* and smoke of the leaves of eucalyptus showed 85.4, 73.2, 74.7 and 72.2% repellency respectively. In the second experiment smouldering *H. suaveolens*, fresh *H. suaveolens*, burning of the bark of *D. oliveri*, smoke of the leaves of *A. indica*, smoke of the infructescence of *E. guineensis*, fresh *O. canum* and fresh *S. occidentalis* showed 83.6, 66.5, 77.9, 76.0, 69.0, 63.6 and 29.4% repellency respectively. All the products tested, except *S. occidentalis* were significantly more effective than the negative control.

LIVE PLANT AS MOSQUITO REPELLENT:

Most of the studies carried out so far have shown repellent effect of plant-based products derived from various plants, but there is no report of any live-intact plant showing repellent action against mosquitoes. However, a recent study has shown that certain plants such as *Lantana camara* and *Lippia uckambensis* repel *An. gambiae* mosquitoes (with an average of 39.7 and 32.4% protection) from human baits in an experimental plant house⁹⁹.

Considering the wealth of floral and plant based resources available in India it was considered worthwhile to select and screen some of the indigenous plants for mosquito Larvicidal properties. The present study has been carried out to determine the mosquito Larvicidal activity of following five indigenous plants against the larvae of *Culex quinquefasciatus*, a principle vector of filariasis in standard laboratory conditions.

PLANTS:

JATROPHA (Euphorbiaceae)

Jatropha curcas L.

[Common Physic Nut; Purging Nut]

Ver. Name.: B. & H.- Bagbherendra, Pahari Erand; Kan.- Adalu Haralu
Dodda haralu; Mal.- Kattayanaku; S.- Kaneranda; Tam.- Kaatu
Amanaku; Tel.- Adavi Amadumu.

Dist: Native of tropical America; occurs almost throughout India and in
Andaman Islands in a semi-wild state.

Use: Nut- purgative; plant – in scabies, eczema, ringworm; twig- as
toothbrush in a swollen gums.

Biolog. Act: Seed-highly toxic; aerial part CNS depressant, diuretic.

Chem. Const.: Seeds contain phorbol derivatives. Plant also contains
curcosones and lathyrane diterpenes.²

JATROPHA (Euphorbiaceae)

Jatropha curcas L.



RICINUS (Eurhorbiaceae)

Ricinus communis L.

[Castor-oil Seed]

Ver. Name; Ass. & B.- Bherenda; B.-Eri; Guj.- Diveli; H. & Mar.- Arandi
Mal.-Avanakka; Or.- Jada; S.-Eranda; Tam.- Amanakku; Tel.
Erandamu.

Dist.: Cultivated and or wild throughout India.

Use: Oil from seed – antirheumatic; oil from seed and young leaf
purgative; castor oil gel- in dermatosis and eczema.

Biolog. Act.: Root and stem – antiprotozoal, anticancer; root, stem and
leaf – hypoglycaemic; leaf- antiviral; seed- diuretic.

Chem. Const.: Coat of beans yielded lupeol and 30- norlupan-3 β -ol-20
one ; roots, stems and leaves contain several amino acids ; flowers
afforded apigenin, chlorogenin, rutin, coumarin and hyperoside. Castor
oil is constituted by several fatty acids².

RICINUS

Ricinus communis L.



Pongamia (Papilionaceae)

Pongamia pinnata (L.) Merr.: syn. *Derris indiaca* (Lam) Bennett

Pongamia glabra Vent. [Pongam Oil Tree; Karanj; Indian Beach
Hungay Oil Tree]

Ver. Name: Ass.- Karchaw; B.- Dahur karanja; Bo., Guj., H., Mar. & P.
Karanj; Delhi- Papri; Kan.- Hongae; Mal.- Pugu, Punnu, Pungam; Or.
Koranjo; Tam.- Ponga, Punngu; Tel.- Gaanuga, Pungu, Kanuga
Krangu.

Dist.: Throughout India, up to 1200 m.

Use: Root- in scrophulous enlargements; Leaf.- carminative
antidiarrhoeal, bechic, in dyspepsia , as preservative for books and
stored grains; stem- bark- in bleeding piles, beri-beri; rind of pod and
seed – tonic, febrifuge, in whooping cough, bronchitis; seed oil
(pongam oil)m – used internally as a cholagogue, stomachic, in
dyspepsia with sluggish liver, used for external applications in scabies
herpes, sores, leucoderma and other skin diseases, applied locally
over chest in pneumonia, cold, chest pain; root and leaf -
antigonorrhoeic, in foul ulcers; leaf and seed- antileprotic; leaf and
seed oil-antirheumatic. Use of karanja oil as a synergist-cum-solvent
for chlorinated insecticides and that of the seed cake as a cheap cattle
feed is suggested.

Biolog. Act.: Essential oil from leaves- antibacterial, antifungal; seed oil-roachicide; karanjin-insecticides; leaf-nematicide.

Chem.Const.: The tree is rich in flavonoids and related compounds. These include simple flavones, furanoflavonoids, chromenoflavones, chromenochalcones, coumarones, flavones glucosides, sterols, triterpenes and a modified phenylalanine dipeptide. Thus seeds and seed oil gave karanjin, pongamol, pongapin and kanjone: seeds in addition gave lanceolatin B, *iso*-pongaflavone and pongol; seed oil also contained *iso*-pongachrome and pongaglabrone. glabrachalcone (a chromenochalcone) was also isolated from the seed oil, which also gave a number of fatty acids-oleic and lino-lenic acids being the major constituents. The heartwood gave chromenochalcones-lanchocarpin, pogachalcone I, pongachalcone II, glabrachromene II and glabrachromene and flavones-ponga-glabrone, kanugin (I), pongachromene and desmethoxykanugin, karangin, pongapin, desmethoxykanugin (II). Stem-bark contained flavones and furanoflavones-karanjin, pongapin, tetra-O-methoxykanugin, kanugin, pinnatin, tetra-O-methylfisetin (III), gamatin, 5-methoxyfurano (2'',3'':7,8) flavone and 5-methoxy-3', 4'-methylenedioxyfurano-flavone and a furanodiketone named glabra I and a furanoflavone-glabra-II,I,II,III and pongachromene were present in roots also. Leaves gave glabrachromenes I and II, karanjin, kanjone, 8-methoxyfurano (4'',5'':6,7) flavone and 7-methoxyfurano (4'',5:6,5) flavone as well as 3'-methoxypongapin¹. The

flowers yielded hydroxyfuranoflavones-pongalabol, iso-pongalabol and its 6-methoxy derivative; two furanoflavones-5-methoxy-3',4'-methylenedioxyfuran (8,7:4'',5'') flavone: two simple flavones- II, III; ϵ -chromenoflavanone-ovalichromene B: two triterpenes-cycloart-23-en-3 β ,25-diol and aurantiamide acetate (a modified phenylalanine dipeptide)².

Pongamia (Papilionaceae)

Pongamia pinnata L.



STRYCHNOS (Strchnaceae)

Strychnos nux-vomica L.

[Snake-Wood;Nux-Vomica: Strychnine Tree]

Ver. Name: B. & H.- Kuchila;B.-Thalkesur,Bo.-Jharakatachura, Kajra Kara; Guj.-Kuchla; H.-Bailewa, Chibbiinge; Kan.-Hem Mushti, Ittangi Kanjira; Kon.- Kazro; Mal.-Kanjiram; Mar.-Jharkhatchura, Kara, Or Kachilal; P.-Kagophale, Hubuljarab; S.Chipita, Dirghapatra, Karaskara Visha Mushu Tam.-Etti, Kagodi, Kanjiriai; Tel.-Mushti, Ushidi, Or. Kachila; Urd.-Azaraki, Kachala.

Dist: Throughout tropical India and monsoon forests up to 360 m.

Use: Leaf- as poultice, in sloughing wounds and ulcers; root and root bark-febrifuge, anticholerin; seed – stimulant, febrifuge, emetic insecticidal, for scalp, in colic; stem-bark and Wood – antiepileptic antidyspepsia; fruit – diurtic , emmenagogue, appeptiser, tonic antiparalytic, astringent, antipyretic, antirheumatic, diuretic, in jaundice, leucoderma, diseases of blood, piles, ulcers, anaemia lumbago ringworm.

Biolog. Act.: Leaf and fruit- spasmolytic. Ouaternary alkaloid extract c root-bark possecess muscle relaxant activity.

Chem. Const.: Plant is rich in alkaloids. Root and seeds contain alkaloids- brucine, strychnine, pseudostrychnine (3-hydroxy-brucine), pseudostrychnine (3-hydroxystrychnine), β -colubrine (vomocine) and *iso*-strychnine (novacine). Root- bark also contain 4-hydroxy-3-methoxystrychnine, 4-hydroxy-strychnine, nor-macusine B, 16-epi-O-metrsylmacusine B, nor-melinine-B, 10-hydroxy-11-methoxystrychnine and 12-hydroxy-11-methoxystrychnine. Seeds also contain α -colubrine, 3-hydroxystrychnine, bromobartenylpalmate, 16-hydroxy- β -colubrine and 3-methoxycajine have also been reported from seeds. Leaves contain *nor*-macusine B, *iso*-strychnine, 19,20-dihydrostrychnine, 12-hydroxystrychnine, 12-hydroxy-11-methoxystrychnine, strychnine Nb oxide, 12-hydroxy-11-methoxystrychnine Nb oxide, 10,11-dimethoxystrychnine Nb oxide, 3-hydroxystrychnine, 3,12-dihydroxystrychnine, 3-hydroxy-10, 11-dimethoxystrychnine and vomocine. Plant also contains prestrychnine. Brucine and Strychnine are also present in stem – bark and leaves (Dictionary of Indian medicinal plants. Published by director, Central Institute of medicinal and aromatic plants.- Akhtar Husain, O P Virman et. al.)²

STRYCHNOS (Strchnaceae)

***Strychnos nux-vomica* L.**



Eichhornia (Pontederiaceae)

Eichhornia crassipes

[Water hyacinth, water orchid]

Ver. Name: Jal parni, Water hyacinth, Water orchid

Dist: Throughout Central America and some parts of India.

Use: plant is used for aquatic pollution control, and it is planted as ornamental plant.

Act.: Water hyacinth can absorb heavy metals from aquatic medium.⁴⁶

Eichhornia (Pontederiaceae)

Eichhornia crassipes



MATERIALS AND METHODS

MATERIALS AND METHODS:

FIELD ACTIVITY AND SAMPLES COLLECTION:

Prevalence and Distribution of Vector in Different Parts of Mumbai:

Survey of breeding places:

A survey of breeding places was carried out in the post monsoon season of the year 2004. Initially, places accumulating polluted water were visited and observed regularly for the period of 2-3 weeks and then the sites, which were regularly treated by the BMC staff, were revisited in order to identify the perpetual breeding places of these mosquitoes. Breeding sites were identified and recorded. The time at which maximum breeding was observed (high number of larvae and egg rafts) after previous treatment was also recorded.

Establishment of stock colonies of mosquitoes in the laboratory:

Larvae collected from some of these breeding sites were brought to the laboratory for establishing stock colony using the following method for collection.

COLLECTION:

Once the site was decided, collections were made periodically with the help of a saucepan of capacity of about 400 ml. The collection was done by dipping the saucepan in the water suddenly and picking

up the water without causing any disturbance to the larvae. They were brought to the laboratory in a clean container with a perforated lid.

The larvae collected were washed with dechlorinated water using nylon filters, identified as to the species and then kept in plastic troughs for further study. Pupae were separated and kept in another jar with its mouth covered with muslin cloth, for emergence. Identification of the larvae and adults was carried out with the help of identification key provided by Barraud⁹. Table no. I shows sites which were regularly visited during the first part of the study.

Out of the many spots located and identified for constant breeding of *Cx. quinquefasciatus* two different sites were selected. They were Wadala, which is near the crack and the city with polluted water accumulations and open drainage system is being subjected to insecticidal pressure because of regular treatment by the BMC and Borivali, situated at a distant end of the city with different ecological set up than the spot at Wadala. It differs from Wadala because it is surrounded by agricultural land as against total human habitation around the Wadala site.

After the selection of sites the collection of samples from these areas was regularly carried out, i.e. every fortnight. The temperature and pH of the water were also recorded at the same time, at the collection sites. The larval collections were made in the early morning to avoid sinking of larvae due to heat of the sun. Adult collections were also made on the same day in the early morning hours.



DISSECTION:

The female mosquito is killed with chloroform and the legs and wings cut from the body which is placed on the slide around the abdomen of the mosquito, and under a X10 lens clean cuts are made to separate head, thorax and abdomen. The severed head is transferred to a separate drop of saline and held in position with a dissecting needle while another fine needle is used to separate the gutter-shaped labium from the other part of the proboscis. If infective filarial worms (about 2 mm long) are present they will emerge from the labium into the saline.

The remainder of the head and also the thorax and abdomen are transferred to fresh drop of saline and carefully teased apart under the high power of a dissecting microscope and examined for immature filarial worms.

Careful examination and staining (Giemsa) of the filarial worms may be necessary to determine whether they are those of *Wuchereria bancrofti*, *Brugia malay* or *Brugia timori*, or filarial worms of animal origin and therefore not of medical importance.^{14,122}

SCREENING OF PLANTS FOR LARVICIDAL ACTIVITY:

SELECTION OF PLANTS:

Review of literature on plant-based insecticides was done and following plants were selected on the basis of their usefulness and medicinal properties. These plants are very common throughout India.

	Plant Name	Family	Part Used
I)	<u><i>Ricinus communis</i></u>	Euphorbiaceae.	Seeds
II)	<u><i>Jatropha curcas</i></u>	Euphorbiaceae.	Seeds
III)	<u><i>Pongamia pinnata</i></u>	Legiminosae.	Seeds
IV)	<u><i>Strychnos nuxvomica</i></u>	Loganiaceae.	Seeds
v)	<u><i>Eichhornia crassipes</i></u>	Pontederiaceae.	Leaves

EXTRACTION PROCEDURE:

The plant material was bought from the different places. *Jatropha curcas* seeds, *Pongamia pinnata* seeds, *Ricinus communis* seeds were collected from fields in Karad, Dist. Stara, Maharashtra, India. *Strychnos nux-vomica* seeds were brought from the local market of Mumbai. Leaves of *Echornia casisipes* was collected from the Rankala lake, Kolhapur, Maharashtra, India. Plant material was shade-dried and ground and extracted by standard Soxhlet extraction method, with the solvent Methanol, Hexane and Chloroform.

All the extracts were then concentrated by solvent evaporation at their respective boiling point, on the water bath.

SCREENING OF MOSQUITO LARVICIDAL ACTIVITY:

Mosquito larvicidal activity was evaluated as per the method recommended by World Health Organization (WHO).¹²³

All experimental exposures were done using 500 ml. Glass beakers. Twenty-five larvae of *Culex quinquefasciatus* were placed in 250 ml. Tap water containing various concentrations of serially diluted extracts, and held 24 hours for mortality count. Three replications were employed for each dilution of extract.

Various solvents employed for extraction of these plants like Hexane, Chloroform, and Methanol were also evaluated for their toxicity to mosquito larvae. Twenty-five larvae were used as control. They were not exposed to any toxicant and were maintained in a 500 ml beaker containing 250 ml water. Larvae incapable of rising to or submerging from the surface upon probing were considered as dead. Total number of larvae pupating and pupae emerging into adults were also recorded when possible. The percentage mortality was corrected by using Abbotts correction formula.

$$\text{Corrected Mortality} = \frac{\% \text{ Test mortality} - \% \text{ Control mortality}}{100 - \% \text{ Control mortality}} \times 100$$

RESULTS

RESULTS:

Prevalence and Distribution of *Culex quinquefasciatus* in Mumbai:

The map of Bombay indicating places where culicine breeding was observed regularly.

Table. I present the details of places surveyed and species of *Culex* found in different localities. It will be seen from the Table that *Culex quinquefasciatus* is the most predominant mosquito species in Mumbai. This mosquito was mainly found to breed in stagnant polluted water, river water, drainage water and also temporary ditches formed in the polluted areas during rainy season. The breeding was found to be highest in the open channel drains and shady areas of Borivli. The breeding sites were scattered in the slum areas as well as in well developed human residential localities. Apart from *Cx. quinquefasciatus* and *Cx. vishnui* were also found to breed in these waters, though their percentage was insignificant. Although breeding of these mosquitoes was low in the stagnant polluted waters it was comparatively higher in cleaner waters with plenty of vegetation.

Out of different areas surveyed for breeding of *Culex quinquefasciatus* two places showing continuous breeding were selected for year round studies. They were:

1. Borivli – Open drains near human residence.
2. Wadala – Shady areas near open drains and human residence.

Collections of larvae and adults were carried out at these two places. Adult collections were made in selected houses throughout the

year. Larval and adult *Cx. quinquefasciatus* densities recorded in Borivali and Wadala are enumerated in Tables II,III & IV. The data on temperature and humidity are also mentioned in the tables.

OBSERVATIONS ON LARVAL DENSITIES OF CULEX QUINQUEFASCIATUS:

Larval density was very high in these two areas, throughout the year. The temperature and humidity factors showed very little variation. However there was reduction in the densities during the rainy season especially during heavy rains. This fall in the density indicates flowing away of the larvae along with rainwater drainage. Records of water temperature and pH indicate that there were no extreme fluctuations in these parameters, which may affect the density of breeding. Temperature ranged between 20°C - 26°C and the pH of water ranged between 6 -7 i.e the water was slightly acidic in nature. These conditions are very common in Mumbai. We come across stagnant polluted drains very frequently. As a result we find that *Cx. quinquefasciatus* is prevalent throughout the city of Mumbai (table. I).

OBSERVATIONS ON ADULT DENSITIES OF CULEX QUINQUEFASCIATUS:

Adult density in each area was measured on the basis of females collected per man hour by carrying out early morning collections. Mosquitoes were found resting in the dark corners of the

houses, under the roof, on the clothing, on the walls etc. Table.IV shows that though the density of adults was high throughout the year it was particularly higher in the months of January to March and November. Biting nuisance was observed maximum in the evenings and early hours of dawn, irrespective of antimosquito measures.

This was recorded by carrying out personal visits and discussions with the residents of these localities.

SURVEY OF ADULT *CULEX QUINQUEFASCIATUS* FOR THE PRESENCE OF FILARIAL WORMS:

Table. IV shows the number of mosquitoes collected from the study areas. Adult density was high throughout the year. Infection with *Wuchereria bancrofti* larvae was not found in the mosquito population

SUSCEPTIBILITY OF *CULEX QUINQUEFASCIATUS* LARVAE TO VARIOUS EXTRACTS:

The larvicidal properties of five plant extracts have been tested against *Culex quinquefasciatus*.

It can be seen from the table-V that all extracts of *Ricinus communis*, *Jatropha curcas*, *Pongamia pinnata* and *Stemodia nuxvomica* were found to be effective at lower concentrations (0.03%) In the case of *Eichornia crassipes* there was no any significant activity Hexane extract of all the four plants were more effective than the methanol and chloroform extracts at the same concentration. Hexane

extract of Pongamia pinnata and Strychnos nuxvomica showed 100% and 97% mortality of mosquito larvae at the end of 24 hours respectively. Standard Pyrethrum extract of the same concentration showed 100% mortality of mosquito larvae.

TABLE I: SURVEY OF BREEDING PLACES:

Place	No. Of Breeding sites surveyed	Species of Culex.
Parel	Five	<i>Culex quinquefasciatus</i>
Borivali	Two	<i>Culex quinquefasciatus</i> <i>Culex vishnui</i>
Masjid Bandar	One	<i>Culex quinquefasciatus</i>
Dadar	Four	<i>Culex quinquefasciatus</i> <i>Culex vishnui</i>
Kurla	Two	<i>Culex quinquefasciatus</i> <i>Culex vishnui</i>
Bhandup	Four	<i>Culex quinquefasciatus</i>
Ghatkopar	Two	<i>Culex quinquefasciatus</i>
Chembur	One	<i>Culex quinquefasciatus</i>
Wadala	Two	<i>Culex quinquefasciatus</i>
Shivari	Two	<i>Culex quinquefasciatus</i>

TABLE II: CULEX QUINQUEFASCIATUS LARVAL DENSITY THROUGH DIFFERENT MONTHS IN WADALA:

Month	Total No. of larvae	Density	Water temp.	PH. Of water	%Culex quinquefasciatus
January	2392	199	22.7	6.4	100
February	2243	186	23	6.1	100
March	1972	164	23.5	6.2	100
April	1631	135	24.9	6.8	100
May	1482	123	25.5	6.3	100
June	796	66	24	6.8	100
July	289	24	24.5	7.2	100
August	1065	88	23.4	7.1	100
September	1307	108	24	6.9	100
October	1829	152	22	7.2	100
November	2175	181	21	6.5	100
December	2047	170	21	6.9	100

TABLE III: CULEX QUINQUEFASCIATUS LARVAL DENSITY THROUGH DIFFERENT MONTHS IN BORIVALI:

Month	Total No. of larvae	Density	Water temp.	PH. Of water	%Culex quinquefasciatus
January	1320	110	21.2	6.8	100
February	1196	99	22.9	6.9	100
March	1158	96	23.5	7.1	99
April	973	81	25.8	7	99
May	501	41	26.4	6.8	100
June	774	64	25	7	99
July	187	15	24.5	7.2	100
August	390	32	25.2	6.9	99
September	899	74	23	7	100
October	1036	86	24.7	6.8	98
November	1722	143	23	7.1	100
December	1568	130	20	6.9	100

**TABLE IV: ADULT *CULEX QUINQUEFASCIATUS*
DENSITY IN DIFFERENT MONTHS STUDY AREAS:**

Months	Wadala			Borivali		
	Total No. of adults collected	No. Time in min.	Density per man hour	Total No. of adults collected	No. Time in min.	Density per man hour.
January	100	105	57	100	115	52
February	100	95	63	100	110	54
March	100	110	54	100	100	60
April	100	85	70	50	55	53
May	100	115	52	50	60	50
June	50	65	46	50	70	43
July	50	75	40	50	65	46
August	50	70	43	50	75	40
September	50	65	46	100	95	63
October	100	95	63	100	105	57
November	100	110	54	100	110	54
December	100	100	60	100	95	63

TABLE V: MOSQUITO LARVICIDAL ACTIVITY OF PLANT EXTRACTS:

PLANT NAME	EXTRACTS	% CONC.	MORTALITY in % (after 24 hrs)
<i>Ricinus communis</i>	Methanol	0.03	64.6 $\pm$ 0.88
	Chloroform	0.03	70.6 $\pm$ 1.76
	Hexane	0.03	86.6 $\pm$ 1.28
<i>Jatropha curcas</i>	Methanol	0.03	42.6 $\pm$ 0.87
	Chloroform	0.03	61.3 $\pm$ 2.89
	Hexane	0.03	88.0 $\pm$ 1.00
<i>Pongamia pinnata</i>	Methanol	0.03	53.3 $\pm$ 1.19
	Chloroform	0.03	89.3 $\pm$ 1.19
	Hexane	0.03	100
<i>Strychnos nuxvomica</i>	Methanol	0.03	58.6 $\pm$ 2.03
	Chloroform	0.03	61.3 $\pm$ 1.19
	Hexane	0.03	97.3 $\pm$ 0.37
<i>Eichornia crassipes</i>	Methanol	0.03	N. S.
	Chloroform	0.03	N. S.
	Hexane	0.03	N. S.
Acetone	Control	2ml.	Nil
Pyrethum standard	Control	0.03ml.	100
Water	Control	-----	Nil

N. S. = Not significant

DISCUSSION

1
2
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DISCUSSION:

An understanding of the entomological parameters constitutes an important element in the study of the bionomics of a vector borne disease in a given terrain both in the presence or absence of vector control measures. Whereas, in most of the mosquito borne diseases the pathogen-vector-host relationships are relatively simple, in filariasis the epidemiological characteristics of this relationship are rather complex. For instance, the amount of exposure to infective mosquitoes is a very important factor for establishment of infection and subsequent manifestation of the disease. It has been estimated that 15,500 infective *Culex quinquefasciatus* bites would be required to produce a case of *Wucheraria bancrofti* infection (Hairston 1968). Further, the survival of infected mosquitoes as well as, the survival of infective larvae of the parasite before entering the body of the definitive host is the crucial problems faced by the parasite for its maintenance of life and perpetuation. Due to some of these considerations, control of filariasis by vector control actions aims at reduction in transmission.

In India recurrent anti mosquito measures are adapted for control of *Culex quinquefasciatus* with the immediate aim to reduce the transmission of infection by reducing the vector population. Anti-adult measures using insecticides, as indoors-residual spray have not been favoured much due to resistance problem.

In the city of Mumbai the disease filariasis is endemic. Its endemicity has been recorded by Basu et. al.¹⁰ and it has been shown

that *Cx. quinquefasciatus* is the vector for filariasis. As a consequence antilarval measures for filariasis control have been in operation for over 30 years. Likewise indoor residual sprays with different insecticides are employed for various purposes for nearly 30-40 years by MCGB and other pest control organizations. In this study specific attempt has been made to highlight various parameters related to the density of the vector and vector control action of selected plants against vector of filariasis.

As we have seen *Cx. quinquefasciatus* constitutes the major mosquito species throughout the city. Along with *Cx. quinquefasciatus* we find presence of *Cx. vishnui* in some areas of the city though their presence is insignificant. Commenting on the density of the vector Bhatia and Wattal (1951) had estimated that the total tolerated density 34 per 10 man hour. Though it was not a precise estimate in the opinion of Wattal (1976) it could be taken as an ideal yard stick as at that density, transmission of filariasis may not be possible. The 10 man hour density for assessment purpose in National filariasis control programme (NFCP) has been classified arbitrarily as low, medium and high as less than 50, 50-83 and above 83 respectively. The range of density encountered per man hour in Mumbai was between 40 to 70. The situation as regards Mumbai reveals that per man hour density is very high throughout the city and exceeds the limits drawn by NFCP for high-level infection. Thus it could be stated that despite control measures the density of vector continues to remain high and the threat of transmission persists despite persistent control measures. The

density of vector species remains high throughout the year shows very little seasonal fluctuations. Wattal (1976) suggested that following entomological parameters should be investigated in filariasis research.

- a) Correlation between the larval and adult densities.
- b) Biting density including seasonal changes.
- c) Anthropophilic index.
- d) Parity rate.
- e) Gonotrophic cycle of the vector under local conditions / seasons.
- f) Average number of 1st, 2nd, 3rd & 4th stages of filarial larvae per infected mosquito.
- g) Susceptibility of local strains to infection.
- h) Change in the ecosystem brought about by developmental activity.

A study of larval density in the study areas revealed that is a definite correlation between larval densities and adult densities. While adult and larval densities remain high throughout the year, there were specific periods when they were considerably higher. The periods showing high larval densities were the months of January, February and November, December. In addition they were continually high during summer months i.e. March and April. During monsoon period larval densities were comparatively low. Likewise adult density also showed a comparatively lower trend. The significance of these parameters is that conditions ideal for transmission of filariasis remain prevalent throughout the year. That Broadly speaking the breeding sites of the vector mosquito can be classified as peridomestic areas, in

and around the houses, like the cess pools, lid off drains from laterines and cattleshed (Borivali) and mainly the open channel drains along with the public breeding grounds like natural valley lines receiving sewage etc. During the rainy months between June and October all these breeding grounds get good and complete washing with almost daily rainfall, throughout the season. During this season the peridomestic areas (Wadala) constitute the major breeding grounds. Hence the density is relatively low. Whatever antilarval measures with larvicides are carried out are prone to be rendered ineffective by continuous and incessant rainfall.

The months following the monsoon present a different picture. The beneficial effect of monsoon on mosquito density is felt till the end of October only. When the rain stops the flow of drains which was fast and fast now slows down. All the drains which were heavily silted up due to the very uneven nature of the city are desilted and cleaned up. At this stage, particularly November onwards a phenomenal increase in *Cx. quinquefasciatus* occurs. This increase during the immediate post monsoon months is due to the following reasons. The drains and water accumulations, which were being flushed now slowdown and organic contents in them also get highly concentrated. The slowing of the drains and the increase in organic contents in them are most favorable for Culicine breeding. Thus we experience increased breeding in the subsequent months.

The temperature and humidity do not constitute a major factor in the life of *Culex quinquefasciatus* in Mumbai, because there is no

significant variation in the range of temperature and humidity in the city area. Likewise observations on the temperature and pH of the breeding water do not show any extreme variations. As a consequence the influence of factors like temperature, humidity and pH do not exert significant influence on mosquito density.

Apart from the climatic conditions, higher densities of the Vector and higher rate of breeding observed throughout the year is the result of vast ecological changes that have been taking place in the city due to developmental activities. Firstly, city limits have been extended beyond Borivali, which was a rural area till recently. The population of the city has reached 10 million mark. The city area today occupies 437 sq.km. The factors related to rapid urbanization have contributed towards creation of slums and shantytowns with ideal mosquito breeding conditions. Industrial and agricultural pollution and inadequate drainage systems have created innumerable breeding sites throughout the city. Particularly the problem has assumed enormous proportion in the suburban areas where proper drainage systems are yet to be established. These are some of the reasons for the abundance of the vector and persistence of the disease despite antimosquito measures and antifilaria treatment extended to the victims of the disease.

In addition to ecological factors it is often observed that the evaluation of antilarval and antiadult measures is not undertaken by the authorities. As a consequence it is very difficult to assess the impact of these measures on mosquitoes in their larval as well as adult stages. This is because the vector species has developed resistance to major

group of insecticides. Some of the insecticides posed grievous health hazards. Insecticides like DDT, the major weapon in our vector control strategy posed serious pollution problem to such an extent that the use of DDT was banned in many parts of the world. This prompted WHO to take up a massive screening program for evaluation of new insecticides. Large numbers of compounds were screened extensively and eventually only few compounds were found to show some promise. The world is facing the danger of running out of compounds for protecting crops and controlling the vectors of diseases.

One of the possibilities to meet this problem is to find out the cheap and effective insecticides, which will be commensurate with the economic conditions of our country. Plant based insecticides have distinct advantage over commercial organic synthetic insecticides, they are easily biodegradable. Most of the synthetic insecticides are being imported and are therefore expensive.

Some herbal products like nicotine obtained from tobacco leaves, anobasine and lupinine the alkaloids extracted from weeds *Anabasis aphylla*, rotenone from *Derris elliptica*¹⁸ and pyrethrum from *Chrysanthemum cinerifolium* flowers⁴¹ have been used as natural insecticides even before the discovery of synthetic organic insecticides³.]Present study shows that all the extracts of selected plants studied except *Eichhornia crassipes* proved to be having larvicidal activity. *Pongamia pinnata* and *Strychnos nux-vomica* proved to be most promising plants possessing larvicidal property achieving 100% and 97% mortality at 0.03 % concentration respectively. Equally

impressive results were obtained with *Jatropha curcas* and *Ricinus communis*. Throughout the course of this studies it was significantly noted that of three extract of each plant material studied. The hexane extract were showing better results from extracts in other solvent. Hexane extracts of *Pongamia pinnata* and *Strychnos nux-vomica* were more toxic than the all other extracts.

As mosquito larvicidal activity is concerned Hexane extract of *Pongamia pinnata*, *Strychnos nux-vomica*, *Jatropha curcas* and *Ricinus communis* (seeds) shows 100%, 97%, 88% and 86% respectively against the vector of filariasis

Chloroform extracts also gives prominent activity, it shows more than 60% mortality at the same concentration. Methanolic extracts of the above plants also shows more than 50% mortality at the 0.03 % concentration against *Culex quinquefasciatus* larvae under standard laboratory conditions.

In this connection all four plants being the easily cultivable and available in our country, their use as mosquito larvicides could be considered. Chemical study of these plants must be carried out for the new insecticidal compounds.

A field trial is necessary to show comparative value of these extracts in relation to current available larvicides. It will be also necessary to work out cost of material if used for field applications and how it will better in comparison to larvicides in vogue.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION:

Filariasis constitutes a major problem in the city of Mumbai. The vector *Culex quinquefasciatus* transmits filariasis. Despite persistence of the disease, no studies have been carried out on its vector and larva control by herbal products.

Considering the magnitude of the problem of filariasis. This study was undertaken to record the prevalence and distribution of *Culex quinquefasciatus* in different areas of Mumbai. Screening of some plant extracts for mosquito larvicidal activity.

In the first phase, survey of larval and adult populations of the vector was undertaken in different selected areas of Mumbai. In this study it was observed that adult density was very high and ranged between 40 - 70 per man hour. In the case of larval population density per 100 ml. of breeding water ranged between 100 to 200 depending on the type of the location and the season of the year. The study showed the prevalence of *Culex quinquefasciatus* throughout the city.

A survey of the breeding sites revealed that Culicine breeding predominantly occurred in stagnant polluted water accumulations like open channel drains, lid off drainages and temporary ditches formed after the monsoon etc. It was seen that percentage of breeding was highest in the open channel drains in all the areas.

Seasonal abundance of *Culex quinquefasciatus* was studied in selected sites located at Wadala and Borivali. These studies showed that although mosquito density per man-hour was high throughout the

year it was highest during the winter months (i.e. November to January).

Likewise larval population studies on *Culex quinquefasciatus* carried out in these areas at fixed locations brought out high larva densities throughout the year. However, higher densities were encountered during the winter months. These ranged between 108 - 199 per 100 ml. in Wdala and 99 - 143 per 100 ml. in Borivali.

Screening plant extract has been carried out to determine the mosquito larvicidal activity. Susceptibility studies of larval *Culex quinquefasciatus* were carried out with extracts of *Pongamia pinnata*, *Strychnos nux-vomica*, *Jatropha curcas* and *Ricinus communis* (seeds). It was observed that Hexane extract of these plants shows more than 80% mosquito larvicidal activity at the 0.03 % concentration which is very good activity compared to standard Larvicide. In these four plants extracts *Pongamia pinnata* and *Strychnos nux-vomica* showed prominent activity against *Culex* larvae at 0.03% concentration.

Most of the plant based products are not as effective as synthetic insecticides and do not produce fast results, their use for mosquito control in a large scale programme under epidemic conditions may not be acceptable. However, the use of indigenous plant based products by individual and communities can provide a prophylactic measure for protection against various mosquito-borne diseases. There is a need for promoting the use of herbal products through community based vector control programme.

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SYNOPSIS

**A SYNOPSIS OF THE THESIS TO BE SUBMITTED TO THE
UNIVERSITY OF MUMBAI FOR THE DEGREE OF MASTER OF
SCIENCE IN APPLIED-BIOLOGY.**

**TITLE OF THE THESIS : “EVALUATION OF SOME INDIGENOUS
PLANT EXTRACTS FOR POSSIBLE
INSECTICIDAL ACTIVITY AGAINST
VECTOR OF FILARIASIS”.**

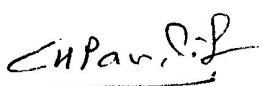
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Introduction:

Insects are responsible for incalculable harm to man in many ways. It would be no great exaggeration to say that insects have been directly or indirectly responsible for more loss of life and destruction of property than that caused by wars, floods, earthquakes, fires and famines in the history of man. Advance in civilization are producing conditions suitable for insect multiplication in many places.

The role of Arthropods-insects, ticks and mites as vector of human, animal and plant diseases, a well-established fact over the years, their increased potential and consequent resurgence of several communicable diseases have received considerable attention in recent years. It is well known that transmission by simuliids of oncocerciasis, sand flies of dermal and visceral leishmaniasis, Glossina of sleeping sickness, Culex of filariasis and encephalitis, Aedes of yellow fever, Anopheles of malaria, fleas of plague, Triatomids of chagasic disease, human louse of typhus fever and relapsing fever, Ticks of haemorrhagic fever and Mites of scrub typhus are continuing unabated in spite of our efforts to control the vectors.¹⁴

Mosquitoes are important vectors of several tropical diseases, including malaria, filariasis, and numerous viral diseases, such as dengue, Japanese encephalitis and yellow fever. In countries with a temperate climate they are more important as nuisance pests than as vectors. Lymphatic filariasis (LF) is ranked as the second most common cause of physical disability and is next only to Malaria among the debilitating vector-borne diseases.

Filariasis is a group of human and animal infectious diseases caused by nematode parasites of order Filariidea, commonly called "Filariae". The nematodes generally called filariae include several hundred species of worms parasitic in the tissue of various vertebrate hosts and several species among them are known to be the causative agents of serious diseases in man.

The filarial parasites may be classified into three main groups with respect to the habitat of the adult worms, i.e. the cutaneous, lymphatic and the body cavity. Once an individual gets infected, he may show variety of symptoms.

depends up on the species of causative organisms, for example, infection with *Wuchereria brugia* cause Lymphatic filariasis (Lymphatics), *Wuchereria bancrofti* causes bancroftian filariasis (Lymphatics), *Brugia malayi* causes Malayan filariasis (Lymphatics), *Brugia timoria* cause timorian filariasis, *Loa loa* causes calabar swelling or loasis (connective tissue), *Mansonella ozzardi* causes ozzardi filariasis, *Mansonella perstans* causes perstans filariasis, and *Mansonella streptosea* cause streptoceral filariasis.^{3,4}

In India 15 States and 5 Union Territories are endemic for bancroftian filariasis, while brugian filariasis is prevalent only in certain regions in Kerala and in some isolated foci in six other States. While an estimated 450 million population are at risk of infection in this Country, these are 31.26 million with microfilariemia, 7.44 million with lymphodema and 12.88 million with chronic manifestation, acute attacks of adenolymphangitis (ADL) are common during the course of this illness. Thus, it is reported that a total of 40.65 million ADL episodes occur annually in the affected population. While these acute attacks by themselves cause considerable short-term disability, they result in significant long term incapacity as well by worsening the underlying lymphoedema. Estimates of health burden suggest that 2.06 million disability adjusted life years (DALYS) are lost in India, due to this disease and the annual wage loss is estimated to be US \$ 811 million.⁶

In India, *Cx. quinquefasciatus* is the principal vector of bancroftian filariasis. It is the most common mosquito prevalent in both urban and rural areas of India.¹³

About 3100 species of mosquitoes belonging to 34 genera arranged in three subfamilies, Toxorhynchitinae, Anophelinae (anophelines) and Culicinae (culicines).² The genus *Culex* comprises 466 species (Busvine 1980). As many as 250 species are reported from India (Dash and Behura, 1985). Several species of the subgenus *Culex* act as vectors of a number of communicable diseases, of which *Culex pipiens* Linn, *Culex comlex* and *Culex vishnui*, group theobal are 'very important'.⁴

Culex quinquefasciatus mosquitoes are highly domestic and are widely distributed throughout the world (Sasa, 1976). The females are night biters and usually highly anthropophilic. The larvae of the species breed usually in open ditches, sewage pools and artificial containers of polluted waters. They occur in a complex form, and are grouped, under several geographical or physiological subspecies, among which the following are of practical importance, these are:

- A) *Cx. P. pipiens* Linnaeus.
- B) *Cx. Quinquefasciatus* say.
- C) *Cx. P. pallens* coquillette.
- D) *Cx. P. molestus* forskal.⁴

Vector control measures, when practicable, are carried out in addition to drug treatment. 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. Insecticides are frequently used to eradicate the mosquitoes. Insecticides are chemically synthesized compounds, as a gas powder and sprays that can be applied on the surface of the plants.¹ Insecticides classified on basis of their origin:

I) Synthetic insecticides:

Synthetic insecticides are chemically synthesized compounds. There are many drawbacks in the use of synthetic insecticides for their use in vector control. Besides the adverse environmental effects that these may cause, the major vectors have become resistant to these compounds. Further these leave chemical residues that are harmful and when biomagnified and cause great deterioration of quality of our environment. The examples of synthetic insecticides are chlorinated hydrocarbons and insecticides of phosphoric acid ester group.

Disadvantages of synthetic insecticides:

1. Emergence of mosquito strains resistant to these insecticides.
2. Toxicity to human beings, damage to aquatic life through leaching of the insecticides into water reservoirs, environment and food chain.

3. Resistance to biodegradation, which causes accumulation of the metabolites in biological system.
4. High cost of production.
5. Destruction of other insects, which are useful thereby causing economic loss and ecological imbalance.

Due to above mentioned drawback, a search for agents that are environmentally safe, biodegradable and target specific insecticides has been directed towards plant kingdom.

II) Plant origin insecticides:

Natural products obtained from plants, animals and microorganisms provide potential alternative to the current pest control agents because these have biodegradable constituents (Swain 1977, Wink 1993). These are target specific, comparatively nontoxic products and potentially suitable for use in integrated pest management programs. Examples of the insecticides from plant origin are nicotine, rotenone, cinerines and pyrethrins. In recent years products based on the Neem tree have attracted wide use.

I) Biocontrol:

Biocontrol has two practices one is a naturally occurring process and another is a purposeful use of one organism to control the other.

Biocontrol can be achieved by following ways:

1. Classical Biocontrol.
2. Inundated release.
3. The Biopesticide approach.
4. Conservation Biocontrol.^{15, 16}

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. Some herbal products such as nicotine obtained from tobacco leaves, *Nicotiana tabacum*, anabasine and lupinine. The alkaloids extracted from Russian weed *Anabasis aphylla*, rotenone from *Derris elliptica* and Pyrethrins from *Chrysanthemum cinerifolium* flowers have been used as natural insecticides even before the

discovery of synthetic organic insecticides. However, the discovery, development and use of synthetic organic chemicals with persistent residual action not only overshadowed the use of herbal products against mosquitoes but also became the major weapon for mosquito control. Since the discovery of DDT, mosquito control approach has been almost completely based on synthetic organic insecticides. But the extensive use of synthetic organic insecticides during the last five decades has resulted in environmental hazards and also in the development of physiological resistance in major vector species. This has necessitated the need for search and development of environmentally safe, biodegradable, low cost, indigenous method for vector control, which can be used with minimum care by individual and communities in specific situation.¹

Considering the wealth of floral and plant based resources available in India it was considered worthwhile to select and screen some of the indigenous plants for mosquito Larvicidal properties. The present study has been carried out to determine the mosquito Larvicidal activity of five indigenous plants against the larvae of *Culex quinquefasciatus*, a principle vector of filariasis in standard laboratory conditions.



Aims and Objectives:

- 1) Prevalence and distribution of *Culex quinquefasciatus* in different parts of Mumbai.
- 2) Dissection of adult female mosquito for *Wuchereria bancrofti* larvae.
- 3) Extraction of the powdered material of the plants has been carried out using increasing polar solvents and evaluates their mosquito Larvicidal activity against *Culex quinquefasciatus*.

Materials and Methods:

1) Prevalence and distribution of Culex Quinquefasciatus in different parts of Mumbai:

A survey of breeding places was carried out in the past monsoon period of 2004. Breeding sites were identified and recorded.

After identifying the breeding sites two sites, known to support the breeding year around, were selected for studies on seasonal abundance (Wadala & Borivali).

i) Larval Studies:

Larval collections were made with the help of a saucepan of 300 ml. capacity and larval density was measured on the basis of number of larvae/100ml. sample. A record of pH & temperature of the breeding source was maintained.

ii) Adult Studies:

Adult collections were carried out at selected sites in the house situated near the breeding places. Collection of adult was carried out during the early morning period. A fixed number of blood fed females were collected and time taken for collection was recorded. Density / man-hour was calculated, 50% of the Collected females were dissected and examined for presence of *Wuchereria bancrofti* larvae.

Collection of larvae and adults was carried out once in a month for a period of 12 months.^{12,13}

2) Plant extraction and there larvicidal activity:

	Plant Name	Family	Part Used
I)	<u><i>Ricinus communis</i></u>	Euphorbiaceae.	Seeds
II)	<u><i>Jatropha curcas</i></u>	Euphorbiaceae.	Seeds
III)	<u><i>Pongamia pinnata</i></u>	Legiminosae.	Seeds
IV)	<u><i>Strychnos nuxvomica</i></u>	Loganiaceae.	Seeds
v)	<u><i>Eichhornia crassipes</i></u>	Pontederiaceae.	Leaves

The above plants material was extracted with solvents like, Hexane Chloroform and Methanol in Soxhlet and extracts were concentrated on the water bath. Extract were subjected to their activity against mosquito larvae as per method recommended by World Health Organization.^{7,2}

3) Mosquito larvicidal testing:

Mosquito Larvicidal (*Culex quinquefasciatus*) activity of various extracts were studied as per the WHO specifications. Twenty-five larvae of third or fourth stage were placed in 500 ml. beakers containing 250 ml. of tap water known amount of various extracts dissolved in 2 ml. of acetone were added to these beakers and the mortality was observed. Acetone and water controls were also kept simultaneously. Each experiment was done in triplicate. Larvae incapable of rising to or submerging from the surface up on probing were considered as dead. The percentage mortality was corrected by using Abbotts correction formula as follows,^{2,10}

$$\text{Corrected mortality} = \frac{\% \text{ Test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

Result and Discussion:

1) Prevalence and distribution of *Culex quinquefasciatus* in Mumbai:

Culex Quinquefasciatus is the most predominant vector species in Mumbai. This mosquito was mainly found breed in drain waters, stagnant polluted waters, and river water, in temporary formed ditches and water reservoirs rich in organic pollutants and sewage. Its prevalence was high throughout the year. Although the breeding was detected throughout the year larval density was low during rainy season. (Table. I,II,III,IV)

In the case of adults the density was high throughout the year. Infection with *Wauchereria bancrofti* larvae was not found in the mosquito population.

2) Susceptibility of *Culex quinquefasciatus* larvae to various extracts:

The larvicidal properties of five plant extracts have been tested against *Culex quinquefasciatus*.

It can be seen from the table-V that all extracts of *Ricinus communis*, *Jatropha curcas*, *Pongamia pinnata* and *Strychnos nuxvomica* were found to be effective at lower concentrations. In the case of *Eichornia crassipes* there was no significant activity. Hexane extract of all the four plants were more effective than the methanol and chloroform extracts. Hexane extract of *Pongamia pinnata* and *Strychnos nuxvomica* showed 100% and 97% mortality of mosquito larvae at the end of 24 hours respectively. Standard Pyrethrum extract of the same concentration showed 100% mortality of mosquito larvae.

Conclusion:

The findings of the present investigation revealed that the *Pongamia pinnata* and *Strychnos nuxvomica* possess remarkable larvicidal activity against *Culex Quinquefasciatus*. The identification of this potential larvicidal plant from the local flora will definitely open the field for further research on this bio pesticide.

Table I: Survey of breeding places:

Place	No. Of Breeding sites surveyed	Species of Culex.
Parel	Five	<i>Culex quinquefasciatus</i>
Borivali	Two	<i>Culex quinquefasciatus</i> <i>Culex vishnui</i>
Masjid Bandar	One	<i>Culex quinquefasciatus</i>
Dadar	Four	<i>Culex quinquefasciatus</i> <i>Culex vishnui</i>
Kurla	Two	<i>Culex quinquefasciatus</i> <i>Culex vishnui</i>
Bhandup	Four	<i>Culex quinquefasciatus</i>
Ghatkopar	Two	<i>Culex quinquefasciatus</i>
Chembur	One	<i>Culex quinquefasciatus</i>
Wadala	Two	<i>Culex quinquefasciatus</i>
Shivari	Two	<i>Culex quinquefasciatus</i>

Table II: Culex quinquefasciatus larval density through different months in Wadala:

Month	Total No. of larvae	Density	Water tem .	PH. Of water	% Culex quin uefasciatus
January	2392	199	22.7	6.4	100
February	2243	186	23	6.1	100
March	1972	164	23.5	6.2	100
April	1631	135	24.9	6.8	100
May	1482	123	25.5	6.3	100
June	796	66	24	6.8	100
July	289	24	24.5	7.2	100
August	1065	88	23.4	7.1	100
September	1307	108	24	6.9	100
October	1829	152	22	7.2	100
November	2175	181	21	6.5	100
December	2047	170	21	6.9	100

Table III: Culex quinquefasciatus larval density through different months in Borivali:

Month	Total No. of larvae	Density	Water temp.	PH. Of water	% Culex quinquefasciatus
January	1320	110	21.2	6.8	100
February	1196	99	22.9	6.9	100
March	1158	96	23.5	7.1	99
April	973	81	25.8	7	99
May	501	41	26.4	6.8	100
June	774	64	25	7	99
July	187	15	24.5	7.2	100
August	390	32	25.2	6.9	99
September	899	74	23	7	100
October	1036	86	24.7	6.8	98
November	1722	143	23	7.1	100
December	1568	130	20	6.9	100



Table IV: Adult *Culex quinquefasciatus* density in different months study areas:

Months	Wadala			Borivali		
	Total No. of adults collected	Time in min.	Density per man hour	Total No. of adults collected	Time in min.	Density per man hour.
January	100	105	57	100	115	52
February	100	95	63	100	110	54
March	100	110	54	100	100	60
April	100	85	70	50	55	53
May	100	115	52	50	60	50
June	50	65	46	50	70	43
July	50	75	40	50	65	46
August	50	70	43	50	75	40
September	50	65	46	100	95	63
October	100	95	63	100	105	57
November	100	110	54	100	110	54
December	100	100	60	100	95	63

Table V: Mosquito Larvicidal activity of plant extracts:

PLANT NAME	EXTRACTS	% CONC.	MORTALITY in % (after 24 hrs)
<i>Ricinus communis</i>	Methanol	0.03	64.6 ± 0.88
	Chloroform	0.03	70.6 ± 1.76
	Hexane	0.03	86.6 ± 1.28
<i>Jatropha curcas</i>	Methanol	0.03	42.6 ± 0.87
	Chloroform	0.03	61.3 ± 2.89
	Hexane	0.03	88.0 ± 1.00
<i>Pongamia pinnata</i>	Methanol	0.03	53.3 ± 1.19
	Chloroform	0.03	89.3 ± 1.19
	Hexane	0.03	100
<i>Strychnos nuxvomica</i>	Methanol	0.03	58.6 ± 2.03
	Chloroform	0.03	61.3 ± 1.19
	Hexane	0.03	97.3 ± 0.37
<i>Echornia crassipes</i>	Methanol	0.03	N. S.
	Chloroform	0.03	N. S.
	Hexane	0.03	N. S.
Acetone	Control	2ml.	Nil
Pyrethum standard	Control	0.03ml.	100
Water	Control	-----	Nil

N. S. = Not significant

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