

POTENCY OF LARVICIDAL PROPERTIES OF PLANT
EXTRACTS AGAINST MOSQUITO LARVAE UNDER
LABORATORY CONDITIONS

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.) VIJAYALAKSHMI KAMATH

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MUMBAI

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NOVEMBER (2002)**

“STATEMENT UNDER THE UNIVERSITY RULE NO. 2190.

As required by the University Regulation No.2190, I wish to state that the work embodied in the thesis titled “Potency of larvicidal properties of plants extracts against mosquito larvae under laboratory condition” forms my own contribution to the research work carried out under the guidance of Dr. (Mrs.) Vijayalakshmi R. Kamath, M.Sc., Ph.D., Department of Zoonosis, Haffkine Institute for Training Research and Testing, Parel, Mumbai. The work in this thesis has not been submitted for any other degree of this or any other university.

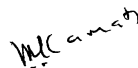
Wherever, the references have been made to previous work of others, it has been clearly indicated as such and included in the ‘ Bibliography ’.



(Ms.Suchita T. Desai)

Signature of the candidate

Certified that the above statement made by the student is correct.



(Dr. (Mrs.) Vijayalakshmi R. Kamath)

Signature of the Guide

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INTRODUCTION

Zoonotic diseases represent a serious threat to the health and well being of human population. There exists a close relationship between man and animals in several diseases, which are intercommunicable between them. Out of about 300 diseases identified as intercommunicable between man and animals, about 100 are of major public health importance¹. Insects are the chief competitors with man for the domination of this planet. Insects destroy man's growing crops and they are responsible for the spread of nearly all the great epidemic fever. Vector-borne disease takes a heavy toll of human life. About one million people die and few hundred millions suffer from insect-borne diseases². Mosquitoes are among the worst of human pests. Mosquitoes come under the family Culicidae, of the order Diptera. There are about 3,200 species of mosquitoes belonging to 34 genera (White 1987)³. Mosquitoes having worldwide distribution and they occur throughout the tropical and temperate regions. Apart from being the vectors, several species of mosquitoes are also voracious biters and cause tremendous annoyance to man. The most important mosquitoes species, which act as vectors, are *Culex*, *Mansonia*, *Aedes* and *Anopheles*, which transmit malaria, dengue, filariasis, and yellow fever.

1. Malaria: -

Malaria is one of the major public health problems in India accounting for millions of new cases each year and affecting mainly poor, rural, tribal and peri-urban people, more so infants and pregnant mother⁴. Even after hundred years of the discovery of mosquito vector of malaria by Sir Ronald Ross in 1897, still continuing research on malaria amply demonstrates concern towards the disease and commitment for development of effective operationally feasible, cost effective and sustainable malaria control tools⁵. Globally, malaria clinical cases are reported as 300-500 million and 1.5-2.7 million deaths annually. The increasing death of environmental change is dramatically changing malaria distribution pattern at local as well as global scales. Malaria situation is worsening with large-

scale epidemics and increasing mortality⁶. Malaria is usually transmitted by the bite of infected female *Anopheles mosquitoes*. Transmission of malaria is affected by climate and geography, and often coincides with the rainy season.

2. Dengue/Dengue Haemorrhagic Fever (DHF) -

Dengue is a mosquito-borne infection that in recent years has become a major international public health concern. Dengue is an arboviral illness commonly transmitted by *Aedes aegypti* mosquitoes. The geographical distribution has greatly expanded and the number of cases has increased dramatically in the past 30 years. It is now endemic in more than 100 countries and threatens the health of about 40% of the world's population (2.5 billion people), particularly in tropical and sub-tropical regions and predominantly in urban and periurban areas. Over 1.2 million cases were reported to WHO in 1998, the greatest number ever for a single year. There are an estimated 50 million infections annually, including 400,000 cases of DHF, a potentially lethal complication first recognized during the 1950s and today a leading cause of childhood mortality in several Asian countries. At present, the only method of controlling or preventing dengue and DHF is to combat the vector⁷.

3. Filariasis -

Lymphatic filariasis of man is due to infection by three lymphatic dwelling filarid, viz *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* transmitted principally by *Culex quinquefasciatus*, *Mansonia* species and *Anopheles barbirostris* mosquitoes respectively. Infection by *B. timori* does not occur in India. More than 1.1 billion people, approximately 18% of the world population, live in areas at risk of infection with lymphatic filarial parasites. Approximately 120 million people in tropical and subtropical area of the world are infected. Of these infections, 90% are caused by *bancrofti* and 10% by *B. malayi*. Almost 25 million men suffer from genital disease (most common hydrocele), an estimated 15 million people the majority of them women – have lymphoedema or

elephantiasis of the leg. The magnitude of infection in children has become much better understood in recent years; indeed, most infections appear to be acquired in childhood, with a long period of subclinical manifestations of adults⁸.

4. Yellow-Fever -

Yellow fever is a viral disease transmitted by infected mosquitoes. In 1998, a total of 303 cases with 117 deaths were reported to WHO; 91% of the cases occurred in the America and 9% were reported from Africa. In 1999, 208 cases and 101 deaths were reported. Of these, all but one case originated in the Americas. During 1998 - 99, 11 countries reported cases of yellow fever to WHO. In Africa, inhabitants of 33 countries are at risk. In the America, yellow fever is endemic in 10 South American countries and 1 Caribbean Island. The majority of the cases during 1998 - 99 were reported from Bolivia, Brazil and Peru⁹.

Mosquitoes breed in practically all forms of water. Any place where water accumulates for a long time can be their breeding place. Hence, it is common to observe the breeding of mosquitoes in all types of weather and conditions, however, adverse. About three thousand species of mosquitoes have been described world-wide.

During the life cycle, mosquitoes undergo a complex metamorphosis. Egg, larva, pupa and adult are the four stage of the mosquito lifecycle. Culencine female mosquito lays eggs in raft in water. First stage larvae emerge when egg embryonation is completed within few days. Culencine larvae hang suspended diagonally from water surface by means of a siphon. Larvae moult four times within seven days and form pupae. It is a very short stage and within two-three days an adult emerges from pupa. (Gillott cedric 1980 Entemology)¹⁰.

Managing the vector control could best control the vector-borne diseases. The mode of control could be environmental, chemical, biological, genetic and

hormonal. In India, besides some physical and environmental methods adopted, the most common method tried to control vector borne diseases in human is by reducing the vector population through use of insecticides which include Chlorinated hydrocarbons, Organophosphates, Carbamates, etc.

1. Organochlorine –

Among the first of the highly effective insecticides, the best-known example of this group is Dichloro Diphenyl Trichloroethane (DDT). DDT is well known for its use as an insecticide and is called as a "Savior of Mankind" because of its efficacy in controlling insect-borne disease in World War II. DDT was the most widely used of the new chemical pesticides. By stopping the transmittal of typhus through fleas, DDT saved millions of lives during the war. It was extensively used during the Second World War to control malaria. Zeidler synthesised it in 1874 but Muller (West and Campbell 1950) discovered its insecticide properties in 1939. DDT occupied a significant position in the field of public health and agriculture.

As an organic synthetic insecticide of the chlorinated hydrocarbon group, DDT was popular due to its low cost, high availability, potency and apparent safety. Most of the DDT sold to the World Health Organisation cost less than 22 cents per pound. After the Second World War, DDT production skyrocketed. Between 1945 and 1955, the production increased 125 million to 600 million pounds. The government supported use of DDT; the chemical industry advocated it aggressively. The Public Health Department held demonstrations of DDT's efficacy and safety. As a result of this positive publicity, public places and private backyards were also sprayed.

Thus, DDT has been used in India and many tropical countries as an insecticide for nearly 30 years in malaria eradication programmes; the control of agricultural pests and for preserving food grains against insect damage¹¹.

It was observed that, DDT is slowly metabolised in biological systems and is very resistant to biodegradation. DDT metabolites accumulated in food chain through biological concentrations in the ecosystem and then entered the human body and got accumulated in adipose tissue and liver.

The other chlorinated ethane derivatives are BHC, Dieldrin, Lindane, and Mirex etc.

2. Organophosphates -

Organophosphates are the most commonly used insecticides. The development of organic phosphorus insecticides had its origin in Germany. One feature of the organic phosphates that has led to their wide usage in agriculture and in the home is that they are much less persistent in the environment than the Organochlorine, such as DDT. The Organophosphates are considerably more toxic to vertebrates than the Organochlorine.

Organophosphate insecticides can be effective whether they are ingested by the pest or are absorbed through the cuticle of the insect. The Organophosphates are used against a wide array of insects and mites.

The toxic action of Organophosphate compounds is the same for insects and mammals. Mode of action is the inhibition of the enzyme cholinesterase crucial for normal nervous impulse transmission. Members of this group of pesticides are cholinesterase inhibitors and among the most acutely toxic of all the pesticides in current use. They are highly toxic by all routes of exposure. The avian toxicity of Organophosphate compounds varies from slightly toxic to highly toxic.

Organophosphate chemicals that are generally nonsystemic and used as to contact insecticides include Diazinon, Malathion, Parathion, Fenthion etc. Parathions are highly toxic to mammals and used extensively on field, crops and vegetables etc. Various commercial modifications of Parathion are used on crops close to harvest and to control insects such as mosquitoes and flies that affects public health. Commonly used Organophosphates in mosquito control are Malathion and Fenthion¹²

2. Carbamates -

Carbamates were developed as commercial pesticides in the 1950s. Although the members of this family of chemicals are effective as insecticides, herbicides and fungicides, they are most commonly used as insecticides. The Carbamates have replaced Chlorinated hydrocarbons as the pesticides of choice for controlling pest on agricultural crops. They control a wide variety of invertebrates eg. mites, spiders and earthworms.

The primary way that insecticidal Carbamate works on both target and non-target species is through the inhibition of the enzyme acetyl cholinesterase. A major difference in the toxicology of organic phosphates and Carbamate insecticides is that the former almost irreversibly phosphorylated the acetyl cholinesterase of tissue, while the Carbamates cause reversible. Carbamate compounds are highly toxic to many types of birds such as red-winged black bird and ring necked pheasant. Carbamate compounds range from highly toxic to moderately toxic for fish such as bluegill sunfish. Carbamates like the Organophosphate are generally not persistent in the environment¹³.

Insecticides of Natural Origin –

Before the development of synthetic organic insecticides, natural substances derived from plants were successfully employed in pest control. The discovery of *Chrysanthemum cinerariaefolium* (Family Asteraceae) marked a

new era in pest management programmes. Various plant and plant products like pyrethrum; nicotine and rotenone were introduced in the market ¹⁴.

Pyrethroids and other botanical pesticides are grouped together not because they have similar toxicological properties like the Organophosphates or Carbamates, but because they all have biological origins. Pesticides may be extracted from naturally occurring materials or they can be synthesised in commercial laboratories. Some plants have developed, over long periods of time, substances that deter plant-eating insects. Researchers have isolated a number of compounds from plants that is effective insecticides or fungicides. Development of these botanical pesticides is one of the fastest growing areas in the pesticide industry.

Due to disadvantages offered by synthetic insecticides, attention was focused on insecticides of natural origin as a possible alternative to synthetic insecticides.

1. Pyrethrum: -

Pyrethrum is obtained from the flower heads of *Chrysanthemum cinerariaefolium* and *Chrysanthemum roseum* (Family Asteraceae). Extracts of the flower heads are now used as an insecticide.

Pyrethrum is very effective against houseflies and mosquitoes. Pyrethrum is completely biodegradable. They have high safety margin to higher animals and are effective against insects at a very low concentration. Consumption of large quantity of pyrethrum powder led to an allergic reaction and dermatitis. Oral ingestion of pyrethrum extract caused adverse effects on the central nervous system (CNS) and also adverse changes in the liver. Pyrethrum is highly toxic to insects, worms and cold-blooded vertebrates such as fish and frog¹⁵.

2. Nicotine: -

Nicotine is obtained as a liquid extract from tobacco dust. Nicotine is completely degradable and has no effect on plants, when it is sprayed. Three compounds make up the bulk of this category nicotine, nor-nicotine and anabasine. Nicotine is by far the most widely used of the group and is also the most potent¹⁶. It affects the nerve's ability to function properly, much like the Organophosphate and Carbamate insecticides, resulting in similar symptoms. Nicotine can cause twitching and difficulties with breathing, convulsions, and death. The use of nicotinoids has been declining. Additionally, they are non-persistent, and are ineffective in cold weather.

3. Rotenone -

The rotenoids are extracted mainly from two species, *Derris* and *Lonchocarpus* members of the bean family. Rotenone is a selective, non-specific botanical insecticide with some acaricidal properties. Rotenone is used in home gardens for insect control for lice and tick control on pets, and for fish eradication as part of water body management.

Rotenoids are highly toxic to fish and some insects, however they are only moderately toxic to mammals. Sign and symptoms of rotenone poisoning include conjunctivitis, dermatitis, pharyngitis, rhinitis, gastrointestinal irritation, nausea and vomiting. Inhalation of dust containing rotenone caused fits and convulsion¹⁷.

Disadvantage of natural insecticides -

Although natural insecticides are less hazardous to mankind as compared to synthetic insecticides, the recent natural insecticides in use have certain disadvantages, which cannot be neglected. Use of these natural insecticides has created certain health hazards. Nicotine is well known to be very

toxic to human beings¹⁸. Rotenoids, which are used in the form of powder, are proved to cause local effects such as conjunctivitis, dermatitis. Contact dermatitis is the most form of pyrethrum poisoning.

Considering the disadvantages of the synthetic and natural insecticides currently in use, attention has been focused to develop a new breed of natural products as potential insecticides. Attempts have been continued to produce new and safer insecticides.

However, in India sufficient attention has not been given to investigate indigenous plants for insecticidal activity in crude or purified form.

Insecticide Resistance: -

World Health Organisation Expert Committee on insecticides (WHO, 1970) defined resistance as "The development of an ability to tolerate the dosage of toxicants which would prove lethal to majority of individuals in normal population"¹⁹. The toxicity of DDT and other groups of synthetic compounds were highly lethal to most of the insects; as a result, the vector control programmes were highly successful. It is now well known that the effect of synthetic insecticides particularly, DDT is transmitted from generation to generation of insects. This transovarian nature of transmission of insecticidal residues caused a pressure of insect population, selectivity exposed to such pressure. As a result of this, soon a genotype called a "resistant strain" developed. In other words, the main attribute of resistant strain was its ability to metabolize the chemical used as insecticides²⁰.

From the originally solitary observation in Greece in 1950 of resistance to DDT by *Anopheles sacharove*, the tally of anopheline species resistant to insecticides is of an increasing order. *Culex pipen fatigans* have developed resistance to malathion in various parts of the world. In West Africa

and Japan culex strains were found resistant to some Organophosphorus and Carbamate insecticides²¹.

Synthetic chemical insecticides like DDT, BHC, malathion, fenthion, phos, chlorpyrifos, etc., are widely used in vector control programmes. After the continuous usage of an array of pesticides over the years, several vector species have developed varying degrees of resistance to many of these insecticides. So far 50 species of anophelines, 44 species of culicines and 43 species of other arthropods of public health or veterinary importance have been reported to be resistant to one or more insecticides (WHO 1986 a; Mehrotra 1992)²²⁻²³. Beside the problem of resistance, the disruption of ecosystem dynamics by their persistent and extensive interactions with both biotic and abiotic components of the ecosphere has led to a radical revision of our concepts of vector control in the past few years.

Management of vector mosquitoes seems to be very difficult because on one hand the vector develop resistance to pesticide and larvicides and on the other hand abundant usage of chemicals enhances the health hazards of human.

Environmental Pollution -

It is considered that the aim of an insecticide is to kill insects, but soon it became evident that as a result of long-term exposure to the insecticide, insect develops resistance. Besides this, the persistent use of insecticide posed several problems related to environmental pollution

The synthetic insecticides are found in environment as the product itself or as metabolites. The presence of these insecticides or its metabolites affects the normal environmental flora and fauna. Organochlorine insecticides are stable and are found in the body fat and blood of the animals and people engaged in pesticide work (Ramchandra et.al; 1973)²⁴. They are easily

incorporated into the food chain. It has been well established than DDT and other chemicals; if remained in the vertebrate body for a long time, are carcinogenic. Many reports have been established that contaminations of insecticides in foodstuffs cause food poisoning. (Griffith and Domnick, 1965)²⁵.

Organo-phosphorus and Carbamate insecticides are less stable as compared to the Organo-chlorine insecticides. Organo-phosphates are known to exert toxic effects by inhibiting cholinesterase activity, thereby allowing accumulation of acetylcholine in the nerves causing disturbances in neurotransmission.

The presence of carbamate insecticides is found to affect the existence of planktonic communities. After the application of Carbamates the population of zooplankton decreases as compared to that of the phytoplankton. A change in the phytoplankton structure was observed²⁶.

REVIEW LITERATURE

Studies on natural products and their efficacy as larvicides during the last decades have indicated them to be possible alternatives to synthetic chemical insecticides. However, more concerted efforts have been made into these studies to make these environment friendly compounds viable for field use and for large-scale vector control operations.

Historically, the commercial development of botanical insecticides is credited to Lady of *Ragusa, Dalmatia*, who noticed dead insects on a discarded bouquet of pyrethrin flowers. She began milling pyrethrum into powder and thus the pyrethrin industry was born (Hartzell and Wilcoxon 1941) ²⁷. Since then, pyrethrin from *Chrysanthemum* flowers and many synthetic derivatives stand prominent as effective pesticides.

In the case of *Acorus calamus*, when the rhizomes were extracted with different solvents like petroleum ether, chloroform and alcohol. The best results against *Culex quinquefasciatus* larvae were obtained with the petroleum ether extract only. (Chavan et. al.) ²⁸

Acorus calamus has been extensively studied for its larvicidal / insecticidal properties. The attention was drawn on insecticidal properties of *Acorus calamus* in 1942. It was reported²⁹ that the powdered rhizomes were very useful against bedbugs, moths, lice, etc In another study it was reported that the solvent extract and steam - volatile principle of rhizomes were toxic to houseflies and mosquitoes. The synergistic activity with DDT against flies was found to be toxic³⁰.

In another study, it is reported that the seed extracts of custard apple (*Annona squamosa*) and neem (*Azadirachta indica*) showed promising activity against household insects. Their synergistic activity was also studied, which was found to be equitoxic to DDT against houseflies³¹.

Essential oils from various plants also showed considerable activity as insecticides. In a review, it is reported that the essential oils from *Acorus calamus*, *Syzygium aromaticum*, *Eucalyptus globulus*, *Cymbopogon citratus* had insecticidal repellent and attractant properties³².

Sujata et. al; (1988)³³ also observed differential susceptibilities with extracts of *Acorus calamus*, *Bambusa arundanasia* and *Madhuca longifolia* against larvae of three species of mosquitoes. *Acorus calamus* extract was most effective against *Culex quinquefasciatus*, while *Bambusa arundanasia* was most toxic against *Anopheles stephensi* larvae. *Madhuca longifolia* extracts had no effect on this species.

Attri and Raviprasad³⁴ reported that toxic property present within the neem oil extract, causing complete inhibition of adult emergence when applied to first instar larvae of *Culex quinquefasciatus*.

It was reported that various parts of *Nerium indicum*, *Lantana camara*, *Scutia indica*, *Eucalyptus globulus*, *Ocimum basilicum*, etc. had promising mosquito larvicidal activity³⁵⁻³⁷.

Deshmukh et. al.³⁸ investigated twenty indigenous plants for their mosquito larvicidal activity. The different plants investigated were *Acorus calamus*, *Adhatoda vasica*, *Allium sativum*, *Aristolochia indica*, *Azadirachta indica*, *Ocimum basilicum*, *Ocimum sanctum*, *Vitex negundo*, etc. Out of the many plants screened *Acorus calamus*, *Allium sativum*, *Ocimum sanctum* and *Artemisa vulgaris* exhibited the most promising larvicidal / insecticidal activity. These plants can be further extensively studied, first to isolate and determine their active principle as an alternative mosquito larvicidal agent.

Mosquito larvicidal activity of essential oil from *Ocimum basilicum*, *Ocimum sanctum* and *Eucalyptus globulus* were studied and reported to have good synergistic activity³⁹.

It has been shown by Chavan et.al⁴⁰ that, the active principle, isolated from the leaves of *Azadirachta indica* possesses better mosquito larvicidal activity than the crude petroleum ether extract. The active principle isolated was a long chain saturated hydrocarbon.

Evans and Kaleysa Raj⁴¹ screened 24 plants for antilarval activity and among them, *Quassai amara* was found to be the most active one.

It has been reported by Chockalingam et.al⁴² that the larvicidal activity of products from 8 different plants (the alkaloids abrine, bixin, harmalin and plumbagin; the flavanoids Karangin and curcumin; piperine and picrotoxic) was determined using 3rd and 4th instar larva of *Culex quinquefasciatus*. Plumbagin, isolated from the root bark of *Plumbago indica*, was found to be the most effective.

Sukumar et. al.⁴³ reported 99 families, 276 genera and 346 species to have insecticidal properties. These included members of the family Solanaceae comprising 5 species belonging to 4 genera, namely *Capsicum frutescens*, *Datura candida*, *Datura stramonium*, *Lycopersicon lycopersicum* and *Nicotiana rustica*.

Tare and Sharma,⁴⁴ had studied the mosquito larvicidal properties of 10-tree seed oil and their common constituents such as fatty acids and terpenoids widely available in India. These were tested against 4th instar larvae of *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi*.

Plant samples of *Acanthus ilicifolius* (root), *Avicennia officinalis* (bark), *Excoecuria agallocha* (bark,fruit), *Lumnitzira racemosa* (bark), *Rhizophora*

apiculata (stilt root), *Salicornia brachiata* (shoot), *Rhizophora mucronata* (stiltroot), *Salicornia brachiata* (shoot) and *Sonneratia apetala* (root) were extracted in acetone and tested for mosquito larvicidal activity against *Aedes aegypti* by Subramonia et.al.⁴⁵ Extract from *Rhizophora apiculata* and *Rhizophora mucronata* were found effective.

The efficacy of *Ipomoea cornea* to control the larvae of *Culex quinquefasciatus* was investigated (Achary, et.al)⁴⁶. The leaf extracts, even at low concentration, (0.1%) showed a potential to disrupt the mosquitoes developmental cycle, resulting in high larval mortalities and indicating a possibility for its use as a larvicide.

Bioassay studies of laboratory colonised larvae and field collected larvae of *Culex quinquefasciatus* with 5 concentrations of pet - ether extract of 12 plants collected in India showed that the extract of *Argemone mexicana*, *Pergularia extensa* and *Withania somnifera* had acute toxicity. The benzene extracts of these plants were tested for their toxic effects on laboratory-colonised larvae and field-collected larvae⁴⁷.

In another study,⁴⁸ it is reported that the petroleum ether extract of two plant species *Piper longum*, and *Zingiber wightianum* were effective against the fourth instar larvae of *Culex quinquefasciatus* and *Culex sitiens*. Therefore, the plant species of *Piper longum* and *Zingiber wightianum* were identified as potent bio-pesticides.

The laboratory studies were carried out by Singh et.al⁴⁹ to ascertain the larvicidal properties of *Solanum nigrum* in three mosquito species, viz. *Anopheles culicifacies*, *Culex quinquefasciatus* and *Aedes aegypti*. Results of this study with the crude extract of the leaves of this plant have exhibited its toxicity to three important disease vector species and warrants further investigations.

Dwivedi and Karwasara⁵⁰ have been reported that the *Ricinus communis* leaf extract possessed strong larvicidal potentiality and could be suggested as one of the agent in integrated management of mosquitoes population suppression.

The above literature clearly indicates the potential of natural products as vector control agents.

MATERIALS & METHODS

AIMS AND OBJECTIVES

India has one of the oldest, richest and the most diverse cultural traditions associated with the use of medicinal plants. There are more than 2000 species of indigenous plants, which are known for their insecticidal or insect repellent activity. After an extensive use of totally or partially synthesised molecules isolated from nature, there is a gradual global shift from synthetic medicines to natural products. India has the necessary infrastructure, expertise, trained manpower, heritage and natural resources to become a major player in the global market for natural products for commercial and therapeutic utilities.

It is believed that India presently has a share of Rs.600 crores in global herbal market and has potential to increase it to Rs.3000 to 4000 crores during the next decades. Keeping in mind all these forthcoming possibilities, things have been rapidly moving not only in India but also all over the world.

The rising cost of petroleum based insecticides, problems of environmental hazards posed by conventional insecticides and increasing resistance exhibited by insect species made it necessary to examine floral wealth for insecticidal activity. However, in India very few investigations have been done to achieve a suitable alternative insecticide from plant origin. Hence, an attempt has been made here to develop a new safer insecticide of plant origin in a country like India. Therefore, it was considered worthwhile to screen some of the promising plants for larvicidal properties. A brief description of plants is given below:



PLAN OF WORK -

1. Different parts of plants under study, were collected, dried and then powdered separately.
2. Extraction of the powdered material of the plant has been carried out using non-polar and polar solvents. Aqueous extracts of the plant material were prepared.
3. Evaluation of solvent free extracts for their mosquito larvicidal activity (*Culex quinquefasciatus*) for 24 hrs. as per WHO specification.
4. Fractionation of the active extracts using chromatographic technique.
5. Evaluation of chromatographic fraction for mosquito larvicidal activity.
6. Residual activity of the active plant extract.
7. Phytochemical studies on the active plant extract.

1. *Andrographis paniculata*

Family - Acanthaceae

English - Green chiretta

Vernacular names: Hindi - Kalamegh , Marathi - Oli kirayat

Distribution - Throughout the plains of India from U.P. to Assam, Madhya pradesh, Andhra pradesh, Tamil Nadu and Kerala; also cultivated in gardens.

Use - Leaf is antispasmodic for loss of appetite in infants, root and leaf is febrifuge, stomachic, tonic, antihelmintic, plant used in jaundice.

Biological activity - The anti-typhoid and anti-fungal activity of the whole plant has already been reported⁵¹. Shoot of *Andrographis paniculata* showed antibacterial activity⁵². A crude substance isolated from methanolic extract of leaves has shown hypotensive activity⁵³. The fractions isolated from the leaves of *Andrographis paniculata* have shown the antimalarial activity⁵⁴.

Chemical constituents - The roots contain the flavonoids, andrographin, panicolin, apigenin-4', 7-dimethyl ether, mono-O-methyl wightin and 5-hydroxy-7, 8, 2', 3'- tetramethoxy flavone and β -sitosterol⁵⁵⁻⁵⁶. The diterpenoids and sesquiterpenoid compounds occurring in *Andrographis paniculata* have been referred to as paniculides and andrographolides⁵⁷. Neoandrographolide is shown to be the β -glucoside⁵⁸.

2. *Andropogon citratus*

Family - Graminae

English - Lemon grass

Vernacular names: Hindi - Gandhatrina, Marathi - Gavatichaha



Endoglyphis periculata (Scutiger)



Endoglyphis periculata

Distribution - Grown in Karnataka, Punjab, Maharashtra and Gujarat.

Use - Leaf used in sudorific, stimulant, antiperiodic, anticatarrhal, essential oil used in carminative and anticholerin.

Biological activity - Essential oil has antifungal activity⁵⁹. Citral is a component of lemon grass oil which is an effective insecticides, fumigant and repellent properties⁶⁰. Pharmacological investigation on the essential oil of *Andropogon citratus* revealed that it exerts a depressant effect on the CNS; it also has analgesic and antipyretic properties⁶¹.

Chemical constituents - Lemon grass yields 0.7% of volatile oil, which consist mainly of citrals a terpene aldehyde. Others constituents of the oil include geraniol, nerol, furfural, citronella, methyleptenone and myrcene⁶²⁻⁶³ as well as the triterpenes cymbopogone and cymbopogonol⁶⁴.

3. *Capsicum frutescens*

Family -Solanaceae

English -Sweet Pepper

Vernacular names: Hindi - Lal Mirch , Marathi - Lavangi mirchi

Distribution - Extensively cultivated throughout the tropical region of India.

Use - Fruit is stimulant, externally rubefacient, antidiarrhoeal antidote for snakebite. In traditional medicine they are employed mainly as a flavoring agent in compound formulations. An ointment prepared with the fruit extract is used topically for skin infections.



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Biological activity - The alkaloid, capsaicin, has been studied extensively, and has been shown to possess antimicrobial, spasmolytic, vasodilation, rubefacient counterirritant, and stimulatory activity on the digestive system⁶⁵. One of the steroidal saponins found in the fruits exhibited antibiotic activity⁶⁶.

Chemical constituents - The fruit contains capsaicin, dihydrocapsaicin, nordihydrocapsaicin, and related alkaloids. The colour of the ripe fruits is due to the presence of carotene, capsanthin and capsorubin as well as steroidal saponins. Vitamins A and C also occur in the plant⁶⁷.

4. *Cinnamomum tamala*

Family - Lauraceae

English -Indian cassis lignea

Vernacular names: Hindi - Tamalpatram , Marathi:- Ranachadal

Distribution - Himalaya from Kashmir to Bhutan and Assam and Khasi hills at 450-2000m.

Use - Leaf is hypoglycaemic, stimulant, carminative, antirheumatic, antidiarrhoeal, antidote for scorpion sting in colic, bark is antigonorrhoeic.

Biological activity - Antifungal activity of the essential oil is reported⁶⁸. Hypoglycaemic action of *cinnamomum tamala*⁶⁹. Tejpat exhibited potent antimicrobial properties⁷⁰.

Chemical constituents - The essential oil obtained from the leaves is reported to contain eugenol, terpin and cinnamic aldehyde. Roots contain oil, which has eugenol, saffrol, benzaldehyde and terpin⁷¹.



Cinnamomum tamala (Lauraceae)

5. *Citrus aurantium*

Family - Rutaceae

English -Sweet orange

Vernacular name: Hindi - Santra, Marathi - Narangi

Distribution - Widely cultivated in India

Use - Fruit is cardiotonic, laxative and stomachic. Dried rind is aromatic, stomachic, tonic, astringent mild carminative and antiscorbutic. Orange water is stimulating and refreshing. Juice is refrigerant and stomachic. Orange is rich in vitamins and is considered to help in digestion.

Biological activity -Citrus aurantium had antifungal activity⁷². Acaricidal activity of crude extracts of citrus of citrus aurantium was observed⁷³.

Chemical constituents The rind of the fruit contains a volatile oil, isomeric with oil of turpentine, gum resin, a fixed oil, hesperidin, a bitter crystalline principle, tannin⁷⁴.

6. *Coriander sativum*

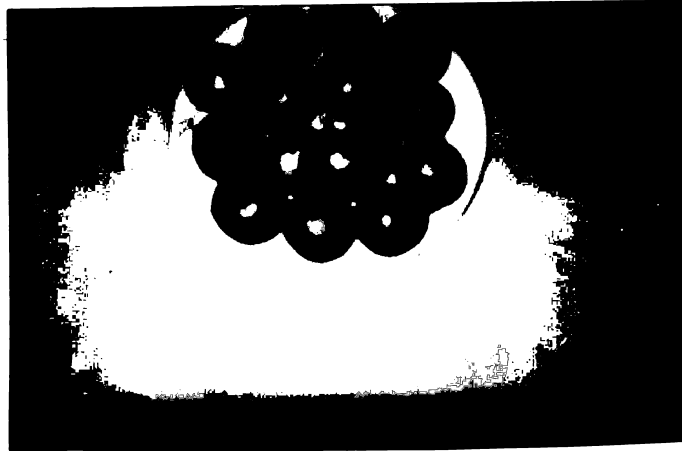
Family -Apiaceae

English -Coriander

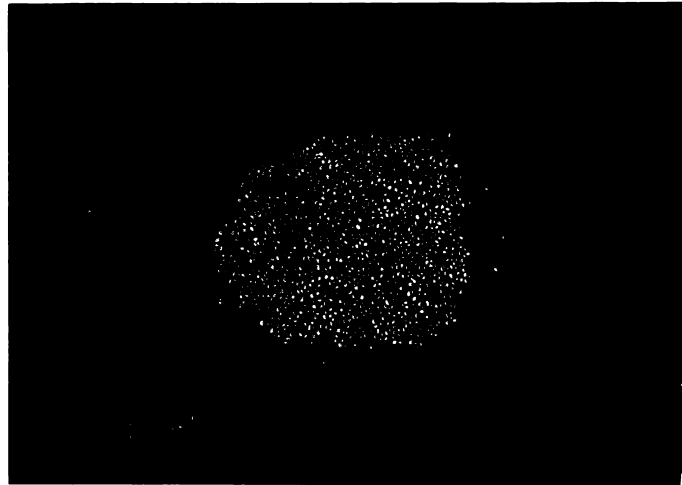
Vernacular names: Hindi - Dhania , Marathi -Dhane

Distribution - A herbaceous plant extensively cultivated in all parts of India for its seeds.

Use - Fruit is stimulant, carminative, diuretic, stomachic, aphrodisiac.



Citrus aurantium (Rutaceae)



Coriander sativum (Apiaceae)

Biological activity - Antimicrobial activity of essential oil from fruit is reported⁷⁵. Inhibitory effect of essential oil on *Aspergillus ochraceus*⁷⁶.

Chemical constituents - Main constituent of essential oil is linalool. Others are α and β -pinene, camphene, sabinene, myrcene, methylheptenol limonene, 1,8-cineole, ocimene, β -phellandrene, γ -terpinene, p-cymene, terpinolene, camphor, linalyl acetate, β -caryophyllene, α -terpineol, geranyl acetate and geraniol⁷⁷.

7. *Cynodon dactylon*

Family - Gramineae English - Bermuda grass

Vernacular names: Hindi - Durva, Marathi - Haryali

Distribution - This elegant perennial grass grows everywhere throughout India.

Use - Plant is astringent, diuretic antiepileptic, antidiarrhoeal, antiepileptic in ophthalmia, cuts and wounds.

Biological activity -The alcoholic extract of the entire plant was found to have antiviral activity against vaccinia virus⁷⁸.

Chemical constituents -The plant contains crude proteins, carbohydrates and mineral constituents oxides of magnesium, phosphorus, calcium, sodium and potassium. Alkaloids are also present⁷⁹.

8. *Elettaria cardamomum*

Family - Zingiberaceae

English - Malabar cardamomum



Cynodon dactylon (Poaceae)



Elettaria cardamomum (Zingiberaceae)

Vernacular names: Hindi - Choti Elachi , Marathi - Veldode

Distribution - South-western India

Use - Seeds-bitter, anticephalagic, antiasthmatic, for foul breath, stimulant, stomachic, carminative, tonic, antiemetic, diuretic in piles, diseases of heart,bladder and kidney.

Biological activity - Its volatile oils have smooth muscle relaxant properties, which contribute to its carminative and antispasmodic action.

Chemical constituents -Chemical constituent of the oil are cineol, terpineol, terpinene, limonene, sabinene and terpineol in the form of formic and acetic acids⁸⁰.

9. *Eleusine coracana*

Family - Graminae

English -Indian millet

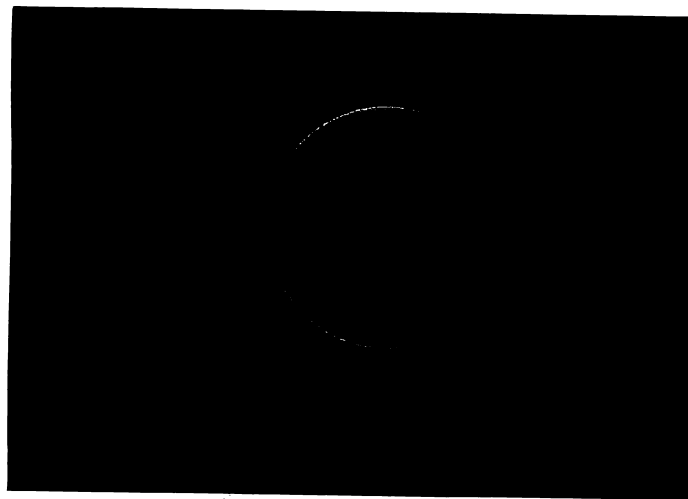
Vernacular names: Hindi - Makra, Marathi - Nachni

Distribution - This cereal is grown almost in all parts of India.

Use - Seeds are tonic, cooling, useful in biliousness and are also astringent.

Biological activity -The plant is reported to be a diaphoretic, diuretic, and vermifuge .

Chemical constituents - The plant yield hydrocyanic acid⁸¹.



Eleusine coracana (Graminae)

10. *Ferula asafoetida*

Family - Apiaceae

English - Asafoetida

Vernacular names: Hindi - Hing , Marathi - Hing

Distribution - Wild in Punjab, Kashmir, Iran and Afghanistan.

Use - Nervous irritation, with mental depression, headache and dizziness; hysteroidal conditions; convulsive disorders from purely functional wrongs of the stomach, gastro-intestinal irritation, with flatulence and palpitation of the heart, dry, deep, choking, bronchial cough.

Biological activity - The odor of asafoetida is imparted to the breathe, sacractions, flatus and gastric eructations. Its properties are stimulant, antispasmodic, expectorant, emmenagogue and vermifuge. Improper in inflammatory conditions of the system, but of marked value in purely functional nervous disorders, with excitability and as a gastric stimulant in gastrointestinal atony with flatulence.

Chemical constituents - Asafoetida contains volatile oil, resin soluble in ether, and a tasteless resin insoluble in ether, sulphate of calcium, carbonate of calcium, oxide of iron and alumina, malate of calcium etc⁸².

11. *Lawsonia inermis*

Family - Lythraceae

English - Samphire

Vernacular names : Hindi - Heena , Marathi - Mehndi

Distribution - Common all over India, cultivated chiefly, as a hedge and garden plant.

Use - The herb is used for the treatment of skin infection. The dry powder leaves are ingredients in the preparation of local cosmetics. They have astringent properties. An ointment prepared from the leaves is used to cure wounds and ulcers. The leaves and roots are believed to have properties that stimulate menstrual discharge.

Biological activity - Leaf extracts show fungicidal properties⁸³. An extractive from the plant has strong antimicrobial activity, which has been evaluated as comparable to the antibiotic activity of sulfonamide and penicillin. Lawson exhibits slight antihemorrhagic properties. The compound also has emmenagogic and oxytocic properties.

Chemical constituents - Naphthoquinones such as lawsone, a 2-hydroxy-1, 4-naphthoquinone, are the major constituents of the leaves. The plant also contains resins and lannatannin⁸⁴.

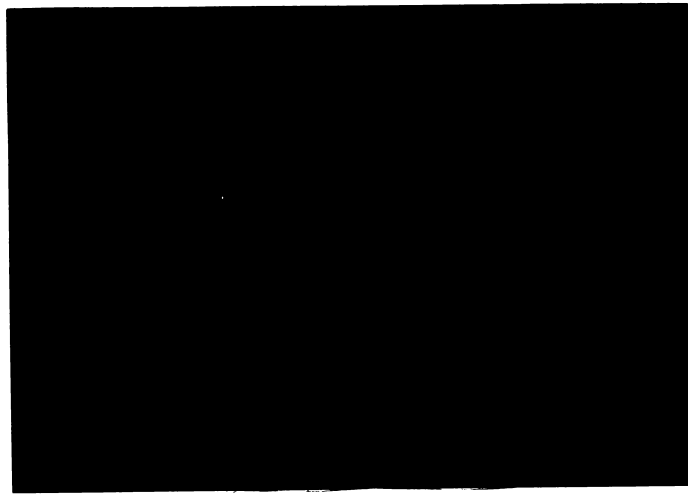
12. *Mentha arvensis*

Family - Lamiaceae

English - Corn mint

Vernacular names: Hindi - Pudina, Marathi - Pudina

Distribution - Western Himalayas, cultivated in Jammu and Kashmir, Uttar Pradesh and Punjab.



Ferula asafoetida (Apiaceae)



Lawsonia inermis (Lythraceae)



Mentha a.

1857

Use - The plant has bad taste and smell, expectorant, tonic to the kidney, useful in disease of the liver and spleen, asthma, pains in the joint. The dried plant is refrigerant, stomachic, diuretic and stimulant.

Biological activity - Effect of volatile constituents menthone, menthol, 1-carvone have been identified as potent anti-insect agents for grain protection⁸⁵. The essential oil from mentha arvensis has observed antiviral⁸⁶ and funfitoxicity effect⁸⁷.

Chemical constituents - β -caryophyllene oxide, α -phellandrene and terpinolene⁸⁸. The essential oil extracted from mentha arvensis were menthol, p-menthone, L- (-)-menthol, isomenthol⁸⁹.

13. *Murraya koenigi*

Family - Rutaceae

English - Curry leaf

Vernacular names: Hindi - Kurry patta , Marathi - Jhirang

Distribution - Throughout India and the Andaman island upto an altitude of 1,500m.

Use - The green leaves are described to be eaten raw for the cure of dysentery. An infusion of the toasted leaves is used to stop vomiting. The plant is credited with tonic and stomachic properties. Leaves are widely used for flavouring curries.

Biological activity - The acetone extract the leaves resulted in the isolation of three bioactive carbazole alkaloids, mahanimbine(1), murrayanol(2), and mahanine(3). All three compounds were



1941

mosquitocidal and antimicrobial and exhibited topoisomerase I and II inhibition activities⁹⁰. Antitermites characteristic of *Murraya koenigii*⁹¹.

Chemical constituents - Ethanolic extract of *Murraya koenigii* leaves afforded a new carbazole alkaloid, which was identified as bismurrayafoline⁹². The major constituent of the leaf oil were α - pinene, α -phellandrene, β -phellandrene, β -ocimene and β -caryophyllene⁹³, dipentene, α -eudesmol; socaryophyllene; β - elemene⁹⁴.

14. *Syzygium aromaticum*

Family - Myrtaceae

English - Clove

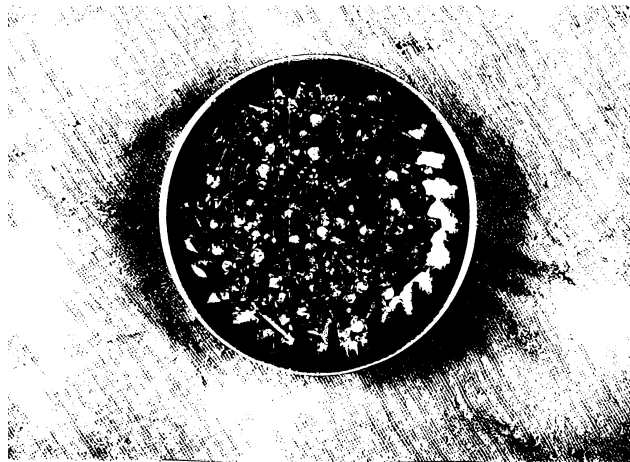
Vernacular names: Hindi - Laung , Marathi - Lavang

Distribution - Cultivated in South India.

Use - Dried flower bud used in antiemetic, stimulant, carminative, in dyspepsia, gastric irritation, essential oil used in antispasmodic, bactericidal, analgesic for hypersensitive.

Biological activity - The toxicity of oil obtained from *Syzygium aromaticum* was found to be an effective against *Anopheles stephensi*⁹⁵. Some natural product, extracts and oil were tested for repellency against *Aedes aegypti*⁹⁶. Antimicrobial activity⁹⁷, Antifungal activity⁹⁸ of *Syzygium aromatic* is reported. Inhibitory effect on hepatitis C virus⁹⁹.

Chemical constituents - It yields 15 - 20 % of volatile oil, which consist of up to 90 % eugenol and about 13 % gallotanic acid¹⁰⁰.



Syzygium cumini (Myrtaceae)

15. *Trigonella foenum graceum*

Family - Papilionaceae

English -Fenugreek

Vernacular names: Hindi - Muthi, Methi , Marathi - Methi

Distribution - Temperate and Mediterranean regions of Africa.

Use - The leaves are used in various folk remedies for the relief of indigestion and general stomach disorders. The seeds are edible and have been used in remedies for rheumatism, chronic cough, and enlargement of spleen and liver and as a general tonic.

Biological activity - A 2% concentration of the saponin fraction showed in vitro spermicidal action against rat and human semen. The alkaloid trigonelline has been shown to counteract the hyperglycemic effect of cortisone given 2 hr before or simultaneously. Nicotinic acid and coumarin, which cooccur in the plant, are also hypoglycemic. The compound was moderately effective in alloxan diabetic rats and in human subjects.

Chemical constituents - The seeds contain trigonelline (N-methylnicotinic acid), choline, nicotinic acid and diosgenin. Essential oil, mucilage and fixed oil are also major components of the seeds. Fenugreek has been shown to be a feasible source of diosgenin, which occurs in the embryo of the seeds¹⁰¹.

16. *Zanthoxylum limonella*

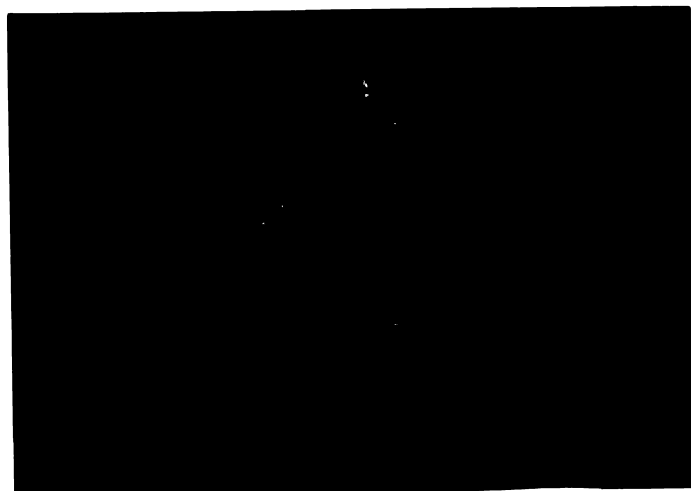
Family - Rutaceae

English - Toothache tree

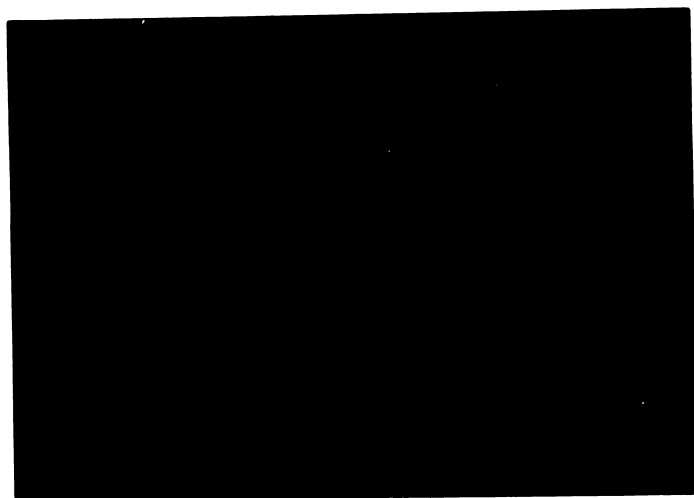
Vernacular names: Hindi - Badrang , Marathi -Tirphal



Trigonella foenum-graceum (Papilionaceae)



Zanthoxylum limonella Seed-coat (Rutaceae)



Zanthoxylum limonella Seed (Rutaceae)

Distribution -Commonly found in the evergreen monsoon forest of the foothills of Assam and Meghalaya and in the eastern and western ghats in peninsular India.

Use -In western and southern India; they are used as a condiment in both sweet and savoury preparations, especially in the preparation of fish curries. The fruits used in asthma and bronchitis, heart, toothache and rheumatism. The pericarps are credited with astringent, stimulant and digestive properties.

Biological activity -The rinds on extraction with ether yield 7.2 % of oil possessing antibacterial activity. Aqueous and alcoholic extracts of the bark have cholinergic, hypoglycemic and spasmolytic activity in preliminary trials¹⁰².

Chemical constituents -Major constituents of volatile essential oil found in these herbs were terpene, hydrocarbons (mono and sesquiterpenes) and oxygenated terpenes. Limonene (phellandrene and 2-undecanone) was present as major constituents in the essential oils of *zanthoxylum limonella*¹⁰³.

Experimental

Methods: -

Collection of plants: -Authentic parts of various plants under study were obtained from local market. Wherever plants were not available, they were purchased from plant dealers and used for experimental purpose.

Extraction of plant material using organic solvent-

The chopped plant parts were sun-dried and powdered in a grinder mixer (80-100 mesh). Extraction of the powdered material of the plant was carried out using non-polar and polar solvents. Aqueous extracts of the plant material were also prepared¹⁰⁴. The dried plant material was first extracted in Soxhlet with petroleum ether (60-80° C) and then with methanol in succession¹⁰⁵. Percent yield of the individual extractives was calculated (Table 1). All these extracts were made free from solvents and then subjected to mosquito larvicidal (*Culex quinquefasciatus*) activity by standard procedure¹⁰⁶.

Aqueous Extraction -

To 5 gm powder 50ml of water was added in 80° C in a stoppered flask. It was shaken well and allowed to stand for 10 minutes. After cooling 2 gm of kieselglehr was added and filtered. The filtrate was transferred to a trade-evaporating dish (7.5 cm in diameter). Water was evaporated on a water bath. Drying for 30 minute and finally dried in a steam oven for 2 hours the residue was weighed. The percentage of water-soluble extractives with respect to the air-dried drug was calculated.

Mosquito Larvicidal Activity Of Various Plant Extracts -

The various plant extracts were subjected to mosquito larvicidal activity, according to WHO specification. Mosquito larvae were collected from suburban areas of Bombay City; these collected larvae were found to be

Culex quinquefasciatus species. These larvae were transferred to fresh water and maintained under laboratory conditions and were fed on dog biscuits and yeast powder in the ratio 2:1.

The test mosquito larvae were third instar or early fourth instar larvae of *Culex quinquefasciatus*. First and second instar larvae were not used because mortality of these stages were greatly affected by changes in extrinsic factors particularly temperature. The late fourth instar larvae were generally not used because of their conversion into pupal stage during the period of experiment. Hence, late third instar / early fourth instar larvae were mostly used for experimental work.

Test Procedure -

All experimental exposures were done using glass beakers with 500ml capacity. Ten glass beakers were arranged in a series. Each beaker was filled with 250ml tap water. The plant extractives were diluted in acetone and 1ml of test concentration solution was pipetted into it under the surface. The glass beaker was then carefully stirred for about 30 seconds, to allow homogenous mixture of the test solution and the tap water. The testing beaker thus prepared was allowed to stand for 15 to 30 minutes. During this period, the batches of 25 larvae were carefully pipetted out into the testing beaker.

Two controls were maintained: one consisted of 1ml of acetone and the other of tap water along with standard pyrethrum pet-ether (0.02%). Three replications were employed for each dilution of the extract under test. After releasing larvae into the testing beaker, the beaker was covered with musclin cloth, to prevent any foreign material from entering the beaker.

Mortality was observed at the end of 24 hours. The mortality was recorded by combining dead and moribund larvae. Dead larvae were those

that could not be induce to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those, incapable of rising to the surface (within a reasonable time) or showing the characteristic diving reaction when the water was disturbed. The larvae that have pupated during the test were discarded.

Out of seventeen plants studied, petroleum ether and aqueous extracts of *Murraya koenigii* showed significant larvicidal activity. This led to undertake chemical investigations on the *Murraya koenigii* in order to isolate and characterize the active constituents responsible for mosquito larvicidal activity. So far, hardly any chemical constituent from *Murraya koenigii* has been reported to possess mosquito larvicidal activity, although the plant has been extensively worked out chemical and other biological activities. Other plant extracts did not show any appreciable larvicidal activity.

Residual activity -

Twenty-five yeast fed larvae of late 3rd or early 4th stage *Culex quinquefasciatus*, were placed in a 500 ml beaker containing 250 ml tap water. The concentrations of 0.24% and 0.28% of extracts were added by dissolving it in 1ml of acetone. The beakers were covered with musclin cloth and kept away from direct sunlight. Water and acetone controls were maintained along with standard pyrethrum pet-ether extract (0.28 %).

At the end of 24hrs, mortality was determined and 25 fresh larvae of *Culex quinquefasciatus* were added to the same experimental beakers. Mortality was counted at the end of every 24hrs and fresh 25 larvae were added everyday to test beaker till the mortality drops down.

Exploratory chemical studies on *Murraya koenigii* -

In view of the promising larvicidal activity demonstrated by the petroleum ether extract of *Muraya koenigii*, it was thought worthwhile to undertake the chemical fractionation of petroleum ether extract of *Murraya koenigii* with a view to isolating and characterizing the active constituents in the plant.

Fractionation of the pet - ether plant extract of *Murraya koenigii* was done using silica gel column chromatography. The glass (length of the column was 7.3 cm and inner diameter was 2.7 cm) column was prepared in petroleum ether using silica gel G (80-120 mesh). The same was allowed to run in petroleum ether for getting uniform packing. 6% of the extract diluted in solvent ether was then charged on the top of the each column and allowed to get adsorbed in the form of the narrow bands. Then the column was eluted using following solvents systems: petroleum ether, benzene, chloroform and alcohol in succession.

All the fractions were collected separately. After every elution, the elute did not show any residue and this criteria was used for changing the solvent system. All these fractions were made free of solvents on water bath and tested for mosquito larvicidal activity.

It was observed that benzene elute showed prominent activity. This fraction produced 99.66 % mortality at 0.24 percent concentration while the remaining elutes did not show any significant results. TLC study using Petroleum-ether: Methanol, 40:60 as a solvent system indicated the presence of single spot.

Benzene-eluted fractions were pooled and subjected to rechromatography. The rechromatographed benzene eluted fraction, which

was yellow in colour, was collected and concentrated. The residue contained a low melting compound M.P. $80 - 83^{\circ}$. On repeated crystallizations from ethyl alcohol, a compound M.P. $83 - 84^{\circ}$ was obtained (Yield: 30 mg / Kg *Murraya koenigii* leaves). It did not give any test for sterols.

IR $\nu_{\max}$ (KBr), (recorded on Perkin Elmer FT – IR – 1600 spectrophotometer), showed 3467 cm^{-1} (OH), 2910 cm^{-1} 2848 cm^{-1} (aliphatic C-H), 1730 cm^{-1} and 1650 cm^{-1} (C=O), 1460 cm^{-1} (C=O), 1460 cm^{-1} , 1250 cm^{-1} , 1052 cm^{-1} (broad C=O, stretch, secondary alcohol) and 800 cm^{-1} .

Mass spectrum of the compound M.P. $83-84^{\circ}$ (recorded on Jeol JMS-D300) showed peaks at 413, 353, 342, 302, 301, 277, 245, 150, 146, 104 and 102 (base peak) with molecular ion peak at 454.

RESULTS & DISCUSSION

Vector control has been achieved using synthetic insecticides for a long time. However, in the long run the multifarious harmful effects of chemical pesticides outweigh their benefits. Several evils are associated with non-judicious and widespread use of synthetic pesticides in vector control. The worldwide concern with the synthetic insecticides due to their ecological incompatibility and effect on non-target organisms' lead to the search of potential natural products.

The various plant parts were extracted with non-polar and polar solvents and these extracts were subjects to mosquito larvicidal activity. Four concentrations of each extract were taken up to percentage mortality of mosquito larvae upto 24 hours.

In case of aqueous extracts, *Citrus aurantium*, *Eleusine coracana*, *Mentha arvensis*, *Murraya koenigii* and *Zanthoxylum limonella* (Seed) have shown most promising larvicidal activity at 0.08 % but the percentage yield (Table 1) of total plant extract was very low to pursue further work.

Petroleum ether extracts of *Andrographis paniculata*, *Andropogon citratus*, *Capsicum frutescens*, *Coriander sativum*, *Ferula asafoetida*, *Trigonella foenum graceum*, *Murraya koenigii*, and *Zanthoxylum limonella* (Seed) were found to be effective at lower concentrations. These extracts showed more than 50 % larvicidal activity at 0.08 % concentration. Hence the concentration has been increased to 0.24 % and it has been seen that *Murraya koenigii* is having maximum potency for larvicidal activity at 24 hours. All other plants did not show 100 % mortality at the highest concentration tested (0.24 %) and did not give promising lead for further work. Larvicidal activity of the petroleum ether and aqueous extract of *Murraya koenigii* were significantly more potent than the all other plants.

None of the methanol extract (Succ.) of plants shows any significant mortality.

It has been observed, that petroleum ether extract of *Murraya koenigii* gave consistent and remarkably well-defined larvicidal activity. Therefore, petroleum extract of *Murraya koenigii* was subjected for residual activity studies (Table-5).

The petroleum ether extract was screened for its residual activity studies. The residual activity was an elongation of the mosquito larvicidal activity. This study was conducted to observe the loss of mosquito larvicidal activity. The residual effects are very important when one observes the development of insecticidal resistance in various species of insects. Organo-chlorinated insecticides remain active in environment for long periods, thereby inducing an ability to withstand the lethal doses of insecticides in insects.

While studying the residual effect, the standard pyrethrum pet-ether extract exhibited 88.33% mosquito larvicidal activity for 0.28% at the end of 96 hours, whereas *Murraya koenigii* pet-ether extract showed 83.33% larvicidal activity for 0.28%. This concludes that *Murraya koenigii* pet-ether extract is giving equitoxic results.

The loss of activity shows that the material does not persist in the environment and thereby does not cause any environmental disturbances. From these studies, it can be seen that the activity had considerably reduced at the end of 96 hours at 0.24%. During the course of residual activity studies, few larvae moulded into pupae. From this it is clear that the petroleum ether extract of *Murraya koenigii* does not cause any serious effect in the metamorphosis.

After chromatographic fractionation of petroleum ether extract (Table-6) of *Murraya koenigii* and evaluating various elutes for their activity it was observed that benzene elute showed prominent activity. This fractionation produced 99.66% mosquito larvicidal activity at same concentration (0.24 %). Remaining elutes did not show any significant result.

Table 1- Percentage yield of the extractives.

Plant (Family)	Parts used	% Yield		
		Aqueous	Petroleum-ether	Methanol (Succ.)
<i>Andrographis paniculata</i> (Acanthaceae)	Leaves	7.90%	10.40%	2.72%
<i>Andropogon citratis</i> (Graminae)	Leaves	3.69%	10.62%	14.26%
<i>Capsicum frutescens</i> (Solanaceae)	Fruit	6.35%	6.62%	41.31%
<i>Cinnamomum tamala</i> (Lauraceae)	Leaves	5.74%	6.53%	24.33%
<i>Citrus aurantium</i> (Rutaceae)	Dried Peel	1.77%	2.04%	43.73%
<i>Coriander sativum</i> (Apiaceae)	Fruit	5.08%	14.49%	20.78%
<i>Cynodon dactylon</i> (Graminae)	Leaves	3.86%	5.06%	12.08%
<i>Elettaria cardamomum</i> (Zingiberaceae)	Fruit	1.82%	7.56%	15.88%

Eleusine corana (Graminae)	Grain	0.86%	1.77%	1.75%
Ferula asafoetida (Apiaceae)	Root	1.57%	6.76%	2.37%
Lawsonia inermis (Lythraceae)	Leaves	3.40%	5.88%	39.77%
Mentha arvensis (Luminaceae)	Leaves	2.38%	4.24%	26.77%
Murraya koenigii (Rutaceae)	Leaves	6.80%	9.80%	12.29%
Syzygium aromaticum (Myrtaceae)	Fruit	11.28%	13.93%	45%
Trigonella foenum graecum (Papilionaceae)	Seed	2.96%	5.87%	18.41%
Zanthoxylum limonella (Rutaceae)	Seed	3.80%	6.00%	3.21%
Zanthoxylum limonella (Rutaceae)	Seed coat	20.84%	4.35%	23.64%

TABLE - 2
Larvicidal effect of the Aqueous extract of plant material on *Culex quinquefasciatus*.

Plant	Percentage mortality at the end of 24 hrs. Mean $\pm$ S.E. n = 10				Percentage mortality in control	
	Aqueous extract concentration.				Acetone	Water
	0.02%	0.04%	0.06%	0.08%		
<i>Andrographis paniculata</i>	1.8 $\pm$ 1.63	1.33 $\pm$ 1.63	1.33 $\pm$ 1.63	2.66 $\pm$ 1.63	1.33 $\pm$ 1.63	2.66 $\pm$ 1.63
<i>Andropogon citratus</i>	1.33 $\pm$ 1.63	1.08 $\pm$ 1.63	3.00 $\pm$ 1.08	4.00 $\pm$ 0.00	3.00 $\pm$ 1.08	3.00 $\pm$ 1.22
<i>Capsicum frutescens</i>	2.66 $\pm$ 1.63	4.00 $\pm$ 0.00	5.33 $\pm$ 1.63	4.00 $\pm$ 2.83	2.66 $\pm$ 1.63	1.33 $\pm$ 1.63
<i>Cinnamomum tamala</i>	4.00 $\pm$ 1.63	6.66 $\pm$ 1.63	8.00 $\pm$ 2.83	9.33 $\pm$ 1.63	3.00 $\pm$ 1.08	4.00 $\pm$ 0.00
<i>Citrus aurantium</i>	16.00 $\pm$ 2.83	29.33 $\pm$ 1.63	38.66 $\pm$ 4.33	49.33 $\pm$ 1.63	3.00 $\pm$ 1.22	2.66 $\pm$ 1.63
<i>Coriander sativum</i>	1.33 $\pm$ 1.63	2.66 $\pm$ 1.63	4.00 $\pm$ 2.83	5.33 $\pm$ 1.63	1.33 $\pm$ 1.63	2.66 $\pm$ 1.63
<i>Cynodon dactylon</i>	4.00 $\pm$ 0.00	5.33 $\pm$ 1.63	6.66 $\pm$ 1.63	8.00 $\pm$ 2.83	4.00 $\pm$ 0.00	3.00 $\pm$ 1.08
<i>Elettaria cardamomum</i>	4.00 $\pm$ 0.00	4.00 $\pm$ 2.83	5.33 $\pm$ 1.63	6.66 $\pm$ 1.63	3.00 $\pm$ 1.22	4.00 $\pm$ 0.00
<i>Eleusine coracana</i>	22.66 $\pm$ 1.63	23.66 $\pm$ 3.19	38.66 $\pm$ 4.33	44.66 $\pm$ 2.16	3.00 $\pm$ 1.08	2.66 $\pm$ 1.63
<i>Ferula asafoetida</i>	3.00 $\pm$ 1.22	5.33 $\pm$ 1.63	11.33 $\pm$ 1.78	13.33 $\pm$ 2.95	1.33 $\pm$ 1.63	4.00 $\pm$ 0.00

<i>Lawsonia inermis</i>	8.66 $\pm$ 1.47	9.33 $\pm$ 1.08	14.66 $\pm$ 1.63	19.00 $\pm$ 1.22	3.00 $\pm$ 1.08	2.66 $\pm$ 1.63
<i>Mentha arvensis</i>	26.66 $\pm$ 1.63	34.00 $\pm$ 4.25	42.66 $\pm$ 4.33	49.33 $\pm$ 1.63	3.00 $\pm$ 1.22	1.33 $\pm$ 1.63
<i>Murraya koenigii</i>	32.00 $\pm$ 2.83	44.00 $\pm$ 2.83	51.00 $\pm$ 1.87	54.33 $\pm$ 2.68	2.66 $\pm$ 1.63	1.33 $\pm$ 1.63
<i>Syzygium aromaticum</i>	4.00 $\pm$ 2.83	6.33 $\pm$ 1.68	10.00 $\pm$ 1.63	11.66 $\pm$ 1.47	3.00 $\pm$ 1.22	2.66 $\pm$ 1.63
<i>Trigonella foenum graecum</i>	4.00 $\pm$ 2.83	6.66 $\pm$ 1.63	8.00 $\pm$ 2.83	10.66 $\pm$ 1.63	2.66 $\pm$ 1.63	1.33 $\pm$ 1.63
<i>Zanthoxylum limonella</i> (Seed)	16.00 $\pm$ 2.83	24.00 $\pm$ 2.83	28.00 $\pm$ 2.83	46.66 $\pm$ 3.27	1.33 $\pm$ 1.63	4.00 $\pm$ 0.00
<i>Zanthoxylum limonella</i> (Seed)	4.66 $\pm$ 2.16	5.33 $\pm$ 1.63	6.66 $\pm$ 1.63	16.00 $\pm$ 2.83	2.66 $\pm$ 1.63	1.33 $\pm$ 1.63
<i>Pyrethrum pet-ether</i> (Standard)	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	1.33 $\pm$ 1.63	4.00 $\pm$ 0.00

TABLE - 3
Larvicidal effect of the Petroleum-ether extract of plant material on *Culex quinquefasciatus*.

Plant	Percentage mortality at the end of 24 hrs. Mean $\pm$ S.E. n=3								Percentage mortality in control	
	Petroleum-ether extract concentration.								Acetone	Water
	0.02%	0.04%	0.06%	0.08%	0.12%	0.16%	0.20%	0.24%		
<i>Andrographis paniculata</i>	26 $\pm$ 2.45	38.66 $\pm$ 1.63	42.66 $\pm$ 1.63	56.00 $\pm$ 0.70	60.00 $\pm$ 2.83	64.66 $\pm$ 4.71	72.66 $\pm$ 2.27	88.00 $\pm$ 2.16	3.00 $\pm$ 1.22	1.33 $\pm$ 1.63
<i>Andropogon citratus</i>	19.66 $\pm$ 1.08	27.00 $\pm$ 1.22	45.33 $\pm$ 1.63	53.33 $\pm$ 3.27	62.00 $\pm$ 3.94	78.00 $\pm$ 2.83	85.00 $\pm$ 2.55	90.66 $\pm$ 3.63	2.66 $\pm$ 1.63	4.00 $\pm$ 2.83
<i>Capsicum frutescens</i>	22.66 $\pm$ 2.16	26.66 $\pm$ 1.63	36.66 $\pm$ 2.95	51.00 $\pm$ 3.94	58.66 $\pm$ 4.33	62.33 $\pm$ 2.95	75.66 $\pm$ 2.48	88.66 $\pm$ 2.48	3.00 $\pm$ 1.22	1.8 $\pm$ 1.63
<i>Cinnamomum tamala</i>	1.33 $\pm$ 1.63	4.00 $\pm$ 0.00	4.00 $\pm$ 2.83	5.33 $\pm$ 1.63	6.66 $\pm$ 1.63	8.00 $\pm$ 2.83	10.66 $\pm$ 1.63	14.66 $\pm$ 1.63	2.66 $\pm$ 1.63	1.33 $\pm$ 1.63
<i>Citrus aurantium</i>	2.66 $\pm$ 1.63	5.33 $\pm$ 1.63	6.66 $\pm$ 1.63	8.00 $\pm$ 2.83	11.33 $\pm$ 2.95	14.00 $\pm$ 2.45	17.33 $\pm$ 1.78	19.00 $\pm$ 1.41	2.66 $\pm$ 1.63	2.66 $\pm$ 1.63
<i>Coriander sativum</i>	16.00 $\pm$ 2.83	29.33 $\pm$ 1.63	38.66 $\pm$ 1.43	52.00 $\pm$ 2.83	77.00 $\pm$ 1.41	85.33 $\pm$ 2.16	89.33 $\pm$ 2.16	92.66 $\pm$ 4.82	1.8 $\pm$ 1.63	2.66 $\pm$ 1.63
<i>Cynodon dactylon</i>	2.66 $\pm$ 1.63	4.00 $\pm$ 0.00	4.00 $\pm$ 2.83	5.33 $\pm$ 1.63	8.33 $\pm$ 0.40	9.33 $\pm$ 1.63	10.66 $\pm$ 1.63	13.33 $\pm$ 2.16	1.33 $\pm$ 1.63	4.00 $\pm$ 2.83
<i>Elettaria cardamomum</i>	1.8 $\pm$ 1.63	1.33 $\pm$ 1.63	2.66 $\pm$ 0.81	4.66 $\pm$ 2.16	6.00 $\pm$ 1.41	8.66 $\pm$ 2.95	15.66 $\pm$ 2.48	17.66 $\pm$ 1.08	3.00 $\pm$ 1.08	2.60 $\pm$ 0.81

Eleusine coracana	3.00 $\pm$ 1.08	4.00 $\pm$ 2.83	5.33 $\pm$ 0.81	7.33 $\pm$ 2.95	10.66 $\pm$ 1.63	12.00 $\pm$ 1.41	16.33 $\pm$ 2.27	19.00 $\pm$ 2.55	2.66 $\pm$ 1.63	3.00 $\pm$ 1.08
Ferula asafoetida	26.66 $\pm$ 2.95	36.66 $\pm$ 2.95	46.00 $\pm$ 1.41	55.00$\pm$1.22	62.33 $\pm$ 2.95	75.66 $\pm$ 2.48	83.66 $\pm$ 1.08	88.66 $\pm$ 2.16	2.60 $\pm$ 0.81	1.8 $\pm$ 1.63
Lawsonia inermis	4.00 $\pm$ 1.63	9.33 $\pm$ 1.63	13.00 $\pm$ 1.22	15.33 $\pm$ 2.16	19.33 $\pm$ 1.63	23.33 $\pm$ 3.56	27.00 $\pm$ 1.22	33.33 $\pm$ 2.95	2.66 $\pm$ 1.63	3.00 $\pm$ 1.08
Mentha arvensis	4.00 $\pm$ 2.83	5.33 $\pm$ 1.63	6.66 $\pm$ 1.63	9.00 $\pm$ 2.55	17.33 $\pm$ 1.78	21.33 $\pm$ 2.16	37.66 $\pm$ 1.78	47.00 $\pm$ 1.22	4.00 $\pm$ 2.83	1.33 $\pm$ 1.63
Murraya koenigii	32.00 $\pm$ 2.83	48.00 $\pm$ 2.83	58.66$\pm$0.81	60.00 $\pm$ 2.83	64.66 $\pm$ 2.18	82.33 $\pm$ 1.78	92.00 $\pm$ 2.83	100.00$\pm$0.00	2.66 $\pm$ 1.63	2.60 $\pm$ 0.81
Syzygium aromaticum	1.8 $\pm$ 1.63	2.60 $\pm$ 0.81	6.33 $\pm$ 0.81	14.00 $\pm$ 1.41	16.00 $\pm$ 1.41	20.66 $\pm$ 2.16	26.66 $\pm$ 2.95	32.33 $\pm$ 1.78	3.00 $\pm$ 1.08	1.8 $\pm$ 1.63
Trigonella foenum graceum	32.00 $\pm$ 2.83	42.66 $\pm$ 4.33	45.33 $\pm$ 1.63	53.33$\pm$3.27	69.33 $\pm$ 1.63	75.66 $\pm$ 2.48	80.00 $\pm$ 1.41	94.33 $\pm$ 2.86	1.33 $\pm$ 1.63	2.60 $\pm$ 0.81
Zanthoxylum limonella (Seed)	16.00 $\pm$ 2.83	24.00 $\pm$ 2.83	38.66 $\pm$ 3.27	58.00$\pm$2.45	77.00 $\pm$ 1.41	88.66 $\pm$ 2.16	91.00 $\pm$ 1.87	93.66 $\pm$ 2.27	1.8 $\pm$ 1.63	3.00 $\pm$ 1.08
Zanthoxylum limonella (SC)	11.33 $\pm$ 1.78	16.00 $\pm$ 2.83	19.33 $\pm$ 1.63	27.33 $\pm$ 2.16	28.66 $\pm$ 0.81	32.00 $\pm$ 2.83	44.66 $\pm$ 2.16	48.33 $\pm$ 2.48	1.33 $\pm$ 1.63	4.00 $\pm$ 2.83
Pyrethrum (Standard)	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	1.33 $\pm$ 1.63	1.8 $\pm$ 1.63

TABLE - 4
Larvicidal effect of the Methanol (Succ.) extract of plant material on *Culex quinquefasciatus*.

Plant	Percentage mortality at the end of 24 hrs. Mean $\pm$ S.E. n = 3				Percentage mortality in control	
	Methanol (Succ.) extract concentration.					
	0.02%	0.04%	0.06%	0.08%	Acetone	Water
<i>Andrographis paniculata</i>	1.33 $\pm$ 1.63	2.66 $\pm$ 0.81	8.33 $\pm$ 0.40	16.33 $\pm$ 2.27	1.33 $\pm$ 1.63	2.66 $\pm$ 1.63
<i>Andropogon citratus</i>	4.00 $\pm$ 0.00	4.00 $\pm$ 2.83	8.66 $\pm$ 2.95	14.00 $\pm$ 2.45	3.00 $\pm$ 1.08	3.00 $\pm$ 1.22
<i>Capsicum frutescens</i>	9.33 $\pm$ 1.08	13.33 $\pm$ 2.95	16.33 $\pm$ 2.27	19.66 $\pm$ 1.08	2.66 $\pm$ 1.63	1.33 $\pm$ 1.63
<i>Cinnamomum tamala</i>	6.66 $\pm$ 1.63	8.00 $\pm$ 2.83	10.66 $\pm$ 1.63	16.00 $\pm$ 2.83	3.00 $\pm$ 1.08	4.00 $\pm$ 0.00
<i>Citrus aurantium</i>	5.33 $\pm$ 1.63	8.00 $\pm$ 2.83	8.66 $\pm$ 1.47	19.00 $\pm$ 1.22	3.00 $\pm$ 1.22	2.66 $\pm$ 1.63
<i>Coriander sativum</i>	1.80 $\pm$ 1.63	4.00 $\pm$ 0.00	5.33 $\pm$ 1.63	8.00 $\pm$ 2.83	1.33 $\pm$ 1.63	2.66 $\pm$ 1.63
<i>Cynodon dactylon</i>	2.66 $\pm$ 1.03	6.66 $\pm$ 1.63	3.00 $\pm$ 1.08	11.66 $\pm$ 1.47	4.00 $\pm$ 0.00	3.00 $\pm$ 1.08
<i>Elettaria cardamomum</i>	4.00 $\pm$ 0.00	4.00 $\pm$ 2.83	9.33 $\pm$ 1.08	16.00 $\pm$ 2.83	3.00 $\pm$ 1.22	4.00 $\pm$ 0.00
<i>Eleusine coracana</i>	4.00 $\pm$ 0.00	8.00 $\pm$ 2.83	11.66 $\pm$ 1.47	13.33 $\pm$ 2.95	3.00 $\pm$ 1.08	2.66 $\pm$ 1.63
<i>Ferula asafoetida</i>	1.80 $\pm$ 1.63	2.66 $\pm$ 1.63	1.33 $\pm$ 1.63	8.66 $\pm$ 1.47	1.33 $\pm$ 1.63	4.00 $\pm$ 0.00

Lawsonia inermis	3.00 $\pm$ 1.08	3.00 $\pm$ 1.22	6.66 $\pm$ 1.63	9.33 $\pm$ 1.63	3.00 $\pm$ 1.08	2.66 $\pm$ 1.63
Mentha arvensis	2.66 $\pm$ 1.63	4.00 $\pm$ 0.00	4.00 $\pm$ 2.83	4.66 $\pm$ 2.16	3.00 $\pm$ 1.22	1.33 $\pm$ 1.63
Murraya koenigii	8.66 $\pm$ 1.47	10.66 $\pm$ 1.63	00 $\pm$ 2.83	26.66 $\pm$ 1.63	2.66 $\pm$ 1.63	3.00 $\pm$ 1.22
Syzygium aromaticum	6.33 $\pm$ 1.08	11.66 $\pm$ 1.47	14.00 $\pm$ 2.45	22.66 $\pm$ 1.63	3.00 $\pm$ 1.22	2.66 $\pm$ 1.63
Trigonella foenum graecum	5.33 $\pm$ 1.63	8.00 $\pm$ 2.83	10.66 $\pm$ 1.63	14.66 $\pm$ 1.63	2.66 $\pm$ 1.63	1.33 $\pm$ 1.63
Zanthoxylum limonella (Seed)	4.66 $\pm$ 2.16	6.00 $\pm$ 1.41	13.33 $\pm$ 2.95	19.00 $\pm$ 1.22	1.33 $\pm$ 1.63	4.00 $\pm$ 0.00
Zanthoxylum limonella (Seed-coat)	2.66 $\pm$ 1.63	4.00 $\pm$ 0.00	8.00 $\pm$ 2.83	13.33 $\pm$ 2.95	1.33 $\pm$ 1.63	1.33 $\pm$ 1.63
Pyrethrum Pet-ether (Standard)	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	1.33 $\pm$ 1.63	2.66 $\pm$ 1.63

Table 5

Residual Activity of Petroleum ether extract of *Murraya koenigii* on *Culex quinquefasciatus* larvae.

Percentage Concentration	Percentage Mortality at hours.Mean $\pm$ S.E.			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.
0.24%	100.00 $\pm$ 1.63	83.66 $\pm$ 1.08	58.66 $\pm$ 0.81	46.00 $\pm$ 1.41
0.28%	100.00 $\pm$ 1.63	94.33 $\pm$ 2.86	69.33 $\pm$ 1.63	55.00 $\pm$ 1.22
Standard Pyrethrum 0.28%	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
Acetone Control	2.66 $\pm$ 1.63	1.33 $\pm$ 1.63	1.33 $\pm$ 1.63	2.66 $\pm$ 1.63
Water control	1.33 $\pm$ 1.63	2.66 $\pm$ 1.63	2.66 $\pm$ 1.63	1.33 $\pm$ 1.63

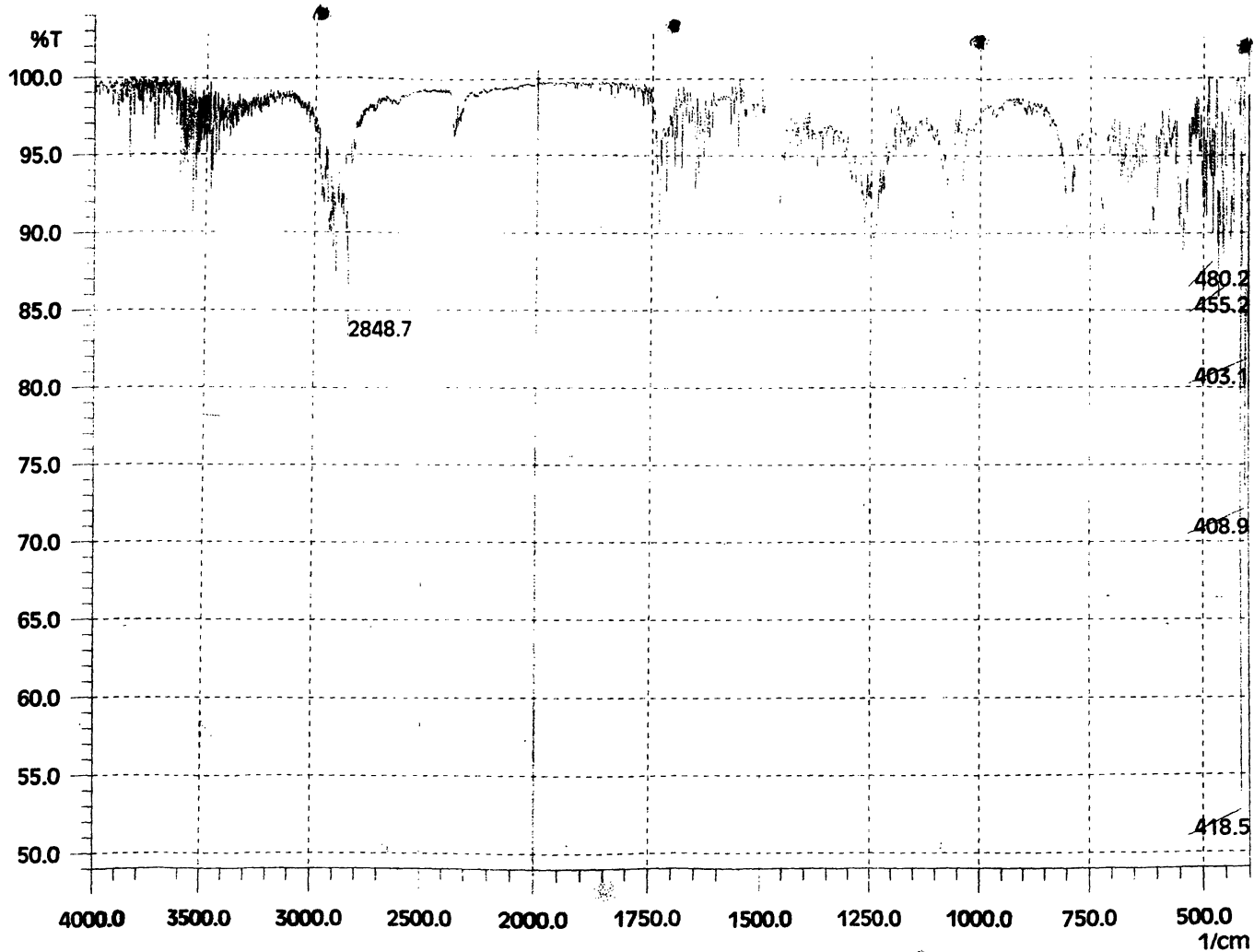
Table 6
Larvicidal effect of silica gel (60-120 mesh) column chromatographic fraction of petroleum ether extract of *Murraya koenigii*.

Fraction	Percentage mortality at the end of 24 hours . Mean± S. E. n=3	
	Percentage Concentration	
	0.20%	0.24%
Petroleum ether elute	38.66±3.27	46.00±1.41
Benzene elute	88.66±2.16	99.66±0.43
Chloroform elute	32.00±2.83	58.66±2.10
Alcohol elute	48.33±2.16	84.33±1.08
Petroleum ether extract	92.00±2.83	100.00±0.00
Acetone control	2.66±1.63	2.60±0.81
Water control	4.00±2.83	1.33±1.63

Table-7
LC₅₀ value of *Murraya koenigii* pet-ether extract

Name of the plant	LC₅₀ Value
<i>Andrographis paniculata</i>	0.06%
<i>Andropogon citratis</i>	0.07%
<i>Capsicum frutescens</i>	0.08%
<i>Cinnamomum tamala</i>	NS
<i>Citrus aurantium</i>	NS
<i>Coriander sativum</i>	0.06%
<i>Cynodon dactylon</i>	NS
<i>Elettaria cardamomum</i>	NS
<i>Eleusine corana</i>	NS
<i>Ferula asafoetida</i>	0.06%
<i>Lawsonia inermis</i>	NS
<i>Mentha arvensis</i>	NS
<i>Murraya koenigii</i>	0.04%
<i>Syzygium aromaticum</i>	NS
<i>Trigonella foenum graecum</i>	0.06%
<i>Zanthoxylum limonella</i> Seed	0.08%
<i>Zanthoxylum limonella</i> Seed-coat	NS

• NS - Non Significant



IR spectrum of Compd. M. P. 83-84°

100 102.11
104.97

%

146.15

277.38

301.56

342.62

302.56

150.24

245.38

353.53

413.81

454.87

0 100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 Da/e

Discussion

Communicable diseases constitute the most important cause for high morbidity and mortality in all age groups of human populations in developing countries. Mosquitoes transmit the most widespread and important human diseases. Attempts were made to completely eradicate this insect. Effectively, long lasting insecticides were developed during the Second World War. These compounds once found widespread use because of the long residual toxicity. These insecticides were found to induce resistance in insects and they were also toxic to mammals.

The advent of insecticides like DDT marked a new stage in the long history of control of vector borne diseases. The global program of malaria eradication initiated by World Health Organization achieved tremendous success and million of people are freed from the diseases. Other vector borne diseases like plague, leishmaniasis had tremendous impact. Unfortunately the situation did not last long. Vectors of diseases started showing resistance. 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. Nearly 1,500 compounds were screened extensively and eventually only five 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. Insecticides from plant products have distinct advantage over commercial organic synthetic insecticides, they are easily biodegradable. Further, most of the synthetic insecticides are being imported and are, therefore expensive.

India is enormously richer in natural resources of innumerable plants having insecticidal properties. This study constitutes screening of plants (Table 2, 3 & 4) for the larvicidal activity against the insects of medical importance.

Of all the plants studied *Murraya koenigii* proved to be most promising plant possessing larvicidal property having **LC₅₀ value = 0.04%** (Table 7). Equally impressive results were obtained with *Andrographis paniculata*, *Andropogon citratus*, *Capsicum frutescens*, *Coriander sativum*, *Ferula asafoetida*, *Trigonella foenum graecum*, *Murraya koenigii*, and *Zanthoxylum limonella* (seed). Throughout the course of this studies it was significantly noted that of eight-plant extract, of each plant studied the petroleum ether of *Murraya koenigii* was more toxic than the all other plants.

As far as mosquito larvicidal activity is concerned *Andrographis paniculata*, *Andropogon citratus*, *Capsicum frutescens*, *Coriander sativum*, *Ferula asafoetida*, *Trigonella foenum graecum*, *Murraya koenigii*, and *Zanthoxylum limonella* (seed). Throughout the course of this studies it was significantly noted that of eight-plant extract, of each plant studied the petroleum ether of *Murraya koenigii* was more effective than the all other plants against *Culex quinquefasciatus* larvae under standard laboratory condition.

In this connection *Murraya koenigii* being the easily cultivable and available plants in our country, its use as mosquito larvicide could

be considered. An exploratory chemical study of *Murraya koenigii* resulted in the isolation of a compound, M.P. 83-84⁰ Chemical tests and spectral data suggested it to be a long-chain fatty compound.

A comparative field trial is necessary to show value of this substance in relation to currently used material. It will be also necessary to work out the cost of material if used for field applications and how it will favor in comparison to larvicides in vogue.

SUMMARY

The growing concern about protecting the environment, recognizing the importance of ecological balance and evidence of changes in the surroundings by extensive and indiscriminate use of pesticides with persistent residues has emphasized the need to review our existing strategies for vector control. Among the several avenues explored for the integrated vector control programmes, the natural products which are safe, easily biodegradable and practically innocuous to non-target species are being investigated world over.

Most of the indigenous plants studied in the present investigations are perennially available in large quantities. The cost involved in the preparation of these extracts is very minimal. More over, solvents used for the extraction can be recovered and recycled. It was observed that only one fifth of the solvent used for the extraction is lost during the concentration of the extract. The extraction of the natural products and their use in control operation appear to be more economical and less polluting to the environment.

The present study has been carried out to determine the larvicidal activity of seventeen indigenous plants against the larvae of *Culex quinquefascitus*, a principle vector of filariasis in standard laboratory conditions. Petroleum ether, methanol (Succ.) and aqueous extracts of the plant material were prepared and screened for larvicidal activity. Among the crude extracts screened, petroleum ether and aqueous extracts of *Murraya koenigii* showed significant activity, but the percentage yield of aqueous plant extracts was very low to pursue further work. The petroleum ether extract of *Murraya koenigii* is having maximum potency for larvicidal activity at 24 hours with LC₅₀ value 0.04 % and residual effect was also encouraging.

The petroleum ether extract of *Murraya koenigii* was further subjected to fractionation. After chromatographic fractionation of petroleum ether extract of *Murraya koenigii* and evaluating various elutes for their activity, it was observed that benzene elute showed prominent activity at same concentration.

Remaining elutes didn't showed any significant results. Chemical fractionation of the petroleum ether extract of *Murraya koenigii* resulted in isolation of a compound M.P. 83-84⁰ possibly a long chain fatty moiety.

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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 : Potency of larvicidal properties of plants extracts
against mosquito larvae under laboratory conditions.

Name of the candidate : Ms. Suchita T. Desai. B.Sc.

Name and Designation

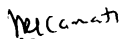
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Number and date

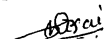
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“POTENCY OF LARVICIDAL PROPERTIES OF PLANT EXTRACTS AGAINST MOSQUITO LARVAE UNDER LABORATORY CONDITIONS”.

INTRODUCTION

One of the major problems faced by the world in public health sector is the eradication of vectors and vector-borne diseases. Amongst the various vectors, responsible for transmitting diseases, mosquitoes are the most prominent blood-sucking arthropods, capable of transmitting epidemic and endemic diseases¹. To combat the danger posed by insects, a number of chemical compounds and plant products have been introduced which are referred to as “Insecticides”.

Various synthetic insecticides are being used to control mosquito population throughout the world. A majority of them fall into three major categories viz., Organo-chlorine, Organo-phosphorus and Carbamate insecticides².

Organochlorines: Among the first of the highly effective insecticides, the best-known example of this group is DDT. Originally seen as a panacea for insect problems, these synthetic neurotoxins have been banned in many countries.

Organophosphates: Extremely toxic to human as well as insects, these substances nonetheless are widely used as they have very short persistence in the environment.

Carbamates: A synthetic version of a toxic compound found in seeds, this class of substances also breaks down quickly in the environment, but in some cases residues have been found to enter the water table.

Continued use of synthetics chemical insecticide-based intervention measures for vector control has resulted in lower efficacy of the insecticides in controlling the medically important disease vectors. In recent years use of

environment-friendly and easily biodegradable natural insecticides of plant origin has received renewed importance for disease vector control. Interest in this field has increased more so, as they are least phytotoxic and do not accumulate chemical residues in flora, fauna and soil. The rising cost of petroleum-based insecticides and increasing resistance exhibited by insect species made it necessary to examine the utility floral wealth for insecticidal activity³.

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 larvicidal properties. The present study has been carried out to determine the larvicidal activity of seventeen indigenous plants against the larvae of *Culex quinquefasciatus*, a principle vector of filariasis in standard laboratory conditions.

PLAN OF WORK

Different parts of plants under study, were collected, dried and then powdered separately. Extraction of the powdered material of the plant has been carried out using non-polar and polar solvents. Aqueous extracts of the plant material were also prepared. The portions of plant extracts have been tested for mosquito larvicidal activity at different intervals of time as per WHO specifications. Side by side, phytochemical studies on these extracts have been undertaken with a view to isolating and characterizing the active principle(s), if any.

MATERIAL AND METHODS

Since several plants have been reported to possess insecticidal properties⁴, it was a difficult job to select the material for study. However, after a literature survey, the seventeen plants were selected for the study (Table 1).

LARVICIDAL TESTING

All experimental exposures were done using 500ml glass beakers. Twenty-five larval mosquitoes, i.e., *Culex quinquefasciatus* were placed in 250ml tap water containing various concentrations of serially diluted extracts and held for 24 hours mortality count. Dead larvae were removed and counted for percentage mortality. Each experiment was carried out in triplicate. Two controls were maintained at a time; one consisted of acetone and the other tap water only. Larvae incapable of rising or submerging from the surface upon probing were considered as dead. The test were carried out under laboratory conditions at room temperature.

Out of the seventeen plants studied, petroleum-ether and aqueous extracts of *Murraya koenigii* showed significant larvicidal activity. This led to undertake chemical investigations on the *Murraya koenigii* in order to isolate the active constituent responsible for mosquito larvicidal activity. So far, hardly any pure chemical constituent has been reported possessing mosquito larvicidal activity from *Murraya koenigii*, although it has been extensively worked out for chemical constituents and other biological activities^{5,6,7,8}. All the other plant extracts did not show any appreciable larvicidal activity.

PRELIMINARY CHEMICAL STUDY OF MURRAYA KOENIGII

Powdered material of *Murraya koenigii* leaves (1.5 Kg) was extracted by using petroleum-ether (60-80°C) and methanol in succession using Soxhlet apparatus. Aqueous extracts were also obtained using conventional procedure. Different concentrations of all plant extracts were prepared in acetone and mosquito larvicidal activity was evaluated as per the method recommended by WHO⁹. Extracts showing promising results were further studied for isolation and characterization of the active constituents. Thin layer and Column chromatography using silica gel (60-

120 mesh) have been employed for isolation of chemical constituents. Preliminary TLC study of the extracts using different solvent systems indicated a presence of number of bands

Column chromatography was carried out using silica gel (60-120 mesh) and eluting the column successively with non-polar and polar solvents. Petroleum-ether extract of *Murraya koenigii* yielded a compound, m.p. 80-83°C from benzene eluate. Phytochemical study on the compound m.p 83°C is in progress for its procurement and identification.

RESULTS AND DISCUSSION

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

In case of aqueous extracts, *Zanthoxylum limonella* (seeds), *Murraya koenigii*, *Mentha arvensis*, *Eleusine coracana* and *Citrus aurantium* have shown most promising larvicidal activity at 0.08 per cent but the percentage yield of total plant extract was very low to pursue further work.

Petroleum-ether extracts of *Capscium frutescens*, *Coriander sativum*, *Trigonella foenum graceum*, *Ferula foetida*, *Murraya koenigii*, *Andrographis paniculata*, *Andropogon citratus* and *Zanthoxylum limonella* (seeds) were found to be effective at lower concentrations. These extracts showed more than 50 percent larvicidal activity at 0.08 percent concentration. Hence, the concentration has been increased to 0.24 percent and it has been seen that *Murraya koenigii* is having maximum potency for larvicidal activity at 24 hours and also the residual effect was encouraging at same concentration. All other plants did not show 100 percent mortality at the highest concentration tested (0.24 percent) and did not give promising lead for further work. Larvicidal activity of the petroleum ether and

aqueous extract of *Murraya koenigii* were significantly more potent than the all other plants. None of the methanol extracts (succ.) of plants shows any significant mortality.

After chromatographic fractionation of petroleum-ether extract of *Murraya koenigii* and evaluating various eluates for their activity, it was observed that benzene eluate showed prominent activity. This fractionation produced 100 percent mortality at 0.24 percent concentration. Remaining eluates did not show any significant results. Further chemical work on this plant is in progress.

The findings of the present investigation revealed that the *Murraya koenigii* 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.

A few relevant references are given below:

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Table 1 – INDIGENOUS PLANTS FOR LARVICIDAL ACTIVITY.

Plant (Botanical name)	Vernacular name	Part of the plant	Family
<i>Andrographis paniculata</i> Burm.f	Kirayat	Leaves	Acanthaceae
<i>Andropogon citratus</i> Stapf.	Lemmon grass	Leaves	Poaceae
<i>Capsicum frutescens</i> Linn.	Bird chilli	Fruit	Solanaceae
<i>Cinnamomum tamala</i> Nees and Eberm.	Tejpatta	Leaves	Lauraceae
<i>Citrus aurantium</i> Linn.	Bitter orange	Dried peels	Rutaceae
<i>Coriandrum sativum</i> Linn.	Coriander	Fruit	Apiaceae
<i>Cynodon dactylon</i> Linn.	Durva	Leaves	Poaceae
<i>Elettaria cardamomum</i> Maton.	Cardamom	Fruit	Zingiberaceae
<i>Eleusine coracana</i> . Gaertn.	Nachni	Grain	Graminae
<i>Ferula foetida</i> Linn.	Asafoetida	Root	Apiaceae
<i>Lawsonia inermis</i> Linn.	Heena	Leaves	Lythraceae
<i>Mentha arvensis</i> Linn.	Pudina	Leaves	Luminaceae
<i>Murraya koenigii</i> Linn.	Curry leaves	Leaves	Rutaceae

<i>Syzygium aromaticum</i> Linn.	Lavang	Fruit	Myrtaceae
<i>Trigonella foenum graceum</i> Linn.	Fenugreek	Seeds	Papilionaceae
<i>Zanthoxylum limonella</i> Linn.	Tirphal	Seeds	Rutaceae
<i>Zanthoxylum limonella</i> Linn.	Tirphal	Seeds coat	Rutaceae

