

EVALUATION OF POSSIBLE LARVICIDAL ACTIVITY OF
SOME SELECTED PLANT EXTRACTS AGAINST AEDES
AEGYPTI

THESIS SUBMITTED
UNIVERSITY OF MUMBAI

FOR THE DEGREE OF
MASTER OF SCIENCE
IN
APPLIED BIOLOGY

SUBMITTED BY
MANGESH S. CHORGE

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

HAFFKINE INSTITUTE FOR TRAINING, RESEARCH & TESTING
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Mr. Mangesh S. Chorge

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Research & Testing.
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(2005)

**DEDICATED
TO
MY PARENTS AND TEACHERS**



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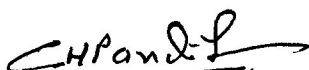
Statement under university Regulation No. 2190

As required by the University Regulation No. 2190, I wish to state that the work embodied in this thesis titled "**Evaluation of possible larvicidal activity of some selected plant extracts against *Aedes aegypti***" forms my own contribution to the research work carried out under the guidance of Dr.(Mrs.) C. H. Pandit at the Haffkine Institute, Mumbai. This work has not been submitted for any other degree of this or any other University. Whenever references have been made to previous works of others, it has been clearly indicated as such and included in the 'Bibliography'.



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Signature of guiding Teacher

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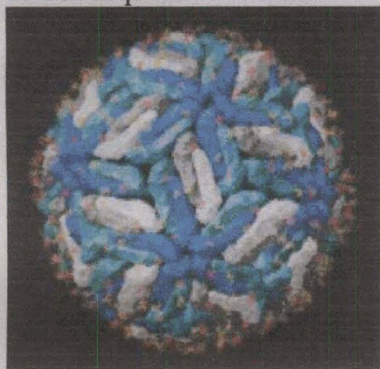
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INTRODUCTION &
REVIEW OF
LITERATURE

INTRODUCTION & REVIEW OF LITERATURE

Vector borne diseases take a heavy toll of human life. About one million people die and few hundred millions suffer from insect borne diseases. The first record of insect as a vector of disease was made by Sir Patric Manson in 1878 when he described the development of filarial worm *Wuchereria bancrofti* in the body of mosquito. In 1897, Sir Ronald Ross announced the discovery of malarial parasite in the mosquito. It is now considered that insects are involved in the transmission of at least 27 diseases of serious consequences to man.³



Dengue virus

Today, dengue is considered the most important arthropod-born viral disease. Dengue is an acute disease caused by four dengue virus serotypes, DEN-1, DEN-2, DEN-3 and DEN-4. All serotypes produce a similar illness characterized by fever, intense headache, nausea and vomiting and induce life long immunity that is specific to the infecting serotype. Its severe form is Dengue Hemorrhagic Fever (DHF). This is characterized by fever, thrombocytopenia, excessive capillary permeability that may lead to Dengue Shock Syndrome (DSS) and with early diagnosis and proper management the Case Fatality Rate (CFR) of DHF is generally under 1% but CFR may be over 10% once shock develops. Dengue virus causes 50-100 million cases of DF and several hundred thousand cases of DHF each year.

Dengue transmission requires the simultaneous presence of three

factors:

- 1) susceptible human 2) a competent mosquito vector and 3) dengue virus. variation in any of these may promote or hinder disease activity. ⁽¹⁾ Since Dengue hemorrhagic fever (DHF) was first described in the mid 1950s, the occurrence of the disease has largely spread. Dengue infection still continues its geographic expansion over Southeast Asia, America, and the Pacific region. A number of complex factors are related to the emergence and the re-emergence of dengue, particularly population growth, unplanned urbanization, and increased travel by airplane which facilitates the expansion of vectors and dispersion of viruses. In India as in most of countries in Southeast Asia, transmission of dengue viruses to humans is ensured mainly by *Ae. aegypti*, a day biter mosquito. ²⁵

Taxonomic classification of *A. aegypti* is as follows:

Phylum - Arthropoda,
Class - Insecta
Sub Class - pterygota
Order - Diptera
Suborder - Nematocera
Family - Culicidae
Sub Family - Culicinae
Genus - Aedes
Species - *Aedes aegypti*

Life cycle and Information on *Aedes aegypti* Mosquitoes

Oviposition: Takes place in the afternoon in dark-colored water containing organic material (e.g., decaying leaves) in dark-colored containers with wide openings. Containers located in the shade are preferred. Females lay their eggs singly on the sides of the container at the water line in batches of 30-50. Egg laying occurs over a period of several days.

Eggs: (Photo on page no.13) Exposure to high humidity at the water line for 2-3 days is required for larvae to hatch from their eggs. However, if the eggs dry out before this development period, they will collapse and the embryos will die. If they remain unhatched above the water line, and the level of humidity is sufficient to permit larval-embryo development, eggs become "curved." This means they are resistant to desiccation and can survive for upwards of six or more months. They can also survive short periods of subfreezing weather. Later, when exposed to water, the eggs will hatch within a day or perhaps even within minutes. The eggs do not all hatch with a single

inundation, however. Instead, they hatch in progressively smaller numbers through a succession of inundations.



Larvae: The 4 larval stages (instars) take 5-10 days for development (some texts say 5-7 days).



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



Adults: The life span for adult mosquitoes is between 2 weeks to a month or more.

Flight Range: Usually 50-100 meters. Ranges have also been expressed as 100 feet to 100 yards, and 25 to 500 yards. However, new studies are indicating that this mosquito may be capable of flying longer distances - as far as 850 meters or half a mile or so.^{27,76}

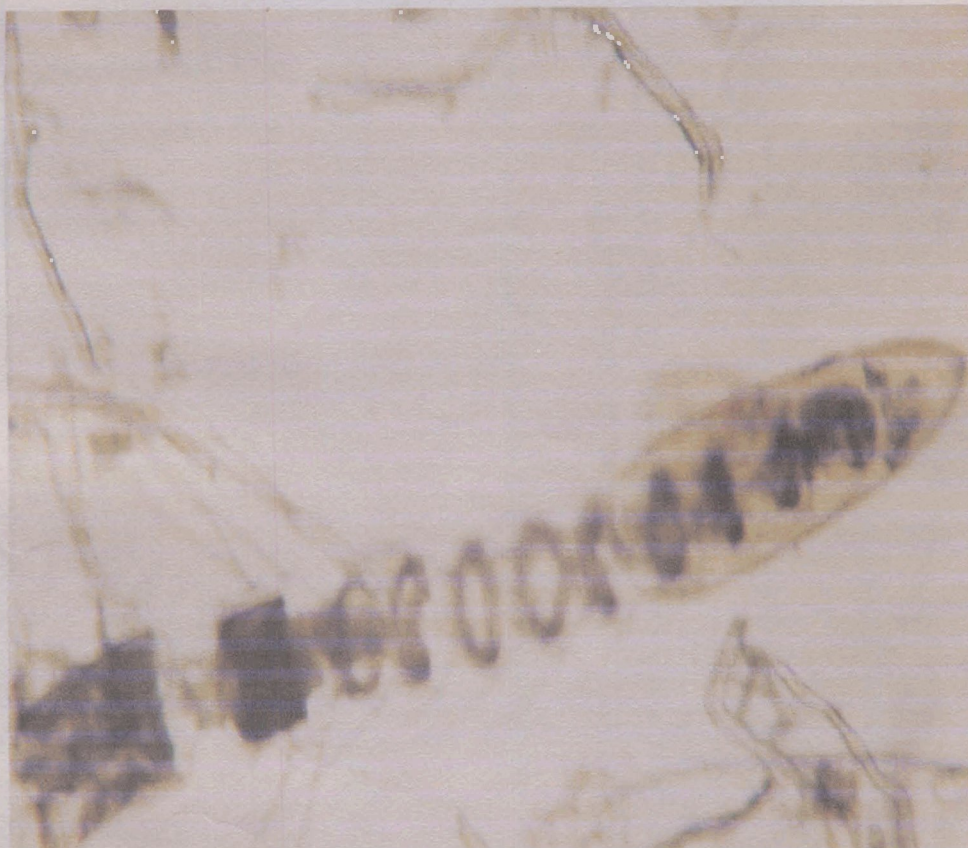
Following are the photographs taken under low power of light microscope:



Egg of *Aedes aegypti*



Embryo inside the Egg



First Instar hatching from the Egg

Prevalence of Disease in India⁷³

In India the dengue outbreak was reported for the first time in Vellore district of Tamil Nadu in south India in the year 1956. In the 1963, first time the virus isolation of DHF was reported (in *Aedes aegypti*) in Calcutta, West Bengal. After that many outbreaks of dengue/DHF have occurred in various parts of India including Visakhapatnam (1964), Madras (1965), Jabalpur (1966), Delhi(1967), Kanpur(1968), Ajmer(1969), Gwalior(1970), Bangalore(1970&71), Jaipur(1973), Jammu(1974) and Delhi (1982).

In Maharashtra in the year 1965 Dengue-4 serotype was reported in Nagpur and Dengue-2&3 serotypes were detected in Miraj (1986). The disease generally occurs in epidemic form during late monsoon and post monsoon periods. However, outbreaks of considerable magnitude have also been reported during pre monsoon & summer season as a result of water scarcity & consignment storage of water leading to building up of the vector population.

In Mumbai city, incidence of dengue was suspected in 1997, where some of the areas of Wadala (E), Kurla (L/Ward), Ghatkopar(N/ward) & other suburban areas were highly suspected. Recently, in 2002 there were some suspected cases of Dengue in New Mumbai areas. In Department of Paediatrics, Lokmanya Tilak Municipal Medical College & General Hospital, Mumbai, from 20 June to 14 November 2002, Of 90 children screened, 16 (17.8 per cent) had dengue. All responded well to the treatment and went home. Two clinical features were significantly associated with dengue, namely arthralgia ($p = 0.020$) and thrombocytopenia ($p = 0.001$). If both these features were present, the chances of the child having dengue increased significantly ($p = 0.001$).⁷⁴

Current Status of Dengue Cases in Mumbai :

Year	2005		2004		2003		2002	
Months	Cases-- Deaths		Cases-- Deaths		Cases-- Deaths		Cases-- Deaths	
January	2	0	0	00	02	00	00	00
February	1	0	03	00	05	00	01	01
March	2	0	01	00	06	00	00	00
April	0	0	02	00	02	00	00	00
May	1	0	00	00	04	00	00	00
June	2	0	03	00	06	00	02	00
July	2	0	04	00	15	00	00	00
August	222	13	08	01	42	00	04	00

September	373	2	21	00	78	02	23	00
October	75	2	40	02	111	06	80	00
November	-		50	01	60	02	22	00
December	-		20	01	03	00	03	00
Total	680	17	152	05	334	10	135	01

Current Status of Dengue Cases in New Mumbai :

Year	2005		2004		2003	
Months	Susp. : Conf.		Susp. : Conf		Susp. : Conf	
January	06	01	03	00	16	03
February	11	0	10	01	05	01
March	05	0	07	00	03	01
April	02	0	05	01	00	00
May	01	0	03	00	00	00
June	09	05	09	05	06	00
July	13	05	29	11	09	02
August	72	16	33	10	34	26
September	62	16	42	11	70	37
October	33	01	58	25	98	04
November	13	No reports	32	06	59	20
December	--		11	00	27	00
Total	227	46	242	70	327	94

Synthetic Insecticides and Health Hazards:

Although synthetic insecticides are proved to be effective in pest control its frequent use causes major problems such as health hazards either by inhalation or ingestion of pesticide or its residue along with the food crop. A review of studies on organo-chlorine insecticides by Attri (1979) indicated that residual DDT in the ecosystem caused shell thinning in carnivorous birds, liver enlargement and increased metabolism of estrogen which leads to serious endocrine imbalance in rats.⁵ Banarjee et al. (1983) showed that DDT fed rats had significantly

higher amount of DDT (and its metabolites), in body fat, liver, and blood as compared to controls.⁶

As a result these insecticides were banned in many advanced countries like USSR, USA, France, etc. In these countries, organophosphorous insecticides replaced the organochlorinated insecticides. Presently malathion, pirimphos-methyl are the most widely used insecticides in many countries like USA and Japan. Even our country spends a considerable amount of foreign exchange on import of these insecticides. Although these insecticides are biodegradable, Du Bois (1971) has shown that these insecticides inhibit cholinesterase, an enzyme indispensable in the nervous system.⁷ Jankyn et al. (1979) implicated these insecticides in hematopoietic dysfunction.⁸

Alternative to Synthetic Insecticides:

Thus, all the different insecticides discussed above suffer from drawbacks such as toxicity and / or high cost. Due to the public concern over the toxicity & environmental impact of conventional synthetic insecticides a novel kind of mosquito control is necessary. In order to take important step in exploration and utilization of naturally occurring products for combating harmful mosquitoes, we decided to evaluate leaves extract of following plants.

- 1) *Annona Squamosa* (Annonaceae)
- 2) *Azadirachta indica* (Meliaceae)
- 3) *Carica Papaya* (Caricaceae)
- 4) *Ficus religiosa* (Moraceae)
- 5) *Ficus Recemosa* (Moraceae)



***Annona Squamosa*, (Annonaceae)** The most widely grown of all the species of *Annona*, the sugar apple, *A. squamosa* L., has acquired various regional names: *anon* (Bolivia, Costa Rica, Cuba, Panama); *anon de azucar*, *anon domestico*, *hanon*, *mocuyo* (Colombia); *anona blanca* (Honduras, Guatemala, Dominican Republic); *anona de castilla* (El Salvador); *anona de Guatemala* (Nicaragua); applebush (Grenadines); *ata*, *fruta do conde*, *fruta de condessa*, *frutiera deconde*, *pinha*, *araticutitaia*, or *ati* (Brazil); *ates* or *atis* (Philippines); *atte* (Gabon); *chirimoya* (Guatemala, Ecuador); *cachiman* (Argentina); *cachiman cannelle* (Haiti); *kaneelappel* (Surinam); *pomme cannelle* (Guadeloupe, French Guiana, French West Africa); *rinon* (Venezuela); *saramulla*, *saramuya*, *ahate* (Mexico); *scopappel* (Netherlands Antilles); sweetsop (Jamaica, Bahamas); *ata*, *luna*, *meba*, *sharifa*, *sarifa*, *sitaphal*, *sita pandu*, custard apple, scaly custard apple (India); *bnah nona*, *nona*, *seri kaya* (Malaya) *manonah*, *noinah*, *pomme cannelle du Cap* (Thailand); *qu a na* (Vietnam); *mang cau ta* (Cambodia); *mak khbieb* (Laos); *fan-li-chi* (China).

Description

The sugar apple tree ranges from 10 to 20 ft (3-6 m) in height with open crown of irregular branches, and some-what zigzag twigs. Deciduous leaves, alternately arranged on short, hairy petioles, are lanceolate or oblong, blunt tipped, 2 to 6 in (5-15 cm) long and 3/4 to 2 in (2-5 cm) wide; dull-green on the upperside, pale, with a bloom, below; slightly hairy when young; aromatic when crushed. Along the branch tips, opposite the leaves, the fragrant flowers are borne singly or in groups of 2 to 4. They are oblong, 1 to 1 1/2 in (2.5-3.8 cm) long, never fully open; with 1 in (2.5 cm) long, drooping stalks, and 3 fleshy outer petals, yellow-green on the outside and pale-yellow inside with a purple or dark-red spot at the base. The 3 inner petals are merely tiny scales. The compound fruit is nearly round, ovoid, or conical; 2 1/3 to 4 in (6-10 cm) long; its thick rind composed of knobby segments, pale-green, gray-green, bluish-green, or, in one form, dull, deep-pink externally (nearly always with a bloom); separating when the fruit is ripe and revealing the mass of conically segmented, creamy-white, glistening, delightfully fragrant, juicy, sweet, delicious flesh. Many of the segments enclose a single oblong-cylindric, black or dark-brown seed about 1/2 in (1.25 cm) long. There may be a total of 20 to 38, or perhaps more, seeds in the average fruit. Some trees, however, bear seedless fruits.

Origin and Distribution

The original home of the sugar apple is unknown. It is commonly cultivated in tropical South America, not often in Central America, very frequently in southern Mexico, the West Indies, Bahamas and Bermuda, and occasionally in southern Florida. In Jamaica, Puerto Rico, Barbados, and in dry regions of North Queensland, Australia, it has escaped from cultivation and is found wild in pastures, forests and along roadsides. The Spaniards probably carried seeds from the New World to the Philippines and the Portuguese are assumed to have introduced the sugar apple to southern India before 1590. It was growing in Indonesia early in the 17 th century and has been widely adopted in southern China, Queensland, Australia, Polynesia, Hawaii, tropical Africa, Egypt and the lowlands of Palestine. Cultivation is most extensive in India where the tree is also very common as an escape and the fruit exceedingly popular and abundant in markets. The sugar apple is one of the most important fruits in the interior of Brazil and is conspicuous in the markets of Bahia.

Climate

The sugar apple tree requires a tropical or near-tropical climate. It does not succeed in California because of the cool winters though in Israel it has survived several degrees below freezing. Generally, it does best in dry areas and it has high drought tolerance. However, in Ceylon it flourishes in the wet as well as the dry zones from sea level to 3,500 ft (1,066 m) elevation. During the blooming season, drought interferes with pollination and it is, therefore, concluded that the sugar apple should have high atmospheric humidity but no rain when flowering. In severe droughts, the tree sheds its leaves and the fruit rind hardens and will split with the advent of rain.

Soil

The sugar apple is not particular as to soil and has performed well on sand, oolitic limestone and heavy loam with good drainage. Water-logging is intolerable. The tree is shallow-rooted and doesn't need deep soil. Irrigation water containing over 300 ppm chlorine has done the tree no harm.

Propagation

Sugar apple seeds have a relatively long life, having kept well for 3 to 4 years. They germinate better a week after removal from the fruit than when perfectly fresh. Germination may take 30 days or more but can be hastened by soaking for 3 days or by scarifying. The percentage of germination is said to be better in unsoaked seeds. While the tree is generally grown from seed, vegetative propagation is practiced where the crop is important and early fruiting is a distinct advantage.

Seedlings may be budded or grafted when one-year old. In India, selected clones grafted on *A. reticulata* seedlings have flowered within 4 months and fruited in 8 months after planting out, compared with 2 to 4 years in seedlings. The grafted trees are vigorous, the fruits less seedy and more uniform in size. *A. senegalensis* is employed as a rootstock in Egypt. *A. glabra* is suitable but less hardy. The sugar apple itself ranks next after *A. reticulata* as a rootstock. In India, budding is best done in January, March and June. Results are poor if done in July, August, November or December unless the scions are defoliated and debudded in advance and cut only after the petioles have dehisced. Side-grafting can be done only from December to May, requires much skill and the rate of success has not exceeded 58.33%. Shield-budding gives 75% success and is the only commercially feasible method.

Inarching is 100% successful. Cuttings, layers, airlayers have a low rate of success, and trees grown by these techniques have shallow root systems and cannot endure drought as well as seedlings do.

Culture

In Egypt, sugar apple trees are spaced at 10 x 10 ft (3x3 m) in order to elevate atmospheric humidity and improve pollination. Palestinian growers were spacing at 16 x 16 ft (5x5 m) but changed to 16 x 10 ft (5x3 m) as more feasible. On light soils, they apply 132 to 176 lbs (60-80 kg) manure per tree annually and they recommend the addition of nitrogen. Commercial fertilizer containing 3% N, 10 % P and 10% K significantly increases flowering, fruit set and yield. Judicious pruning to improve shape and strength of tree must be done only in spring when the sap is rising, otherwise pruning may kill the tree. Irrigation during the dry season and once during ripening will increase fruit size.

Cropping and Yield

Seedlings 5 years old may yield 50 fruits per tree in late summer and fall. Older trees rarely exceed 100 fruits per tree unless hand-pollinated. With age, the fruits become smaller and it is considered best to replace the trees after 10 to 20 years. The fruits will not ripen but just turn black and dry if picked before the white, yellowish or red tint appears between the rind segments, the first signs of separation. If allowed to ripen on the tree, the fruit falls apart.

Keeping Quality

In India, mature fruits treated with 50-60 g carbide ripened in 2 days and thereafter remained in good condition only 2 days at room temperature, while those packed in straw ripened in 5-6 days and kept well for 4 days.

Storage trials in Malaya indicate that the ripening of sugar apples can be delayed by storage at temperatures between 59° and 68°F (15°-20°C) and 85-90% relative humidity, with low O₂ and C₂ H₂. To speed ripening at the same temperature and relative humidity, levels of O₂ and CO₂ should be high. Storing at 39.2°F (4°C) for 5 days resulted in chilling injury.

Pests and Diseases

In Florida and the Caribbean, a seed borer (chalcid fly), *Bephratelloides cubensis*, infests the seeds and an associated fungus mummifies the partly grown fruits on the tree. This has discouraged many from growing the sugar apple, though in the past it was a fairly common dooryard fruit tree. Similar damage is caused by *B. maculicollis* in Colombia, Venezuela and Surinam, by *B. ruficollis* in Panama, and *B. paraguayensis* in Paraguay. The soft scale, *Philephedra* sp., attacks leaves and twigs and deposits honeydew on which sooty mold develops. Ambrosia beetles lay eggs on young stems and the larvae induce dieback during the winter.

The mealybug is the main pest in Queensland, Australia, but is easily controlled. The green tree ant is a nuisance because of the nests it makes in the tree. Bird and animal predators force Indian growers to cover the tree with netting or pick the fruits prematurely and ripen them in straw.

A serious leaf blight in India is caused by the fungus *Colletotrichum anthoniicola*. In 1978 a new fruit rot of sugar apple was observed in India, beginning with discoloration at one end which turns brown or black in 4 or 5 days, and 2 or 3 days later the entire fruit starts to rot. Later, the fruit is covered with gray-black mycelium and spherical bodies. The isolated fungus was identified as the *Colletotrichum* state of *Glomerella cingulata*.

Food Uses

The ripe sugar apple is usually broken open and the flesh segments enjoyed while the hard seeds are separated in the mouth and spat out. It is so luscious that it is well worth the trouble. In Malaya, the flesh is pressed through a sieve to eliminate the seeds and is then added to ice cream or blended with milk to make a cool beverage. It is never cooked.

Toxicity

The seeds are acrid and poisonous. Bark, leaves and seeds contain the alkaloid, anonaine. Six other aporphine alkaloids have been isolated from the leaves and stems: corydine, roemerine, norcorydine, norisocarydine, isocorydine and glaucine. Aporphine, norlaureline and dienone may be present also. Powdered seeds, also pounded dried fruits serve as fish poison and insecticides in India. A paste of the seed powder has been applied to the head to kill lice but must be kept away from the eyes as it is highly irritant and can cause blindness. If applied to the uterus, it induces abortion. Heat-

extracted oil from the seeds has been employed against agricultural pests. Studies have shown the ether extract of the seeds to have no residual toxicity after 2 days. High concentrations are potent for 2 days and weaken steadily, all activity being lost after 8 days. In Mexico, the leaves are rubbed on floors and put in hen's nests to repel lice.

Other Uses

The **seed kernels** contain 14-49% of whitish or yellowish, non-drying oil with saponification index of 186.40. It has been proposed as a substitute for peanut oil in the manufacture of soap and can be detoxified by an alkali treatment and used for edible purposes. The leaves yield an excellent oil rich in terpenes and sesquiterpenes, mainly B-caryophyllene, which finds limited use in perfumes, giving a woody spicy accent.

Fiber extracted from the bark has been employed for cordage. The **tree** serves as host for lac-excreting insects.

Medicinal Uses: In India the crushed leaves are sniffed to overcome hysteria and fainting spells; they are also applied on ulcers and wounds and a leaf decoction is taken in cases of dysentery. Throughout tropical America, a decoction of the leaves alone or with those of other plants is imbibed either as an emmenagogue, febrifuge, tonic, cold remedy, digestive, or to clarify the urine. The leaf decoction is also employed in baths to alleviate rheumatic pain. The green fruit, very astringent, is employed against diarrhea in El Salvador. In India, the crushed ripe fruit, mixed with salt, is applied on tumors. The bark and roots are both highly astringent. The bark decoction is given as a tonic and to halt diarrhea. The root, because of its strong purgative action, is administered as a drastic treatment for dysentery and other ailments.

Food Value Per 100 g of Edible Portion*

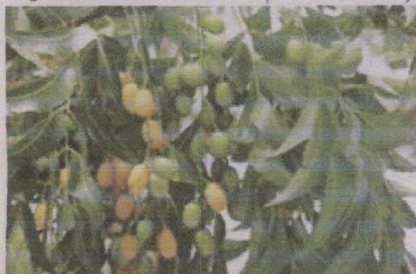
Calories	88.9-95.7 g
Moisture	69.8-75.18 g
Fat	0.26-1.10 g
Carbohydrates**	19.16-25.19 g

Crude Fiber	1.14-2.50 g
Protein	1.53-2.38 g
<i>Amino Acids:</i>	
Tryptophan	9-10 mg
Methionine	7-8 mg
Lysine	54-69 mg
<i>Minerals:</i>	
Ash	0.55-1.34 mg
Phosphorus	23.6-55.3 mg
Calcium	19.4-44.7 mg
Iron	0.28-1.34 mg
<i>Vitamins:</i>	
Carotene	5-7 I.U.
Thiamine	0.100-0.13 mg
Riboflavin	0.113-0.167 mg
Niacin	0.654-0.931 mg
Ascorbic Acid	34.7-42.2 mg

*Minimum and maximum levels of constituents from analyses made in the Philippines, Central America and Cuba.

**The average sugar content is 14.58% and is about 50-50 glucose and sucrose.⁷⁵

Azadirachta indica (Meliaceae) (Neem) :



Azadirachta indica has been used for centuries as the country store of developing nations. Earliest reference to it is in Sanskrit writings that are over 4,000 years old.⁵⁶

Parts of this tree have been used for medicine, shade, building materials, fuel, lubrication, and most of all as pesticides. It is the use of this tree as an insecticide that now draws interest from industrialized countries. It is seen as an environmentally safe alternative to synthetic pesticides. To date over 195 species of insects are affected by this trees extracts at concentrations ranging from 0.1 to 1,000 ppm, and insects that have become resistant to synthetic pesticides are controllable with these extracts.⁵⁸

THE NEEM TREE

Azadirachta indica, commonly referred to in many countries as the neem tree, is a member of the Meliaceae family. This broad-leaved evergreen can reach heights of 30 meters with a trunk girth of 2.5 meters and live for over two centuries. Its deep root system is well adapted to retrieving water and nutrients from the soil profile, but this deep root system is very sensitive to waterlogging. The neem tree thrives in hot, dry climates where shade temperatures often reach 50 degrees celsius and annual rainfall ranges from 400 to 1,200 millimeters. The tree can withstand many environmental adversities including drought and infertile, stony, shallow, or acidic soils. The neem produces ellipsoidal drupes, that are about two centimeters in length, borne on axillary clusters. These fruits contain kernels that have high concentrations of secondary metabolites⁶¹. There is evidence, but no scientific correlation, that trees grown in climates with lower rainfall produce kernels with higher content of metabolites⁶⁴.

The neem tree is believed to have originated in Assam and Burma of South Asia, but other reports suggest various areas of Pakistan, Sri Lanka, Thailand, Malaysia, and Indonesia⁶¹. The tree also grows well in other tropical and

subtropical areas around the world ⁶⁸. This is very important to commercial neem extract production so that a broad raw material base for industrial refinement can be established. Neem trees have successfully been established in Australia, Haiti, West Africa, the Dominican Republic, Ecuador, Puerto Rico, the Virgin Islands, and in the continental United States in Florida, California, Oklahoma, and Arizona ^{51,64,68}. The trees growing in Arizona are part of a breeding and selection program aimed at developing a variety that will be frost tolerant to temperatures as low as 18 degrees below zero celsius. Such a development would allow this tree to be established in many more regions. Cultivation of the neem tree is also an important consideration as the tree is established in new regions. Very little problems arise in vegetative propagation. Transplanting seedlings, saplings, or root suckers achieves a high success rate ⁶¹. Seeds are more desirable to use when transporting a long distance for ease of packing, however, minor problems have been observed when growing these trees from seeds. It was found that dry or unripe seeds would rot in soil. Large scale establishment of neem trees required germination in sand, transplanting to clay pots after a month, and then planting in the field when the seedlings reached 30 to 45 centimeters in height ⁵¹.

NEEM CHEMISTRY

The chemicals that have pesticidal activity can most efficiently be extracted from neem seed kernels. Neem trees begin their reproductive stage at about three to five years of age but don't become fully reproductive until they are ten years old. From this time on, the tree will yield an average of about 20.5 kilograms of fruit per year, with maximum production reaching 50 kilograms per year ⁶¹.

Of the fruit yield, only about ten per cent is attributed to seed kernels, and desired biologically active compounds comprise only ten grams per kilogram of kernel weight. This means that an adult neem tree will only produce about 20 grams of pesticidal compounds in a season ⁶⁵.

Many biologically active compounds can be extracted from neem, including triterpenoids, phenolic compounds, carotenoids, steroids, and ketones. The tetranortriterpenoid azadirachtin has received the most attention as a pesticide because it is relatively abundant in neem kernels and has shown biological activity on a wide range of insects. Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtin-A to azadirachtin-G with azadirachtin-A being present in the highest

quantity and azadirachtin-E regarded as the most effective insect growth regulator⁶⁵. Many other compounds have been isolated that show antifeedant activity as well as growth regulating activity on insects. Polar and non-polar extractions yield about 24 compounds other than azadirachtin that have at least some biological activity^{51,64}. This cocktail of compounds significantly reduces the chances of tolerance or resistance developing in any of the affected organisms. However, only four of the compounds in neem have been shown to be highly effective in their activity as pesticides: azadirachtin, salannin, meliantriol, and nimbin⁶¹. These compounds can be extracted by many methods. Leaching with water is the oldest method and is still used by some firms to selectively extract azadirachtin. On the other hand, most companies are using more non-polar solvents to obtain a more varied mixture of chemicals. Hexane, pentane, ethanol, methanol, esters, and dichloromethane are used in extractions as well as mixtures of these solvents with water⁶¹. Once extracted, several separation techniques are often incorporated to isolate compounds. For instance, in the isolation and identification of 7-deacetyl-17 α -hydroxyazadiradione, researchers used insect bioassays to guide reverse phase HPLC fractionation, IR spectrum analysis, ¹³C NMR and ¹H NMR spectrum analysis, and mass spectrum to determine the structure of the active compound. By using laboratory techniques, it is possible to closely mimic azadirachtin as it has been identified from the neem tree. Anderson et al. (1990) and Kolb et al. (1991) each describe processes in which they synthesized roughly half of separate ends of the azadirachtin molecule. These subunits form a compound that has similar but less activity than the natural molecule. The activity of synthetic azadirachtin compares close enough to natural products to verify that azadirachtin is the primary toxic compound in neem⁶⁸. Because of the great number of reactions involved in each process, synthetic azadirachtin will be very costly to produce. For this reason, companies developing azadirachtin as a commercial pesticide are working with natural products. W. R. Grace & Co., NPI, and Safer Ltd. are all trying to produce low cost, yet effective, neem-based pesticides⁶⁸. Research is discovering that initial by-products of azadirachtin extraction have significant efficacy on pests also. Neem seed oils have detrimental effects on viruses, mites, and early larval stages of some insects, while the solid seed residue has enough residual chemical content to have activity on soil borne fungal pathogens and plant parasitic nematodes⁶⁵.

NEEM EFFECTS

The mode of action of neem extracts is not understood very well. It is quite possible that the different chemicals or different ratios of chemicals found in neem trees have varied effects on insects. There is also evidence given in many research studies, that insect species react quite differently to compounds from the neem tree. More research has been conducted to find the primary mode of action of azadirachtin than of any other chemical in the neem tree. This is because of interest in it as a product for commercial use. Azadirachtin alone probably has several modes and sites of action. Primary of which is an interference with the neuroendocrine system in insects which controls the synthesis of ecdysone and juvenile hormone. It has been indicated by Schmutterer (1988) that interference involves the inhibition of the release of these hormones. Indication of this was an accumulation of large quantities of stainable neurosecretory material in the corpora cardiaca of *Locusta migratoria*. In this insect, azadirachtin regulated juvenile hormone titer to prevent vitellogenin production in females, causing sterility. This and other research has convinced many people that azadirachtin definitely has antihormonal activity. However, other evidence indicating that control of hormone concentrations is controlled indirectly leads to the conclusion that azadirachtin is not a true antihormone.

The effect of azadirachtin as an antihormone on juvenile hormone titer was also investigated in the variegated cutworm by Koul et al. (1991). Their goal was to either eliminate or reproduce the effect of azadirachtin on metamorphic abnormalities

by artificially raising the concentrations of juvenile hormones I and II or BEPAT, a juvenile hormone esterase inhibitor. They were unable to achieve any desired outcome, but ligation experiments did indicate that the region of activity was in the head capsule. The possibility was proposed that an inhibition of the synthesis of a neurosecretory protein could alter titer levels⁵⁴. So while azadirachtin has activity on hormone levels, it may be an indirect relationship indicating that azadirachtin is not a true antihormone. Evidence at this time is not conclusive on the matter of primary mode and site of action, and researchers involved admit that much more investigation is necessary to unwind the mystery⁶³. Other research has indicated a more direct role in the inhibition of molting. Direct cytotoxic effects on imaginal discs and epidermal cells result in primary lesions that prevent molting⁵⁴. Azadirachtin has also been proved to be a chitin synthesis inhibitor, but the role of this inhibition as the primary mode of action has not been investigated⁶³.

Neem extracts have many effects on insects. The antifeedant and growth regulating effects are the most valued in pest management as these are the

most intense effects on the widest range of insects. Other secondary effects that have been studied include repellency, antioviposition, sterility, fecundity reduction, loss of flying ability, disrupting sexual communication, and reducing guttural motility^{61,64}.

EFFICACY STUDIES

Research has shown that many organisms are sensitive to neem extracts. These include insects from several orders, mites, nematodes, snails, fungi, and viruses⁶¹. Insect control is now the primary use of neem and has been found to be effective against insects by several methods stated earlier. The growth regulation and feeding deterrence of azadirachtin are receiving the most attention, and other effects are studied secondarily as the experiment enables. This is not so much because these responses are less important, but not as many insects show sensitivity. Insects have shown the most sensitivity to azadirachtin as a growth regulator. Metamorphic stages are affected in such a way that death often occurs during the molting process. These results are not only dose dependent, but also, response increases with earlier larval stages. Not all species react the same though. A few insects show no mortality or metamorphic abnormalities until the final molt to an adult insect, at which time very high rates of death are observed. Molting inhibition can be seen at very low topical and ingestion rates, one ppm. Even though this was in a laboratory, field rates are effective at rates much lower than those required to elicit other responses⁶⁴. Antifeedant effects have received much attention especially in crops that suffer from excessive insect damage. Response by insects to neem extract applications varies greatly across the spectrum of sensitive insects. Even within an order this can be seen. The desert locust is believed to be the most sensitive insect to antifeedant effects of azadirachtin, but the migratory grasshopper feeds undeterred on cabbage treated with 500 ppm, a rate that would deter many other insect species. Growth reduction as a preliminary indication of food refusal can be seen at 0.1 ppm azadirachtin, but antifeedant activity often requires higher concentrations, usually over 200 ppm. In *Rhodnius prolixus*, antifeedant activity is observed at 600 times the amount needed to disrupt development. Gustatory and non-gustatory sensilla as well as reduced guttural motility may contribute to deterrent responses^{51,64}. With a large number of organisms being affected by neem extracts, concern was expressed for the welfare of beneficial organisms under management programs using

neem tree extracts. It has been found, however that predator and parasitoid insects are relatively unaffected when their life cycle involves exposure to neem extracts. Evidently, azadirachtin does not affect these insects in the same way or not enough chemical is taken up in their diet to cause behavioral or metamorphic abnormalities. Some parasitoids showed slight toxic affects when emerging from treated mummified hosts, but these parasitoids were likely exposed to a much higher dose than normal. Further longevity studies are warranted to determine if extracts have any effects on reproduction or alter fitness of natural enemies. The effects of neem on other desirable organisms have led to similar conclusions. In a study conducted by Shapiro et al.(1994), mortality of the gypsy moth was evaluated in the presence of a virus pathogen and also when the moth and virus were subject to neem treatments. Not only did the extracts have no adverse effect on viral activity but, when applied concurrently, moths died sooner. A neem product has shown no toxicity to honeybee workers at rates of 500 ppm. Earthworms actually benefit from soil application of neem by-products with increased weight gain and more progeny⁶⁵. And spiders, butterflies, ants, and ladybugs also show no detrimental effects from exposure to neem tree extracts⁶¹.

APPLICATION PROBLEMS

One of the main problems of using neem treatments is the durability of azadirachtin in field conditions. The activity of neem-based products subsides rapidly, lasting four to eight days, meaning that many applications will likely be needed in a season. The primary means of this is photodegradation by ultra-violet light. But leaf pH can also affect detoxification rates, and rain can wash residue off leaf surfaces. Derivation of natural product stabilizes azadirachtin and may provide an avenue for greatly increasing its residual activity. Also, activity can be extended in plants, such as potato and tomato, that demonstrate systemic activity. This protects azadirachtin from light and through translocation enables protection of new growth which is often preferred by insects. Systemic activity in plants also relates to a greater chance of phytotoxicity. Potato, onion, cabbage, and chrysanthemum have demonstrated various types and extent of phytotoxicity. In most instances this is undesirable, but the stunting that occurs on chrysanthemums can actually take the place of plant growth regulators that are sprayed for the same effect on plants grown in greenhouses. Azadirachtin content in neem kernels and quickness of activity are further considerations in the commercialization of neem extracts. To provide a consistent product, refining kernels with similar levels of compounds is essential. On the contrary, a Canadian company

discovered that samples of neem oil from Indian sources ranged from undetectable amounts, less than 50 ppm, of azadirachtin to 6,800 ppm. Farmers using synthetic pesticides also are used to quick acting chemicals. They may not be patient enough to wait for the activity of neem-based products to produce results ⁶⁵.

Carica Papaya (Caricaceae):



Common Names: Papaya, Papaw or Paw Paw (Australia), Mamao (Brazil), Tree Melon.

Related Species: Babaco (*Carica pentagona*), Mountain Papaya (*C. pubescens*), Chamburo (*C. stipulata*).

Origin: The papaya is believed to be native to southern Mexico and neighboring Central America. It is now present in every tropical and subtropical country.

Adaptation: Papayas have exacting climate requirements for vigorous growth and fruit production. They must have warmth throughout the year and will be damaged by light frosts. Brief exposure to 32° F is damaging and prolonged cold without overhead sprinkling will kill the plants. Cold, wet soil is almost always lethal. Cool temperatures will also alter fruit flavor. Papayas make excellent container and greenhouse specimens where soil moisture and temperature can be moderated.

Description:

Growth Habit: The papaya is a short-lived, fast-growing, woody, large herb to 10 or 12 feet in height. It generally branches only when injured. All parts contain latex. The hollow green or deep purple trunk is straight and cylindrical with prominent leaf scars. Its diameter may be from 2 or 3 inches to over a foot at the base.

Foliage: The leaves emerge directly from the upper part of the stem in a spiral on nearly horizontal petioles 1 to 3-1/2 feet long. The blade, deeply divided into 5 to 9 main segments, varies from 1 to 2 feet in width, and has prominent yellowish ribs and veins. The life of a leaf is 4 to 6 months.

Flowers: The five-petalled flowers are fleshy, waxy and slightly fragrant. Some plants bear only short-stalked female flowers, or bisexual (perfect) flowers also on short stalks, while others may bear only male flowers, clustered on panicles 5 or 6 feet long. Some plants may have both male and female flowers. Others at certain seasons produce short-stalked male flowers, at other times perfect flowers. This change of sex may occur temporarily during high temperatures in midsummer. Male or bisexual plants may change completely to female plants after being beheaded. Certain varieties have a propensity for producing certain types of flowers. For example, the Solo variety has flowers of both sexes 66% of the time, so two out of three plants will produce fruit, even if planted singly. How pollination takes place in papayas is not known with certainty. Wind is probably the main agent, as the

pollen is light and abundant, but thrips and moths may assist. Hand pollination is sometimes necessary to get a proper fruit set.

Fruit: There are two types of papayas, Hawaiian and Mexican. The Hawaiian varieties are the papayas commonly found in supermarkets. These pear-shaped fruit generally weigh about 1 pound and have yellow skin when ripe. The flesh is bright orange or pinkish, depending on variety, with small black seeds clustered in the center. Hawaiian papayas are easier to harvest because the plants seldom grow taller than 8 feet. Mexican papayas are much larger than the Hawaiian types and may weigh up to 10 pounds and be more than 15 inches long. The flesh may be yellow, orange or pink. The flavor is less intense than that of the Hawaiian papaya but still is delicious and extremely enjoyable. They are slightly easier to grow than Hawaiian papayas. A properly ripened papaya is juicy, sweetish and somewhat like a cantaloupe in flavor, although musky in some types. The fruit (and leaves) contain papain which helps digestion and is used to tenderize meat. The edible seeds have a spicy flavor somewhat reminiscent of black pepper.

CULTURE

Location: Papayas like to be warm with both sunshine and reflected heat, so the hottest place against the house where nothing else seems happy is an ideal location. They also like to be as free from wind as possible, although this is not as critical as their need for sun. Papayas can be grown successfully in shade, but the fruit is rarely sweet. They are best planted in mounds or against the foundation of a building where water can be controlled.

Soils: Papayas need a light, well-drained soil. They are easily killed by excess moisture. The soil needs to be moist in hot weather and dry in cold weather. Since this is the opposite of India's rain pattern, in addition to good drainage, plastic coverings to prevent over-wetting in winter may also be worthwhile. Papayas do not tolerate salty water or soil.

Irrigation: Watering is the most critical aspect in raising papayas. The plants should be kept on the dry side to avoid root rot, but also need enough water to support their large leaves. In winter the plant prefers to remain as dry as possible. A plant that has been injured by frost is particularly susceptible to root rot.

Fertilization: The fast-growing papaya requires regular applications of nitrogen fertilizers but the exact rates have not been established. Feed monthly

and adjust according to the plant's response. They can take fairly hot organic fertilizing such as chicken manure if used with deep irrigation after warm weather has started. Phosphorus deficiency causes dark green foliage with a reddish-purple discoloration of leaf veins and stalks.

Pruning: Papayas do not need to be pruned, but some growers pinch the seedlings or cut back established plants to encourage multiple trunks.

Frost Protection: Papayas need warmth and a frost-free environment, but can often withstand light freezes with some kind of overhead protection. This can be provided by building a frame around the plants and covering it with bedding, plastic sheeting, etc. when frost threatens. Electric light bulbs can also be used for added warmth. Potted specimens can be moved to a frost-secure area. Prolonged cold, even if it does not freeze, may adversely affect the plants and the fruit. Mexican papayas are more hardy than Hawaiian varieties.

Propagation: Papayas are normally propagated by seed. To start a plant, extract the seeds from ripe papayas and wash them to remove the gelatinous covering. They are then dried, dusted with a fungicide and planted as soon as possible (the seeds lose their viability rapidly in storage). Plant the seeds in warm (80° F), sterile potting mix. Seeds should be planted in sterile soil as young papaya seedlings have a high mortality rate from damping off. Potting soil can be sterilized by mixing 50-50 with vermiculite and placing in an oven at 200° F for one hour. Under ideal conditions the seeds may germinate in about two weeks, but may take three to five weeks. Gibberellic acid can be used to speed up germination in some seasons. Seedlings usually begin flowering 9 - 12 months after they germinate. Seedling papayas do not transplant well. Plant them in large containers so the seedlings will have to be transplanted only once, when they go into the ground. Transplant carefully, making sure not to damage the root ball. To prevent damping off, drench the potting mix with a fungicide containing benomyl or captan. Set the plants a little high to allow for settling. A plastic mulch will help keep the soil warm and dry in wet winter areas, but remove it as soon as the weather becomes warm. Plant at least three or four plants to insure yourself of having females or plant hermaphroditic plants. Papaya plants can also be grown from cuttings, which should be hardened off for a few days and then propped up with the tip touching moist, fertile soil until roots form. Semihardwood cuttings planted during the summer root rapidly and should fruit the following year.

Pests and diseases: Thrips, mites and white flies as well as In red spider and fruit spotting bugs are potential problems in some areas. The plants may also be attacked by mildew, anthracnose, root rot and various virus diseases. Fruit flies often ruin the fruit in Florida and Hawaii. Nematodes can attack the roots and are often a factor in the decline of individual plant. Gopher damage can be avoided by planting in wire baskets. Papaya plants should probably be replaced every 4 years or so.

Uses

Papaya is cultivated for its ripe fruits, favored by tropical people, as breakfast fruit, and as an ingredient in jellies, preserves, or cooked in various ways; juice makes a popular beverage; young leaves, shoots, and fruits cooked as a vegetable. Latex used to remove freckles. Bark used for making rope. Leaves used as a soap substitute, are supposed to remove stains. Flowers eaten in Java. Papain, the proteolytic enzyme, has a wealth of industrial uses. It has milk-clotting (rennet) and protein digesting properties. Active over a wide pH range, papain is useful in medicine, combatting dyspepsia and other digestive disorders. In liquid preparations it has been used for reducing enlarged tonsils. Nearly 80% of American beer is treated with papain, which digests the precipitable protein fragments and then the beer remains clear on cooling. Papain is also used for degumming natural silk. But most of the papain imported in the U.S. is used for meat-tenderizers and chewing gums. Also used to extract the oil from tuna liver. Cosmetically it is used in some dentifrices, shampoos, and face-lifting preparations. Used to clean silks and wools before dying, and to remove hair from hides during tanning. It is also used in the manufacture of rubber from Hevea ⁴⁴. Recently, the FDA has cleared chymopapain for intradiscal injection in patients with documented herniated lumbar intervertebral discs whose signs and symptoms have not responded to conservative therapy over an adequate period of time. Fruit and seed extracts have pronounced bactericidal activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Shigella flexneri* ⁴⁰.

Folk Medicine

The juice is used for warts, cancers, tumors, corns, and indurations of the skin. Sinapisms prepared from the root are also said to help tumors of the uterus. Green fruit said to be eccholic. Vermifugal seeds said to quench thirst. Leaves poulticed onto nervous pains and elephantoid growths. Roots said to cure piles and yaws. In Asia, the latex is smeared on the mouth of the uterus as eccholic. The root infusion is used for syphilis in Africa. Leaf smoked for

asthma relief in various remote areas. Javanese believe that eating papaya prevents rheumatism. Dietary papaya does reduce urine acidity in humans. Flowers have been used for jaundice. Experimentally papaya is hypoglycemic. Inner bark used for sore teeth. Latex used in psoriasis, ringworm, and prescribed for the removal of cancerous growths in Cuba.. Latex used locally as antiseptic. Seeds considered alexeritic, abortifacient, counter-irritant, emmenagogue, and anthelmintic. Infusion of roots said to remove urine concretions. Young leaves, and to lesser degree, other parts, contain carpain, an active bitter alkaloid, which has a depressing action on heart. Plant is strong amoebicide. Latex, used as dyspepsia cure, is applied externally to burns and scalds ⁴⁶.

Toxicity

Externally the latex is irritant, dermatogenic, and vesicant. Internally it causes severe gastritis. Some people are allergic to the pollen, the fruit, and the latex. Papain can induce asthma and rhinitis. The acrid fresh latex can cause severe conjunctivitis and vesication. According to Morton (1977) the latex will digest tissue and cause sores under rings and bracelets, while it has been used internally for malicious poisoning. Mitchell and Rook (1979) report a yellowing of the palms and soles caused by eating papaya. Anaphylaxis is reported in about 1% of cases of chymopapain injections.

Description

Erect, fast-growing, usually unbranched tree or shrub, 7-8 m tall, with copious latex, trunk about 20 cm in diameter, soft, leaves clustered near top of plant, alternate, long-petiolate, blade suborbicular, to 80 cm long, palmately 7-11-lobed; lobes glabrous, toothed, flat; plants dioecious in nature, some monoecious cultivars; flowers aromatic, male in drooping axillary panicles to 80 cm long, with a 5-toothed green calyx and 5-toothed cream to yellow corolla; stamens 10; female flowers solitary or cymose in axils or below leaves, with 5 yellow nearly free petals to 5 cm long; ovary with 5 stigmas; fruit a large yellow to greenish-orange berry, oblong about 7.5 cm long and bitter in wild types, up to 45 cm long, with flesh 2.5-5 cm thick, sweet, juicy and of orange color in cultivars; seeds numerous cavity, rounded, blackish, about 0.6 cm in diameter, each enclosed in a gelatinous membrane (aril). Flowers and fruits nearly continuous all year.

Distribution

Native to Central America, papaya has been carried throughout the tropics, where it is extensively cultivated, and as far north and south as 32° latitude. Almost weedy in some areas of tropics.

Ecology

Ranging from Warm Temperate Dry to Moist through Tropical Very Dry to Wet Forest Life Zones, papaya is reported to tolerate annual precipitation of 6.4 to 42.9 dm (mean of 42 cases = 19.2), annual temperature of 16.2 to 26.6°C (mean of 42 cases = 24.5), and pH of 4.3 to 8.0 (mean of 33 cases = 6.1). Papaya is a tropical plant, killed by frost; does not tolerate shade, waterlogging, or strong winds, and may require irrigation in dry regions. It recuperates very slowly from serious root or leaf injury (Malo and Campbell, s.d.). Grows best below 1,500 m in well-drained rich soil of pH 6-6.5.

Cultivation

Seeds retain their viability for 2-3 years when kept air-dry in airtight containers. Seeds may be sown in coldframes or boxes during January or in the open in March. Early planting is much to be desired to make a vigorous plant before the beginning of following winter. Seeds germinate in 2-3 weeks. When 2 or 3 true leaves have formed, seedlings should be transplanted, spacing them 5-7.5 cm apart in seedbed. When plants are 7.5-10 m tall, they can be set in their permanent places in the field. Usual planting distance is about 3-4 m apart each way, giving about 1750 trees to the hectare. In selecting plants for field planting, the more vigorous growing plants are usually the males and may be safely discarded except for a few. By planting 2 or 3 plants in a hill, there is a chance for further selection and elimination of excessive males when first flowers appear, about one male plant to each 25 or so females is sufficient. Transplants must be watered and shaded. Mulch gives much better results than clean culture, keeping down weeds, preserving moisture, shading the soil from hot summer sun, and preventing the burning out of humus and nitrates in the top soil layer. Heavy applications of stable manure or commercial fertilizers can often be used with profit. Attempts at grafting and rooting shoots have not been successful on a commercial scale.

Harvesting

Trees from seed sown in early spring should fruit the following winter and continue to bear some fruit nearly every month in the year thereafter. Bloom to maturity is 5-8 months. Average life of a papaya grown under cold conditions is 2 or 3 years, but trees may live in the wild 25 years or more. Yield declines after the first few years. All inferior and wild male trees in a region should be destroyed so that their pollen cannot fertilize blossoms of trees from which seed is to be selected. Seed for new plantings should be saved from perfect-flowered plants whenever possible. For fresh fruit, they are harvested just as yellowing commences. Fruits should be twisted gently, the harvester wearing cotton gloves so as not to bruise fruit surface. On trees

where fruits are not crowded, fruits are cut off with a knife. Fruit should be placed directly in a picking tray that is well padded on sides and bottom with shredded paper or other soft material. The blossom end is more resistant to bruising and so fruit should be packed with this end down. Since papayas are injured by chilling, they should be kept at about 7°C with relative humidity of 85-90%, and may be kept for 7 to 21 days under these conditions. They should be ripened at 21-26.5°C as needed for marketing. Papain is harvested, like opium, by tapping the unripe fruits. Latex drips into a suitable container and is sun dried or oven dried at 55 to 60°C. The Same fruits are tapped in different cuts at weekly intervals. Tapped fruits are ultimately edible, so that both fruit and latex are harvested.

Biotic Factors

The papaya fruit-fly (*Toxotrypana curvicauda*) is a serious pest in some areas, so that bagging fruit while still quite small is the only means for protecting it. This most important insect pest, punctures the rind of fruit to lay eggs. Use of bags is only feasible in small plantings; in commercial plantings, selection of another area at least 2 miles from old plantations or wild stands usually gives protection for a few years. Papaya whitefly (*Trialeurodes variabilis*), hornworms and corn earworms (*Heliothis zea*) may also be pests. In Florida, the papaya webworm *Romalopalpia dalera*, produces webs around the fruits and stems therein (Malo and Campbell, s.d.). Soil used for seedbeds should be absolutely free from nematodes that cause root knot.

It contains many biologically active compounds. Two important compounds are chymopapain and papain, which are supposed to aid in digestion. Papain also is used to treat arthritis. The level of the compounds vary in the fruit, latex, leaves, and roots. In addition, plant parts from male and female trees differ in the quantity of the compounds. For example, phenolic compounds tend to be higher in male trees than female trees. The quantity of fresh papaya latex and dry latex (crude papain) also vary with the sex of the tree and the age of the tree. Female and hermaphrodite trees yield more crude papain than male trees and older fruit yields more than younger fruit. However, the activity of the papain is higher in the extracts from the younger fruit than the older fruit. The following list of compounds found in parts of *Carica papaya*.²⁶

COMPOUNDS	PART OF PLANT(<i>Carica papaya</i>)
Alkaloids	1,300-4,000 ppm in leaves
Butanoic acid	As much as 1.2mg/kg in fruit pulp
Methyl butanoate	As much as 18% of the volatile components in the fruit
Carpaine	Leaves (1,000-1,500 ppm), bark, roots, and seeds
Dehydrocarpaines	1,000 ppm in leaves
Pseudocarpaine	100 ppm in leaves
Chymopapain-a and b	Latex and exudate
Flavonols	0-2,000 ppm in leaves
Benzylglucosinolate	Found in all parts of the plant, but highest in young leaves
Linalool	As much as 94% of the volatile components in the fruit
Cis- and trans-linalool oxide	Fruit
Alpha-linolenic acid	250-2,238 ppm in fruit
Papain	Fruit and 53,000 ppm in latex and exudate
Alpha-phellandrene	Fruit
Tannins	5,000-6,000 ppm in leaves
Alpha-terpinene	Fruit
Gamma-terpinene	Fruit
4-terpineol	Fruit
terpinolene	Fruit

***Ficus Recemosa (Moraceaea)*, (Umbar):**The alcoholic extract of the stem bark of the plant possessed antiprotozoal activity.



Biochemistry:

Glycosides are present in the plant. 8-sitosterol and lupeol have been isolated from the petroleum ether extract of the root-bark of the plant. Tannins are also present. Psoralens have also been detected.

It is astringent dysentery drug. It is a good remedy for excessive vermifuge and an anti-appetite. The extract of fruit is used in diabetes, leucoderma and nienorrhagias. It is used ocally to relieve inflammation of skin wounds, lymphadenitis, in sprains and fibrositis. the alcoholic extract of the stem bark of the plant possessed antiprotozoal activity.^{28,30}

Ficus religiosa (Moraceaea) (Peepul)

Kingdom	<u>Plantae</u> – Plants
Subkingdom	<u>Tracheobionta</u> – Vascular plants
Superdivision	<u>Spermatophyta</u> – Seed plants
Division	<u>Magnoliophyta</u> – Flowering plants

Class	<u>Magnoliopsida</u> – Dicotyledons
Subclass	<u>Hamamelidae</u> –
Order	<u>Urticales</u> –
Family	<u>Moraceae</u> – Mulberry family
Genus	<u>Ficus</u> L. – fig
Species	<u>Ficus religiosa</u> L. – peepul tree



Nomenclature:

'*Ficus*' is the latin word for 'Fig', the fruit of the tree. '*Religiosa*' refers to 'religion', because the tree is sacred in both Hinduism and Buddhism and is very frequently planted in temples and shrines of both faiths. '*Bodhi*' or its short form 'Bo' means 'supreme knowledge' or 'awakening' in the old Indian languages.

'*Pipal*' relates (I believe) to the same ancient roots which give rise to English words like 'Pip' and 'Apple', and therefore mean something like 'fruit-bearing tree'.

'*Ashwattha*' and '*Ashvattha*' come from an ancient Indian root word "Shwa" means 'morning' or 'tomorrow'. This refers to the fact that Ashwattha is the mythical Hindu world tree, both indestructible and yet ever-changing: the same tree will not be there tomorrow.

Description:

A medium sized tree with a relatively short trunk (often about the height of human being) and a large crown with wonderful wide spreading branches. The tree is semi- or fully deciduous in monsoon climates. It becomes leafless for a brief period in dry habitats. The trunk has smooth grey bark and with age this trunk becomes irregularly shaped, fluted and often has low buttresses. The bark can have brownish specks and peel off in irregular rounded flakes.

It has too many possible beneficial herbal uses to fully enumerate. A few of its uses include: as an astringent, as an antidiarrheal, as an antidysenteric and as a laxative. The fruit and the leaves and the bark and even

the latex are used to prepare herbal remedies. The young tree can be epiphytic. (Epiphytic plants can have aerial roots and do not require soil to grow. This strategy gives young plants many advantages such as plenty of light. Water is obtained mostly via air humidity. Nutrients, such as nitrogen, are also derived from the air and occasionally from decomposing matter such as leaves and dead insects. The roots seek out cracks and crevices where soil, water and rotting organic matter accumulate. In natural circumstances most epiphytic plants may be attached to tree bark, as something to hold on to, but not in any way feeding off the tree. This is probably because the seeds have been excreted by birds who visited this tree. Rocks or buildings are other places the young plants may be found. Once the roots reach the ground they switch over to growing as normal tree.

Leaves:

When the leaves first appear their colour is red-pinkish, but then they turn deep green and grow to about 12 to 18 cm long (5-7 inches). They are attached to long flexible stalks, which makes them rustle, flutter and dance in the slightest whiff of wind. The foliage can often be dense. The alternate leaves are heart-shaped, shiny with an elegant tail-like tip, which is often called a "drip-tip", guiding water efficiently down to the soil. This prevents the sometimes heavy monsoon rain from collecting on the leaves for prolonged periods, which could make them rot in very warm weather. The leaves have 6-8 pairs of side-veins and a further network of very fine veins. This delicate venation and the ability of the leaf to disintegrate easily in water are both clearly illustrated in the greeting cards which are sometimes made with Peepal leaves. The leaves are soaked for 8 hours (in warm countries) in a bowl of water and then washed carefully under running water until only the veins remain. This skeleton leaf is dried and stuck on a darker background.

Flowers:

The small red flowers appear in February. The tree is dependant on its associated pollinator wasp, *Blastophaga quadraticeps* to set seed.

Fruit:

The tree fruits in May/June and bears a small flat-topped figs (12-13mm or ½ inch in diameter), which appears in pairs in the angles of the leaves on the twigs (or above the scars in the bark left by fallen leaves). They have 3 basal bracts, are green at first and ripen to a blackish purple (may have reddish dots). The fruiting tree becomes a treat for many different birds and animals.

Habitat:

Ficus religiosa is known to be a native Indian tree, and thought to be originating mainly in Northern and Eastern India, where it is widely found in uplands and plane areas and grows up to about 1650 metres or 5000 ft in the mountainous areas.

It is also found growing elsewhere in India and throughout the subcontinent and southern Asia, especially in Buddhist countries, wild or cultivated. After at least 3000 years of veneration and cultivation, it is of course difficult to tell exactly which trees are indigenous and which are not.

It is a familiar sight in Hindu temples, Buddhist monasteries and shrines, villages and at roadsides. People also like to grow this sacred tree in their gardens. In urban situations where there is little room one often finds the tree growing in Bonsai form. Many places derive their name from the tree and one can find villages such as Piprahi and Piperbandha. *Ficus religiosa* has also been widely planted in many hot countries all over the world from South Africa to Hawaii and Florida, but it is not able to naturalise away from its Indian home, because of its dependence on its pollinator wasp, *Blastophaga quadraticeps*. An exception to this rule is Israel where the wasp has been successfully introduced.

Cultivation:

Each Fig species has an associated species of agaonid wasp to pollinate its flowers. This means that it will only freely propagate itself in its native areas where this wasp is present, unless the wasp gets successfully introduced. In turn the wasps will only lay their eggs in the Figs they are associated with. The pollinator wasp for *Ficus religiosa* is *Blastophaga quadraticeps*.

Wherever pollinator wasps are not present, the seeds are therefore not viable and the trees must be propagated from cuttings. Once established *Ficus religiosa* can be a very long-lived tree. There is also a veteran tree in Bombay which is even older at about 3000 years.

Known Medicinal Uses of the Tree:**Bark**

- The bark is cooling and astringent.

- An aqueous extract of the bark shows anti-bacterial activity against *Staphylococcus aureus* and *Escherichia coli*.
- The bark is useful in inflammations and glandular swellings of neck.
- An infusion or decoction of the bark is used with some honey for the treatment of gonorrhoea, ulcers, skin diseases and scabies.
- Freshly burnt ashes of the bark are steeped in water and given to cure obstinate cases of hiccups, resistant to other home remedies. This preparation also stops nausea. The healers simply burn the bark, collect the ash and store it for future use.
- A decoction of milk boiled with dried bark is said to be a good aphrodisiac.
- Unani practitioners blow the powder of the dried bark into the rectum through a pipe in cases of anal fistula and inflammatory swellings as an absorbent. The astringent property of the bark will be helpful here.
- The dried bark powder is also sprinkled over unhealthy ulcers and wounds to promote granulation.
- A standard compound preparation, Pancha Valkaladi Tailum, containing several herbs including the decoction of the bark of Peepal is used as an external application in cases of eczema, leprosy and rheumatism.
- Leaves and bark are astringent and laxative and are employed together to relieve diarrhea and dysentery and also to help reduce bleeding.

Root bark

- Root bark is good for stomatitis, to clean ulcers, and it is astringent in leucorrhoea and promotes granulations.
- According to Unani system of medicine, root bark is aphrodisiac and also good for lumbago.

Root

- Roots are said to be good for gout.
- The roots are chewed to prevent gum disease.

Fruits

- The fruit is laxative, promotes digestion, is aphrodisiac, and checks vomiting.
- Ripe fruits are alexipharmic (*an antidote or defensive remedy against poison, venom or infection*), are good for foul taste, thirst, and heart disease,
- The powdered fruit is taken for asthma. It is said that the powder taken with water for two weeks cures asthma.

Seeds

- The seeds are cooling, laxative, refrigerant.
- Seeds are useful in urinary troubles.

Leaves (Some of the indication below may seem contradictory, but the therapeutic action is very likely due to combining ingredients and quantities given, etc.)

- The leaves are used with "ghee" (a clarified butter) as a poultice and applied to boils and to swollen glands in mumps.
- As female tonic and also as preventive, the traditional healers of Chhattisgarh plains suggest the female patients to use the leaves of Pipal with cow milk regularly, both during attack and disease free time. They instruct them to boil the leaf in milk and drink it, once in a day.
- The leaves and bark together are employed to relieve diarrhea and dysentery and to reduce bleeding.

- The leaves alone are used to treat constipation.
- The leaves and young shoots together are purgative (strong laxative).
- In some areas licking honey placed on peepal leaves is believed to cure speech irregularities.

Latex (milky juice in the stems)

- The latex is used in the same way as other Ficus species, including application to hemorrhoids, warts, and aching joints. This should never be used internally and must be handled carefully.

Animal Fodder:

The fruits, tender leaves and twigs of the tree are commonly lopped to provide cattle and elephant fodder. Some Customs and beliefs associated with the Tree: The traditions and beliefs surrounding this venerable tree go back into the mists of time. There is an Upanishadic story of the Pippala tree as the Tree of Supreme Knowledge, which is the great-grandmother to the Bible story of Adam and Eve eating from The Tree of Life. Ficus religiosa is said to have its mythical origin in the personality of Indra, the ruler of the skies, but many of the Hindu Gods have equally close associations with the tree. It has been called the Tree of eternal life whose roots originate in heaven and whose branches spread on Earth to bring blessings to humankind. We will explore the religious and philosophical significance of the tree a bit more on page no. 27 (so as not to overload this page too much) and concentrate here on listing some of the practical side of the beliefs, which has lead to a great variety of customs.

Vedic Boat

"In Vedic times, boats were made of peepal wood. " I wonder if this usage is related to the tree being connected with the passage of souls to the world of the Dead, because further down we see that the tree is associated with Death, Ancestors and Ghosts. Many cultures all over the world have looked on special trees as vehicles to connect our world with the so called "Otherworld" and the residence of dead souls and ancestors in trees (as well as the trees' own spirits) is universal.

Inspiration

"The peepal has inspired artists and sculptors for centuries to create graphic designs and sculptural friezes which stylise its branches as a symbol of a rich life. " The leaves of some of the temple trees are highly prized as relics which

pilgrims take home with them. The leaves, which are about the size of a hand, have also been widely used as a canvas for small paintings, sometimes describing mythical or religious themes or also just rural scenes.

Fertility

"The *ashwattha* symbolises the continuity of life because the tree itself lives and grows for hundreds of years. Childless couples devoutly believe in its powers and worship it, tying threads of white, red and yellow silk around it to pray for progeny and rewarding parenthood." "Women circumambulate the peepal tree to be blessed with children or to gain a desired thing or person." Watering the *bodhi* tree enhances another aspect of its magic: the power of fertility. Villagers come to the *bodhi* tree observes Godakumbura and having made due observances, pray for a male child, thus continuing the original fertility image. Godakumbura enumerates a number of factors which seem to assert the fertility aspect of this tree. "When we consider the history of the *bodhi* tree, we notice that from the very beginning of its recorded history it was attended by females... Emperor Asoka had sent it to Ceylon accompanied by the Theri Sanghamitta and Bhikkunis, making a total of twelve. Along with the large retinue that was sent to attend on the *bodhi* tree, Asoka also sent four royal maidens to pour water on the tree during the festivals that took place at the port. The *bodhi* tree was sprinkled with water by virgins of the Ksatriya, Vesya and Brahmana clans... At Anuradhapura, the duty of attending on the *bodhi* tree fell to the nuns, the order which was founded by Theri Sanghamitta. The four royal maidens who were appointed by the king to sprinkle water on the *bodhi* tree were called *perahara bisavu* (Bath Maidens). The 'Sinhala Bodhivamsaya' (The Chronicle of the Bodhi tree) describes in detail the institution of *perahara biso* "Saying that four royal virgins should pour water on the Bodhi-tree with golden and silver pitchers, the king decked them with every kind of ornament and appointed them to the office of *Peraharabiso*. All four of these royal virgins entered the king's palace with great splendour. The position of the *Peraharabiso* maidens was somewhat like that of the Vestal Virgins in ancient Rome. Today, however, this ritual is conducted not by maidens but by monks and laymen. *Bodhi-rujas*, offering of vows to the *bodhi* has become today extremely popular. The *bodhi* tree which makes rains fall, crops grow and blesses women with offspring is, thus, tended with care and veneration by the Sri Lankan Buddhists."

Marriage

The Banian (*Ficus benghalensis*) tree is "supposed to be a male tree, while the Aswath-tha or Peepul (*Ficus religiosa*) is looked upon as a female, whence

the lower orders of the people plant them side by side and perform the ceremony of matrimony with a view to connect them as man and wife". "Bengal, peepal and banyan trees are married." "In Awadh, if a girl's horoscope predicts widowhood, she is first married to a peepal tree on Chaitra Krishna or Ashwin Krishna tritiya. In olden days, when remarriage was forbidden for girls, young widows were married to the peepal tree and then allowed to remarry." "People belonging to Dhantale caste who reside in Maharashtra, Karnataka and Madhya Pradesh use a branch of the peepal tree in the marriage ceremony. The branch, along with a pot of water, is placed between the bride and groom. The village deity is installed under the peepal tree which also provides a shaded place to hold the panchayat."

More healing properties

"Watering the *bodhi* tree even at other times is a common rite observed in all temples. Folklore has it that giving life to the *bodhi* tree by watering it is similar to giving life to a being who is in need of it. When someone is taken fatally ill, it is the custom for one of his relatives to visit the *bodhi*, water it seven times on seven days and make vows on behalf of the sick for speedy recovery." "The air purification properties of Pipal tree are also mentioned in ancient Indian literatures".

Death, ancestors and ghosts

"Peepal tree is also believed to be the abode of Lord Yama (god of death) and ancestors. Offerings made at its roots are believed to reach them. In Rajasthan, if a man dies, his son pours 300 buckets of water at the root of a peepal tree and circumambulates it five times so that his father's soul would rest in peace. In Uttar Pradesh, Bihar and Madhya Pradesh, water, milk and sesame seeds are offered to ancestors in an earthen pot hung on the peepal tree." "The natives of rural areas associate Pipal tree with Bhoot (ghost) and according to them, this tree is home of ghosts. This is the reason that during night time, they hesitate to go near these trees. "The Munja ghost is also believed to stay on peepal tree." The Thai people also believe that ghosts live in the tree and it is said that their whispers can be heard in the rustling leaves of the tree.

Rites of passage

"A peepal tree is planted to the east of the house or temple. Eight, 11 or 12 years after the tree has been planted, the upanayan ceremony is performed for the tree. A round platform is constructed around the tree. Different gods like Ashwattha, Narayan, Vasudev, Rukmini, Satyabhama are invoked and

worshipped. All the rituals of the upanayan ceremony are performed and then the tree is married to the basil plant."

Gods and the Tree

"Once, Agni (the fire god) left the land of the gods, took the form of Ashwattha and resided on the peepal tree for a year. Since then, Peepal is also known as Ashwattha."

"According to *Padmapuran*, Vishnu turned into a peepal tree because of a curse by sage Ambarish." Once, all the gods decided to visit Shiva. However, Narad informed them that it was an inappropriate time for a visit as Shiva and Parvati were in solitude. But Indra did not heed the advice and assured the gods that there was nothing to fear when he was there to protect them. Narad reported Indra's arrogance to goddess Parvati. She cursed the gods that they, along with their wives, would turn into trees. When the gods asked for forgiveness, she promised that as trees, they would attain fame. Thus Indra turned into a mango tree, Brahma became a palash tree and Vishnu turned into a peepal tree."

Peepal and the Neem tree

In Tamil Nadu, peepal and neem trees are planted so close to each other that they mix up as they grow. A naga idol is placed under them and worshipped. This is believed to bless the worshipper with wealth. Women take an early morning bath and circumambulate these trees

Other Rituals

Chakkiliyan people in Tamil Nadu place the Gangamma ghatam under a peepal tree near a river and worship it for ten days." "Tribals in Bengal call the peepal tree as Vasudev (a Hindu God). They water the plant in the month of Vaishakh and at times of difficulty. Peepal tree is planted in the temples of Shani and Hanuman. The tree is worshipped on Saturday, especially in the month of Shravana, because goddess Lakshmi sits under the tree on this day. Any person who waters the tree is believed to earn merit for his progeny, his sorrows are redeemed and diseases cured. The peepal tree is also worshipped to escape from contagious diseases and enemies. Another popular ritual connected with the Bodhi-tree is the lighting of coconut-oil lamps as an offering (*pahan-puja*), especially to avert the evil influence of inauspicious planetary conjunctions. The other aspects of this ritual consist of the offering of flowers, milk-rice, fruits, betel, medicinal oils, camphor, and coins. These coins (designated *panduru*) are washed in saffron water and separated for offering in this manner. The offering of coins as an act of merit-acquisition

has assumed ritualistic significance with the Buddhists of the island.Another part of the ritual is the hanging of flags on the branches of the tree in the expectation of getting one's wishes fulfilled.....Bathing the tree with scented water is also a necessary part of the ritual. So is the burning of incense, camphor, etc. Once all these offerings have been completed, the performers would circumambulate the tree once or thrice reciting an appropriate stanza.

Worship of the tree

The *Vibhanga Commentary* (p.349) says that the bhikkhu who enters the courtyard of the Bodhi-tree should venerate the tree, behaving with all humility as if he were in the presence of the Buddha. Thus one of the main items of the daily ritual at the Anuradhapura Bodhi-tree (and at many other places) is the offering of alms as if unto the Buddha himself. A special ritual held annually at the shrine of the Anuradhapura tree is the hanging of gold ornaments on the tree. Pious devotees offer valuables, money, and various other articles during the performance of this ritual. To the Buddhists, the Bodhi-tree became a sacred object belonging to the *paribhogika* group of the threefold division of sacred monuments, while the ordinary veneration of trees, which also exists side-by-side with the former in Sri Lanka, is based on the belief already mentioned, i.e. that there are spirits inhabiting these trees and that they can help people in exchange for offerings.³⁰

AIMS AND OBJECTIVES

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1. Study of vector population:-

- A. To identify the study areas.**
- B. To Collect and identify the larvae of *aedes aegypti* from selected study areas.**
- C. To calculate the Container Index.**

2. Evaluations of possible larvicidal activity of leaves extracts of selected plants.

- A. To collect and identify selected plants taxonomically:**
- B. To prepare leaves extracts of selected plants using solvent:**
- C. To evaluate the leaves plant extracts for activity against larvae of *A.aegypti* in vitro:**
- D. Residual activity of leaves extracts:**
- E. Adsorption column chromatography of active extract:**
- F. Calculations:**

MATERIAL AND METHODS



MATERIAL AND METHODS:

1. Study of vector population:-

A. To identify the study area:

Airoli is identified as a study area in New Mumbai on the basis of information available on suspected outbreaks of dengue and also on the basis of number of reasons : A number of construction sites with water storage tanks, auto garages with a piles of old tyre are present in these areas. These areas are heavily populated. A number of discarded tins, coconut shells, broken pots, bottles & plastic sheets lie scattered in & around the service quarters of these areas, which during the monsoon serve as potential breeding sites for *A. aegypti*.

B. To Collect and identify the larvae of *Aedes aegypti* from selected study areas: ⁽²⁾*Aedes* larvae can easily be distinguished in the field by their distinctive jerky wriggling movement. They are generally found clustered in dark corners of containers, still identification of the larvae in laboratory was necessary. Siphon tube is shorter in *Aedes aegypti* larvae than larvae of *Culex*. *Aedes aegypti* larvae has only one pair of subventral tufts on siphon tube. We have covered 250 houses in the selected area in different localities. The type of containers were searched as follows, barrels, plastic drums, metallic containers, etc. Collected larvae transported to the laboratory in clean containers, Identification of larvae is done using entomological keys and colony is maintained and blood fed on guinea pig.

C. To calculate the container Index of *Aedes aegypti* :

Following parameter is used.

$$\text{Container Index} = \frac{\text{No. of positive containers}}{\text{Total no. of containers screened}} \times 100$$

2. Evaluations of possible larvicidal activity of selected plant extracts.

A. To collect and identify selected plants taxonomically:^{9, 10}

1) *Annona squamosa* (Annonaceae); Custard Apple, a small evergreen tree, occurs wild; it is also cultivated throughout India because of its fruits. Leaves simple, alternate, bifarious; fruits yellowish green; seeds hard. Distributed throughout the India

2) *Azadirachta indica* (Meliaceae) (Neem): A medium to large size tree, 15-20 m in height, leaves compound, imparipinnate, leaflets, subopposite, serrate, very oblique at base. This tree is distributed throughout the India, deciduous forest and is also widely cultivated.

3) *Carica papaya* (Caricaceae) : A small soft wooded short lived lactiferous tree up to 8.0 m in height with a straight cylindrical stem bearing characteristic leaf-scars throughout and with tuft of leaves at the top; leaves deeply lobed, palm-like with long hollow petiole. Distributed and cultivated throughout the India

4) *Ficus racemosa* (Moraceae), (Umbar) : Deciduous tree, leaves dark green, ovate or elliptic. Distributed throughout India.

5) *Ficus Religiosa* (Moraceae) (Peepul) : A large deciduous tree. Leaves are shiny, cordate, long petioled ovate. Distributed throughout the India

B. To prepare extracts of selected plants using solvent:²¹

About 20 gram of powdered, air dried leaves of selected plants, placed in a conical flask. Macerated with 100ml of methanol for 6 hours, shaking frequently then allowed to stand for 18 hours. Filtered & concentrated at

40°C on water bath. Accurately weighed extract diluted in water to give different concentrations.

C. To evaluate the plant extracts for activity against larvae of *A. aegypti* in vitro:³

Ten beakers of 500 ml capacity were arranged in a series. Each beaker will be filled with 250ml of water. Different aliquots of the extracts were added to beakers. After thirty min. twenty five third instar larvae of *Aedes aegypti* were placed in these beakers and the larval mortality was recorded after 24 hrs. Temephos (50%EC) served as a standard and two controls were maintained, one was water and other was water with the solvent concentration used in dilution of extract.

D. Residual activity of extracts:³ Emulsions containing individual extract in concentration higher than those showing 100% larvicidal activity in 24 hrs. were tested for continued mosquito larvicidal activity until mosquito larvicidal activity lowered to 60% mortality in each case. The general design of experiment was as described in the earlier experiment.

E. Adsorption column chromatography of active extract:³

Silica gel (60-120 mesh) for column chromatography and column of size (33cms x 4cms) were used for the purpose. The slurry was prepared in benzene alone, extract in the concentration of 6% was loaded on the column. After adsorption, benzene-methanol mixture (99:1) was passed through the column. 10 ml fractions were collected until a fraction did not show any visible spots when subjected to TLC. Similar fractions were collected thereafter by passing benzene-methanol mixtures in ratios, 80:20, 50:50, 20:80 and 0:100 sequentially. Fractions from individual solvent mixtures elutes were pooled on the basis of their R_f values on TLC and subjected to estimation of mosquito larvicidal activity

F. Calculations: Bioassays were carried out in triplicate. The mean value was recorded as mortality after 24 hrs. The corrected mortality was calculated by Abbot's formula:¹³

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

100 - Control mortality (%)

Lc 50 value is calculated according to methods outlined by Finney 1964: Concentration of extract is converted to ppm and then it is converted in to ln log. Mortality in % plotted on Y axis and ln log on X axis. Value obtained for 50% mortality is recorded and converted in to ppm as Lc 50 value

RESULTS

RESULTS

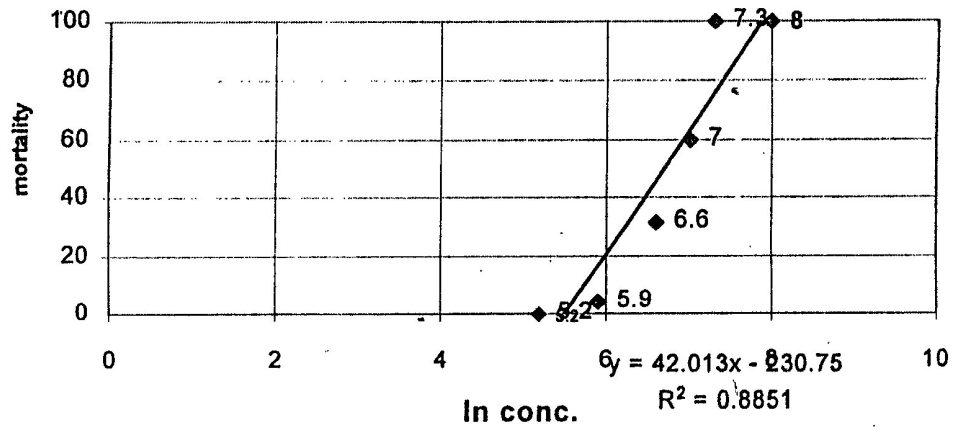
We have covered 250 houses in the selected area in different localities. The type of containers were searched as follows, barrels, plastic drums, metallic containers, etc. Total 100 containers were checked and out of that 10 containers were positive for *Aedes* larvae (Container Index = 10).

The methanol (most polar solvent) extracted most of the bio-active material from the leaves of selected plants. Out of five selected plants two plants showed promising activities those are *Annona Squamosa* with 100% mortality after 24 hrs at the concentration of 0.16% and *Azadirachta indica* with 100% mortality at the concentration of 0.2%. *Carica Papaya*, *Ficus racemosa* and *Ficus religiosa* didn't show any promising activity. Temephos standard showed 100% mortality at 0.01% concentration.

Mortality of *Aedes aegypti* larvae with leaves extract of *Annona Squamosa* after 24hrs.

Concentration (%)	0.32	0.16	0.12	0.08	0.04	0.02	control
Corrected mortality(%)	100	100	60	32	4	0	0

Annona squamosa leaves extract vs Mortality



6.5	50%
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Lc 50 at 665ppm

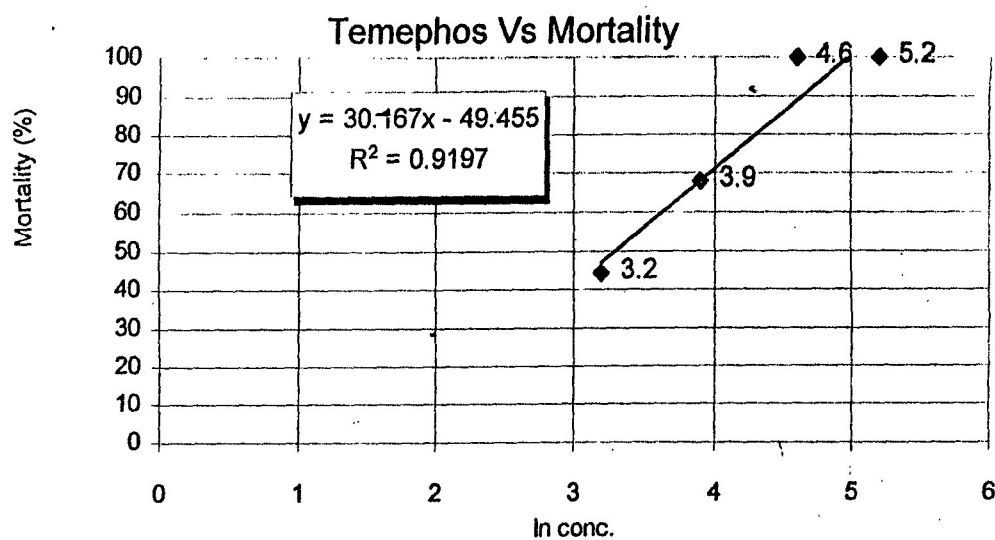
ln conc.	8	7.3	7	6.6	5.9	5.2	
Corrected Mortality(%)	100	100	60	32	4	0	

Mortality of *Aedes aegypti* larvae with Temephos(50%EC) after 24hrs.

Temephos(50%EC) concentration (%)	0.02	0.01	0.005	0.0025	0.0012
Corrected mortality(%)	100	100	68	44	28

50%	3.3
Lc 50	27ppm

ln concentration	5.2	4.6	3.9	3.2	2.4
Corrected mortality(%)	100	100	68	44	28



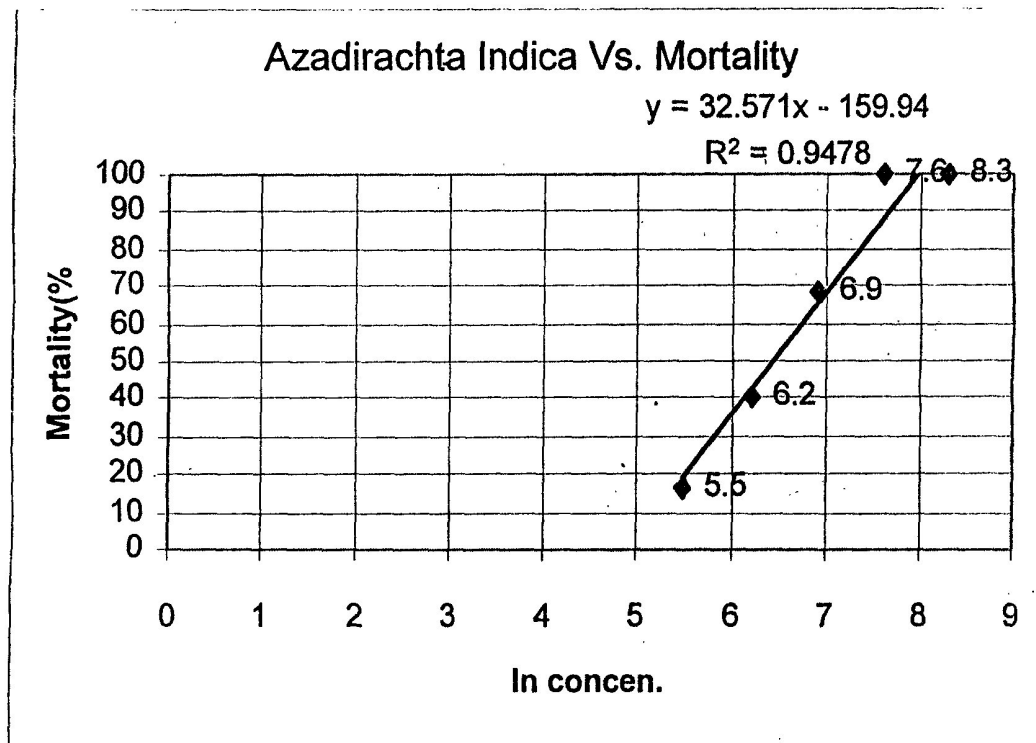
Mortality of *Aedes aegypti* larvae with leaves extract of *Azadirachta indica* after 24hrs.

Concentration (%)	0.4	0.2	0.1	0.05	0.025	control
Corrected mortality(%)	100	100	68	40	16	0

ln Concentration	8.3	7.6	6.9	6.2	5.5	control
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ln Concentration	8.3	7.6	6.9	6.2	5.5	control
Corrected mortality(%)	100	100	68	40	16	0

50% at 6.6

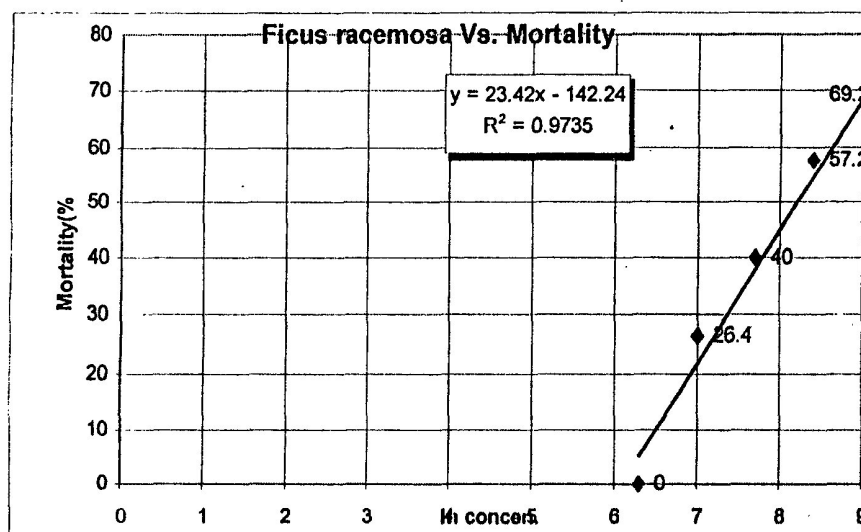


Lc 50 at 735ppm

Mortality of *Aedes aegypti* larvae with leaves extract of *Ficus racemosa* after 24hrs.

Concentration (%)	0.96	0.48	0.24	0.12	0.06	control
Corrected mortality(%)	69	57.21	40	26.4	0	0

ln Concentration	9.2	8.4	7.7	7	6.3	control
Corrected mortality(%)	69.2	57.21	40	26.4	0	0

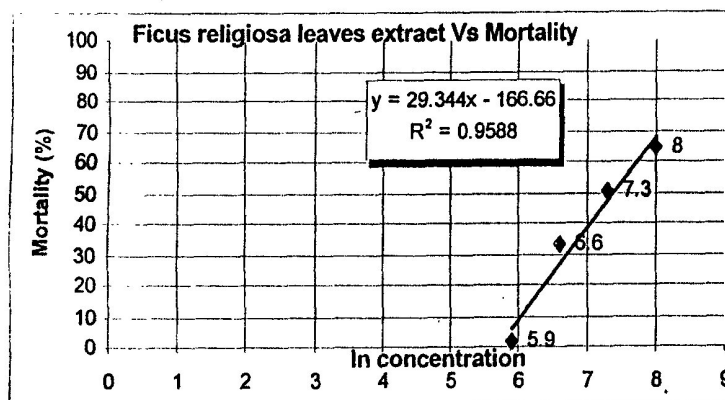


50%	8.1
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Lc 50 at 3294ppm

Mortality of *Aedes aegypti* larvae with leaves extract of *Ficus religiosa* after 24hrs.

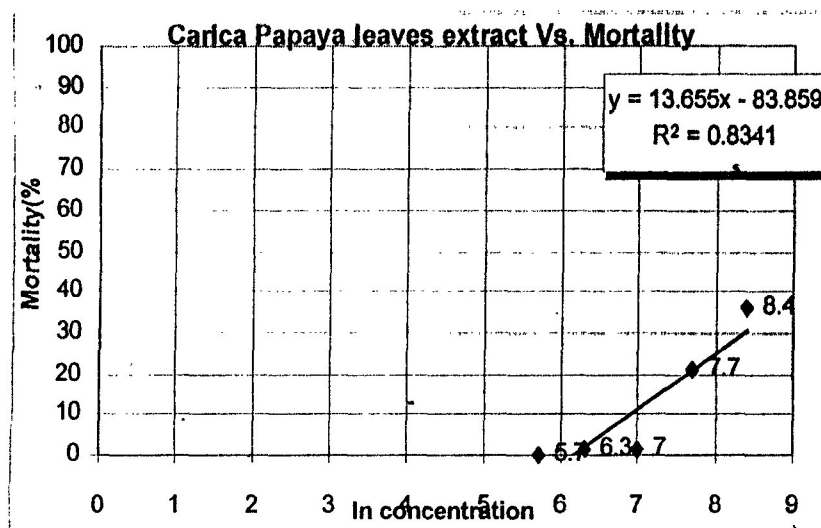
Concentration (%)	0.32	0.16	0.08	0.04	control
Corrected mortality(%)	64	50.64	33	1.33	0
ln Concentration	8	7.3	6.6	5.9	control
Corrected mortality(%)	64	50.64	33	1.33	0



50%	7.5
Lc50	1808ppm

Mortality of *Aedes aegypti* larvae with leaves extract of *Carica papaya* after 24hrs.

Concentration (%)	0.48	0.24	0.12	0.06	0.03	control
Corrected mortality(%)	36	21.32	1.33	1.33	0	0
ln Concentration	8.4	7.7	7	6.3	5.7	control
Corrected mortality(%)	36	21.32	1.33	1.33	0	0



Residual activity of these extracts is 92 hrs for 0.16% concentration in the case of *Annona squamosa* and 0.2% concentration in the case of *Azadirachta indica*. The activity however falls thereafter to 60% on the 5th day. Temephos standard at 0.2% concentration showed 100% mortality for 2 days and 60% mortality up to 6 days.

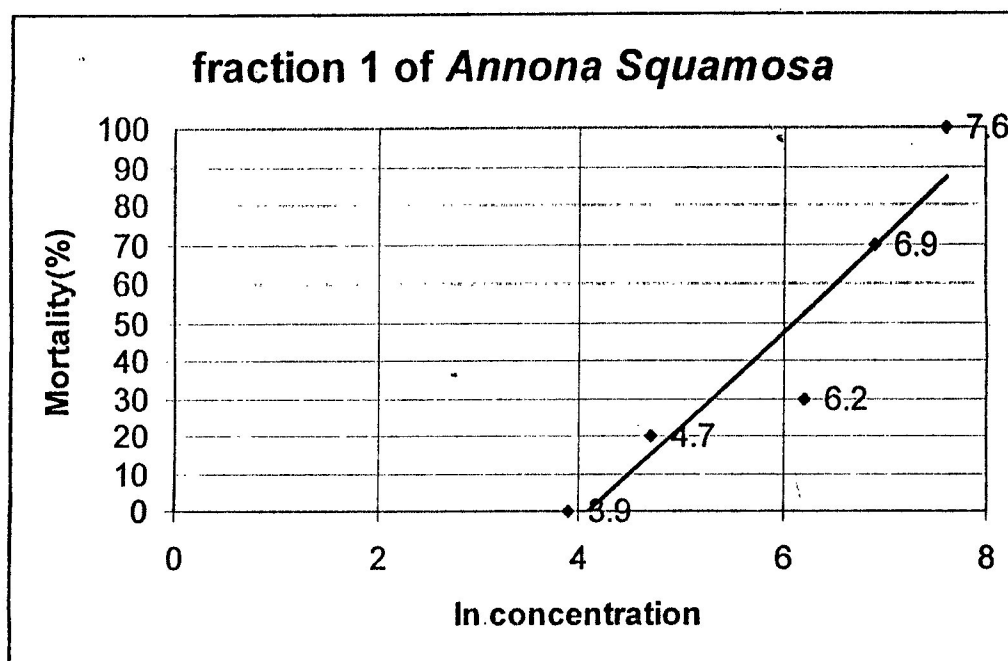
Silica gel column chromatography resulted in four active fractions out of total nine showing 0 to 100% activity, indicating thereby that this column fails to isolate single fraction with increased activity.

Fractions of leaves extract of *Annona Squamosa* obtained after Silica gel column chromatography are as follows:

Fraction No.	Mobile Phase		Colour of fraction
	Chloroform	Methanol	
1	100 : 0		Yellow
2	80 : 20		Dark Brown
3	50 : 50		Dark green
4	20 : 80		Green
5	0 : 100		Pale green
6	50 : 100		Colourless
7	60 : 40		Dark green
8	100 : 50		Colourless
9	100 : 00		Greenish yellow

Mortality of *Aedes aegypti* larvae with fractions of leaves extract of *Annona Squamosa* after 24hrs.

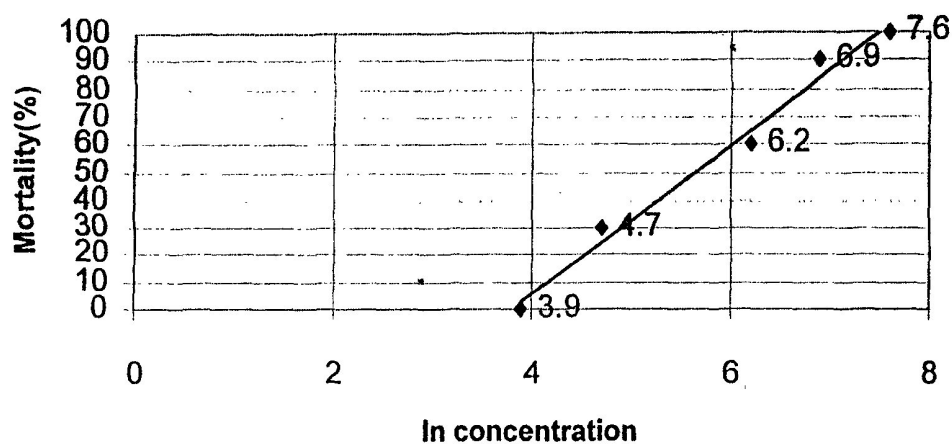
Concentration of Fraction 1 (%)	0.2	0.1	0.05	0.025	0.012	Control	Lc50 Value in PPM
Mortality (%)	100	70	30	20	0	0	500
Concentration of Fraction 2 (%)	0.5	0.25	0.12	0.05	0.012	Control	Lc50 Value in PPM
Mortality (%)	100	60	40	10	0	0	1800
Concentration of Fraction 3 (%)	0.2	0.1	0.05	0.025	0.012	Control	
Mortality (%)	30	20	0	0	0	0	
Concentration of Fraction 4 (%)	0.5	0.25	0.12	0.05	0.012	Control	
Mortality (%)	0	0	0	0	0	0	
Concentration of Fraction 5 (%)	0.5	0.25	0.12	0.05	0.012	Control	
Mortality (%)	0	0	0	0	0	0	--
Concentration of Fraction 6 (%)	0.2	0.1	0.05	0.025	0.012	Control	
Mortality	0	0	0	0	0	0	--
Concentration of Fraction 7 (%)	0.5	0.25	0.12	0.05	0.012	Control	
Mortality	0	0	0	0	0	0	--
Concentration of Fraction 8 (%)	0.5	0.25	0.12	0.05	0.012	Control	Lc50 Value in PPM
Mortality	40	10	00	00	0	0	--
Concentration of Fraction 9 (%)	0.2	0.1	0.05	0.025	0.012	Control	Lc50 Value in PPM
Mortality	100	90	60	30	0	0	225



ln dose	mortality
7.6	100
6.9	70
6.2	30
4.7	20
3.9	0

Concentration of Fraction I (%)	0.2	0.1	0.05	0.025	0.012	Control	Lc50 Value in PPM
Mortality (%)	100	70	30	20	0	0	500

fraction 9 of *Annona Squamosa*



In dose	mortality
7.6	100
6.9	90
6.2	60
4.7	30
3.9	0

Concentration of Fraction 9 (%)	0.2	0.1	0.05	0.025	0.012	Control	Lc50 Value in PPM
Mortality	100	90	60	30	0	0	225

Fractions of leaves extract of *Azadirachta indica* obtained after Silica gel column chromatography are as follows:

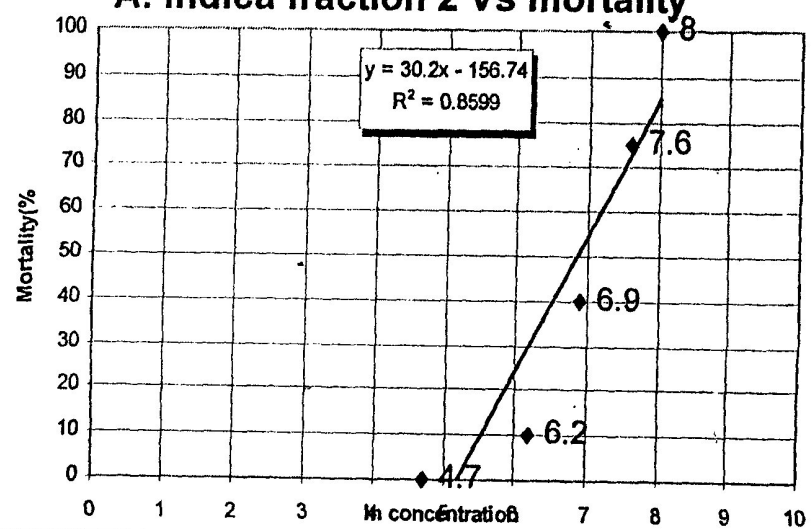
Fraction No.	Mobile Phase		Colour of fraction
	Benzene	Methanol	
1	100 : 0		Yellow
2	80 : 20		Dark yellowish green
3	50 : 50		Orange
4	20 : 80		Yellowish dark green
5	0 : 100		Dark green

Mortality of *Aedes aegypti* larvae with fractions of leaves extract of *Azadirachta indica* after 24hrs.

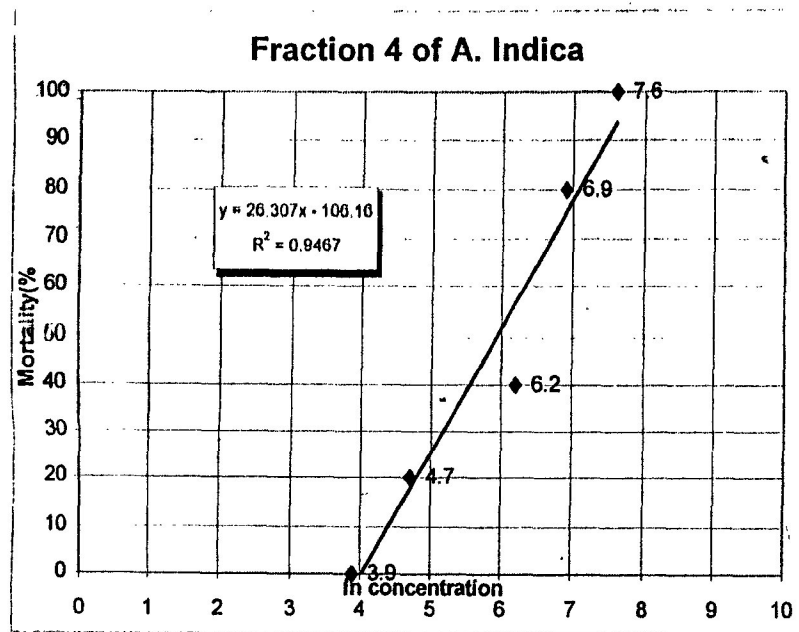
Concentration of Fraction 1 (%)	0.2	0.1	0.05	0.025	0.012	Control	Lc50 Value in PPM
Mortality (%)	50	35	20	0	0	0	
Concentration of Fraction 2 (%)	0.3	0.2	0.1	0.05	0.012	Control	Lc50 Value in PPM
Mortality (%)	100	75	40	10	0	0	897 ppm
Concentration of Fraction 3 (%)	0.2	0.1	0.05	0.025	0.012	Control	
Mortality (%)	30	20	0	0	0	0	
Concentration of Fraction 4 (%)	0.2	0.1	0.05	0.012	0.005	Control	Lc50 Value in PPM
Mortality (%)	100	80	40	20	0	0	403ppm
Concentration of Fraction 5 (%)	0.2	0.1	0.05	0.025	0.012	Control	Lc50 Value in PPM
Mortality(%)	100	70	50	35	0	0	500ppm



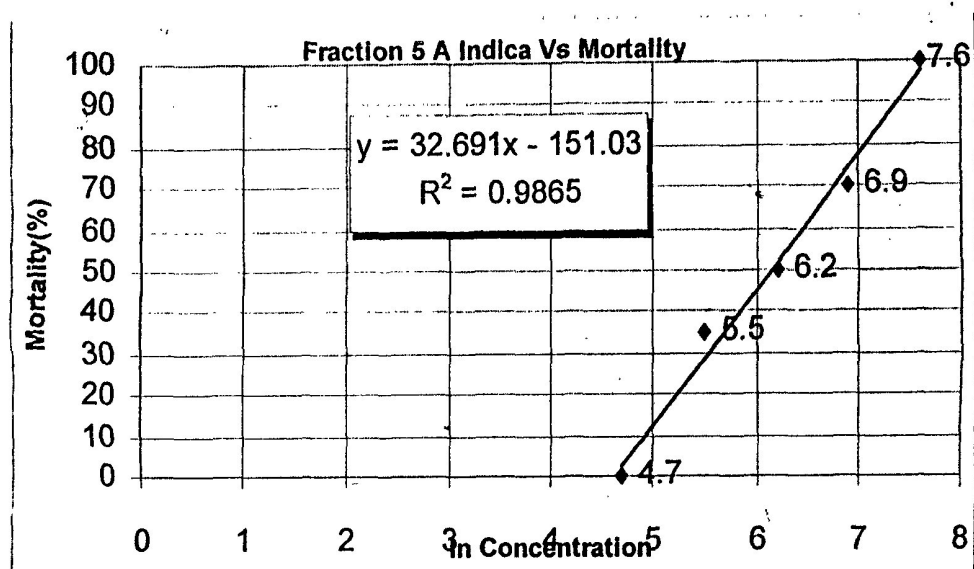
A. Indica fraction 2 Vs mortality



ln dose	mortality
8	100
7.6	75
6.9	40
6.2	10
4.7	0
6.8	50%
lc50	897.84ppm



In dose	mortality
7.6	100
6.9	80
6.2	40
4.7	20
3.9	0
50%	6
Lc50	403.42ppm



ln dose	mortality
7.6	100
6.9	70
6.2	50
5.5	35
4.7	0
6.2	50%
Lc50	500ppm



DISCUSSION

DISCUSSION

Originally *Aedes* mosquito introduced from Africa and it has adapted well to urban environment. *Aedes aegypti* has repeatedly been found to be associated with dengue outbreak in India. Recently newspapers were full of reports of dengue cases all across the Mumbai city. For example the news in one of the leading news paper 'DNA' on Tuesday, November 08, 2005 was ; "An eight-year-old girl died of dengue at the Hinduja National Hospital on Sunday." "She fell ill on November 1, She was suffering from headache and recurring fever, and vomiting whatever she ate," said her mother. On Wednesday, October 26, 2005 01:30:01 am TIMES NEWS NETWORK had following news; "Dengue, which caused a large number of deaths in the aftermath of the floods, appears to be on the rise again. Two deaths have been reported from a reputed south Mumbai hospital last week, while in Kandivili, a 43-year-old housewife died a couple of days ago of the disease. From south Mumbai's upmarket homes to suburban sprawls, cases of the deadly dengue disease with its, at times, severe complications such as internal bleeding and shock, are showing up across the city. Data given in tables on the page no.16 suggests that there were considerable increase in deaths due to Dengue after the July 26, 2005 cloudburst. Doctors believe water stored at construction sites could be the breeding ground for the *Aedes* mosquito which spreads the dengue virus." In conclusion of this we must agree that, as there is no specific treatment against dengue virus infection, vector control is the best method to combat dengue disease in times to come. Moreover exploration and utilization of natural substances as an alternative to synthetic pesticides may be the best way to achieve this aim. Beside it on page No. 13,14 and 15 original photographs of early development of Embryo towards first instar indicates our interest in studying various possibilities to arrest or modify mosquito development in more ecofriendly way.

Among selected Five medicinal plants, extracts from the leaves of *Annona squamosa* and *Azadirachta indica* are good inhibitors of mosquito breeding with 100% mortality at 0.16% and 0.2% concentrations respectively. *Azadirachta indica* fraction no. 2, 4, 5 and *Annona squamosa* fraction no. 1, 2 and 9 shown promising activity.

Among these two plants *Annona Squamosa* deserve special attention and need to be discussed in detailed. When the green, reticulated segments of the fruit split and show a pinkish tinge, the fruit is ripe and edible. The fleshy pulp is slightly sweet with a custard-like consistency. It is a favorite of

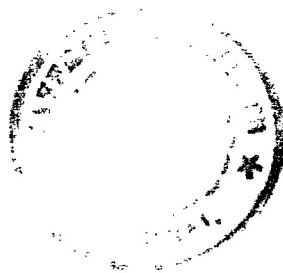
children. All the parts of this plant are reported to have medicinal value. Literature survey indicated that leaves of this plant are rich in aporphines. The seeds are used by the tribals of Madhya Pradesh State in India to induce abortion. Extracts of ground seeds from *Annona squamosa* revealed interesting insecticidal properties. By an activity-monitored fractionation, different acetogenins, called annonins and annonacin, were determined to be the active components.³⁷ The bark extracts of *Annona squamosa* yielded a new bioactive acetogenin, squamotacin, and the known compound, molvizarin, which is new to this species. Compound 1 is identical to the potent acetogenin, bullatacin, except that the adjacent bis-tetrahydrofuran (THF) rings and their flanking hydroxyls are shifted two carbons toward the "γ"-lactone ring. Compound 1 showed cytotoxic selectivity for the human prostate tumor cell line (PC-3), with a potency of over 100 million times that of Adriamycin.³

Even if temephos with 100% mortality at 0.01% concentration seems to be more effective, above extracts are water soluble, environmentally safe and non toxic to human being as compared to temephos (50%EC).

Public health pests have been increasingly the focus of researchers, environmentalists and industry. Because "Natural pesticides, or pesticides derived from natural products, support both crop production and the environment by being effective in pest control, less toxic to non target organisms and biodegradable at the same time, they may be safer than synthetic pesticides. Repeated use of a single synthetic pesticidal ingredient can result in resistance amongst the target population: where as natural products in plant defense mechanisms often consist of a variety of toxins which make adaptation of the predator unfavorable. There is need for studies that will provide the basis for developing more effective prevention strategies, including bioenvironmental factors responsible for mosquito breeding and new mosquito control measures.

We hope this study would enable such an approach.

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SYNOPSIS

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

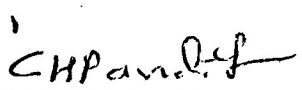
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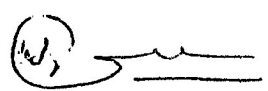
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INTRODUCTION

Dengue is an acute disease caused by four dengue virus serotypes, DEN-1, DEN-2, DEN-3 and DEN-4. The principal vector is '*Aedes aegypti*' a mosquito with worldwide distribution in tropical and many subtropical areas. All serotypes produce a similar illness characterized by fever, intense headache, nausea and vomiting and induce life long immunity that is specific to the infecting serotype. Its severe form is Dengue Hemorrhagic Fever (DHF). This is characterized by fever, thrombocytopenia, excessive capillary permeability that may lead to Dengue Shock Syndrome (DSS) and with early diagnosis and proper management the Case Fatality Rate (CFR) of DHF is generally under 1% but CFR may be over 10% once shock develops.

Dengue transmission requires the simultaneous presence of three factors:

- 1) susceptible human 2) a competent mosquito vector and 3) dengue virus. variation in any of these may promote or hinder disease activity. ⁽¹⁾

In India the dengue outbreak was reported for the first time in Vellore district of Tamil Nadu in south India in the year 1956. In the 1963, first time the virus isolation of DHF was reported (in *Aedes aegypti*) in Calcutta, West Bengal. After that many outbreaks of dengue/DHF have occurred in various parts of India including Visakhapatnam (1964), Madras (1965), Jabalpur (1966), Delhi (1967), Kanpur (1968), Ajmer (1969), Gwalior (1970), Bangalore (1970 & 71), Jaipur (1973), Jammu (1974) and Delhi (1982).

In Maharashtra in the year 1965 Dengue-4 serotype was reported in Nagpur and Dengue-2 & 3 serotypes were detected in Miraj (1986). The disease generally occurs in epidemic form during late monsoon and post monsoon periods. However, outbreaks of considerable magnitude have also been reported during pre monsoon & summer season as a result of water scarcity & consignment storage of water leading to building up of the vector population. The *A. aegypti* is a day biter mosquito. ⁽⁴⁾

In Mumbai city, incidence of dengue was suspected in 1997, where some of the areas of Wadala (E), Kurla (L/Ward), Ghatkopar (N/ward) & other suburban areas were highly suspected. Recently, in 2002 there were some suspected cases of Dengue in New Mumbai areas. Although synthetic

insecticides are proved to be effective in pest control its frequent use causes major problems such as health hazards either by inhalation or ingestion of pesticide or its residue along with the food crop. A review of studies on organo-chlorine insecticides by Attri (1979) indicated that residual DDT in the ecosystem caused shell thinning in carnivorous birds, liver enlargement and increased metabolism of estrogen which leads to serious endocrine imbalance in rats.⁵ Banarjee et al. (1983) showed that DDT fed rats had significantly higher amount of DDT (and its metabolites), in body fat, liver, and blood as compared to controls.⁶

As a result these insecticides were banned in many advanced countries like USSR, USA, France, etc. In these countries, organophosphorous insecticides replaced the organochlorinated insecticides. Presently malathion, pirimphos-methyl are the most widely used insecticides in many countries like USA and Japan. Even our country spends a considerable amount of foreign exchange on import of these insecticides. Although these insecticides are biodegradable, Du Bois (1971) has shown that these insecticides inhibit cholinesterase, an enzyme indispensable in the nervous system.⁷ Jankyn et al. (1979) implicated these insecticides in hematopoietic dysfunction.⁸

Thus, all the different insecticides discussed above suffer from drawbacks such as toxicity and/ or high cost. Due to the public concern over the toxicity & environmental impact of conventional synthetic insecticides a novel kind of mosquito control is necessary. In order to take important step in exploration and utilization of naturally occurring products for combating harmful mosquitoes, we decided to evaluate leaves extract of following plants.

- 1) *Annona Squamosa* (Annonaceae)
- 2) *Azadirachta indica* (Meliaceae)
- 3) *Carica Papaya* (Caricaceae)
- 4) *Ficus religiosa* (Moraceae)
- 5) *Ficus Recemosa* (Moraceae)

Annona Squamosa, (Annonaceae) All the parts of this plant are reported to have medicinal value. Literature survey indicated that leaves of this plant are rich in aporphines. The seeds are used by the tribals of Madhya Pradesh State in India to induce abortion.

Azadirachta indica (Meliaceae) (Neem) leaves have shown potential in the following areas :

- 11 Studies indicate that tender leaves are effective in Parasitic infections.
- A 10% aqueous extract of tender leaves has been found to possess anti-viral properties.
- Studies on plasma clotting time using Russel's viper venom have proved that the leaf extract contains a clotting inhibitor. This justifies its use in the treatment of poisonous bites.
- A total extract of Neem leaves has shown potential as a potent Hepatoprotective agent
- Water extract of Neem leaves have shown significant antiulcer activity
- Essential oil from fresh leaves has a mild fungicidal action
- Neem leaf extract shows significant Anti-inflammatory effect
- Neem leaf extract have shown reduction in the frequency and severity of stress-induced gastric mucosal lesions.
- Intraperitoneal administration of Neem leaf, bark and seed extracts revealed immuno-stimulatory properties of Neem, which are responsible for their anti-HIV effect.

Carica Papaya (Caricaceae), It contains many biologically active compounds. Two important compounds are chymopapain and papain, which are supposed to aid in digestion. Papain also is used to treat arthritis. The level of the compounds vary in the fruit, latex, leaves, and roots. In addition, plant parts from male and female trees differ in the quantity of the compounds. For example, phenolic compounds tend to be higher male trees than female trees. The quantity of fresh papaya latex and dry latex (crude papain) also vary with the sex of the tree and the age of the tree. Female and hermaphrodite trees yield more crude papain than male trees and older fruit yields more than younger fruit. However, the activity of the papain is higher in the extracts from the younger fruit than the older fruit.

Ficus Recemosa (Moraceae), (Umbar) the alcoholic extract of the stem bark of the plant possessed antiprotozoal activity. ***Ficus religiosa (Moraceae) (Peepul)*** has too many possible beneficial herbal uses to fully enumerate. A few of its uses include: as an astringent, as an antidiarrheal, as an antidysenteric and as a laxative. The fruit and the leaves and the bark and even the latex are used to prepare herbal remedies. It was therefore decided to study the ecological aspects of *Aedes aegypti* as a vector of dengue & possible control with selected indigenous plant extracts.

AIMS AND OBJECTIVES

1. Study of vector population:-

- A. To identify the study areas.**
- B. To Collect and identify the larvae of *aedes aegypti* from selected study areas.**
- C. To calculate the Container Index.**

2. Evaluations of possible larvicidal activity of leaves extracts of selected plants.

- A. To collect and identify selected plants taxonomically:**
- B. To prepare leaves extracts of selected plants using solvent:**
- C. To evaluate the leaves plant extracts for activity against larvae of *A.aegypti* in vitro:**
- D. Residual activity of leaves extracts:**
- E. Adsorption column chromatography of active extract:**
- F. Calculations:**

MATERIAL AND METHODS:

1. Study of vector population:-

B. To identify the study area:

Airoli is identified as a study area in New Mumbai on the basis of information available on suspected outbreaks of dengue and also on the basis of number of reasons : A number of construction sites with water storage tanks, auto garages with a piles of old tyre are present in these areas. These areas are heavily populated. A number of discarded tins, coconut shells, broken pots, bottles & plastic sheets lie scattered in & around the service quarters of these areas, which during the monsoon serve as potential breeding sites for *A. aegypti*.

B. To Collect and identify the larvae of *Aedes aegypti* from selected study areas: ⁽²⁾*Aedes* larvae can easily be distinguished in the field by their distinctive jerky wriggling movement. They are generally found clustered in dark corners of containers, still identification of the larvae in laboratory was necessary. We have covered 250 houses in the selected area in different localities. The type of containers were searched as follows, barrels, plastic drums, metallic containers, etc. Collected larvae transported to the laboratory in clean containers, Identification of larvae is done using entomological keys and colony is maintained and blood fed on guinea pig.

D. To calculate the container Index of *Aedes aegypti* :

Following parameter is used.

$$\text{Container Index} = \frac{\text{No. of positive containers}}{\text{Total no. of containers screened}} \times 100$$

2. Evaluations of possible larvicidal activity of selected plant extracts.

A. To collect and identify selected plants taxonomically:^{9, 10}

1) *Annona squamosa* (Annonaceae); Custard Apple, a small evergreen tree, occurs wild; it is also cultivated throughout India because of its fruits. Leaves simple, alternate, bifarious; fruits yellowish green; seeds hard. distributed throughout the India

2) *Azadirachta indica* (Meliaceae) (Neem): A medium to large sized tree, 15-20 m in height, leaves compound, imparipinnate, leaflets, subopposite, serrate, very oblique at base. This tree is distributed throughout the India, deciduous forest and is also widely cultivated.

3) *Carica papaya* (Caricaceae) : A small soft wooded short lived lactiferous tree up to 8.0 m in height with a straight cylindrical stem bearing characteristic leaf-scars throughout and with tuft of leaves at the top; leaves deeply lobed, palm-like with long hollow petiole. Distributed and cultivated throughout the India

4) *Ficus racemosa* (Moraceae), (Umbar) : Deciduous tree, leaves dark green, ovate or elliptic. Distributed throughout India.

5) *Ficus Religiosa* (Moraceae) (Peepul) : A large deciduous tree. Leaves are shiny, cordate, long petioled ovate. Distributed throughout the India

B. To prepare selected leaves extracts using solvent:²¹

About 20 gram of powdered, air dried leaves of selected plants, placed in a conical flask. Macerated with 100ml of methanol for 6 hours, shaking frequently then allowed to stand for 18 hours. Filtered & concentrated at 40°C on water bath. Accurately weighed extract diluted in water to give

different concentrations.

C. To evaluate the leaves extracts for activity against larvae of *A. aegypti* in vitro:³

Ten beakers of 500 ml capacity were arranged in a series. Each beaker will be filled with 250ml of water. Different aliquots of the extracts were added to beakers. After thirty min. twenty five third instar larvae of *Aedes aegypti* were placed in these beakers and the larval mortality was recorded after 24 hrs. Temephos (50%EC) served as a standard and two controls were maintained, one was water and other was water with the solvent concentration used in dilution of extract.

D. Residual activity of extracts:³ Emulsions containing individual extract in concentration higher than those showing 100% larvicidal activity in 24 hrs. were tested for continued mosquito larvicidal activity until mosquito larvicidal activity lowered to 60% mortality in each case. The general design of experiment was as described in the earlier experiment.

E. Adsorption column chromatography of active extract:³ Silica gel (60-120, mesh) for column chromatography and column of size (33cms x 4cms) were used for the purpose. The slurry was prepared in benzene alone, extract in the concentration of 6% was loaded on the column. After adsorption, benzene-methanol mixture (99:1) was passed through the column. 10 ml fractions were collected until a fraction did not show any visible spots when subjected to TLC. Similar fractions were collected thereafter by passing benzene-methanol mixtures in ratios, 80:20, 50:50, 20:80 and 0:100 sequentially. Fractions from individual solvent mixtures elutes were pooled on the basis of their R_f values on TLC and subjected to estimation of mosquito larvicidal activity

F. Calculations: Bioassays were carried out in triplicate. The mean value was recorded as mortality after 24 hrs. The corrected mortality was calculated by Abbot's formula :¹³

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

Lc 50 value is calculated according to methods outlined by Finney 1964.

RESULTS

We have covered 250 houses in the selected area in different localities. The type of containers were searched as follows, barrels, plastic drums, metallic containers, etc. Total 100 containers were checked and out of that 10 containers were positive for aedes larvae (Container Index = 10).

The methanol (most polar solvent) extracted most of the bio-active material from the leaves of selected plants. Out of five selected plants two plants showed promising activities those are *Annona Squamosa* with 100% mortality after 24 hrs at the concentration of 0.16% and *Azadirachta indica* with 100% mortality at the concentration of 0.2%. *Ficus racemosa* and *Ficus religiosa* didn't show any promising activity. Temephos standard showed 100% mortality at 0.01% concentration.

Mortality of *Aedes aegypti* larvae with leaves extract of *Annona Squamosa* after 24hrs.

Concentration (%)	0.32	0.16	0.12	0.08	0.04	0.02	control
Corrected mortality(%)	100	100	60	32	4	0	0

Mortality of *Aedes aegypti* larvae with Temephos(50%EC) after 24hrs.

Temephos(50%EC) concentration (%)	0.02	0.01	0.005	0.0025	0.0012
Corrected mortality(%)	100	100	60	32	4

Residual activity of these extract is 92 hrs for 0.16% concentration in the case of *Annona squamosa* and 0.2% concentration in the case of *Azadirachta indica*. The activity however falls thereafter to 60% on the 5th day. Temephos standard at 0.2% concentration showed 100% mortality for 2days and 60% mortality up to 6 days. Silica gel column chromatography resulted in four active fractions out of total nine showing 0 to 100% activity, indicating thereby that this column fails

DISCUSSION

Aedes aegypti has repeatedly been found to be associated with dengue outbreak in India. Originally Aedes mosquito introduced from Africa, it has adapted well to urban environment.

The extract from the leaves of *Annona squamosa* is a good inhibitor of mosquito breeding with 100% mortality at 0.16% concentration. Even if temephos with 100% mortality at 0.01% concentration seems to be more effective, *Annona squamosa* extract is water soluble, environmentally safe and non toxic to human being as compared to temephos (50%EC).

Public health pests have been increasingly the focus of researchers, environmentalists and industry. Because "Natural pesticides, or pesticides derived from natural products, support both crop production and the environment by being effective in pest control, less toxic to non target organisms and biodegradable at the same time, they may be safer than synthetic pesticides. Repeated use of a single synthetic pesticidal ingredient can result in resistance amongst the target populations: where as natural products in plant defense mechanisms often consist of a variety of toxins which make adaptation of the predator unfavorable. There is need for studies that will provide the basis for developing more effective prevention strategies, including bioenvironmental factors responsible for mosquito breeding and new mosquito control measures.

We hope this study would enable such an approach.

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