Materials and Methods
MATERIALS AND METHODS

DOMESTIC WELLS

Due to urbanisation, people in the cities enjoy maximum benefits, which is being provided by science, as development technologies. In case of domestic wells, most of them are replaced with motor drawn than with hand drawn; and in some houses, these wells are not being used and are not closed, as they have started using bores. The partially used and unused wells are the primary sources for multiplication of mosquitoes in Chennai City. Many common wells in the city were used continuously by people, and the absence of mosquito population was recorded. Most of the domestic wells in houses are not provided with proof for insects; they also lack fishes or insects, which could control mosquito population to some extent.

These wells are used to tap underground water resources for domestic purposes. The wells are usually brick-lined and the water may be hand drawn or pumped out. Hand drawn wells are generally left open to draw water (Plate 1a). Observation for this study was made on the immatures, breeding in domestic wells located in Perambur, Chennai.

Sampling of immatures in wells was undertaken with the help of a well sampling net (well net). Russell et al. (1963) used the weighted cone
Plate 1

a - Domestic Well
b - Well Net
shaped net, which was gently lowered into a well and carefully pulled against the walls of the wells after a short interval. A similar well net was designed for this study. The well net used for this purpose was made up of a cone shaped muslin bag attached to a galvanized iron ring frame of 20 cms diameter. The net was kept suspended to the iron ring frame at equal distance to each other, the other end of the strings being joined together about 18 inches above the net. A length of nylon rope was tied at this point to enable sampling at different well depths (Plate 1b).

Samplings were undertaken in 10 domestic wells. Five samples were taken from each well. Samples were collected by pulling the well net through a distance of 1 metre along the sides of the wall. Care was taken to avoid repeated samplings in the same area. Instarwise immature count for each sample was recorded. Fourth instar larvae and pupae were collected in a container and brought to the laboratory and identified after adult emergence. The water temperature was recorded for the wells sampled. The emerged mosquitoes were identified from the keys of Christophers (1933) and Barraud (1934).

WATER STORAGE CISTERN

The mosquito breeding pattern in cistern was carried out at Nugambakkam in Chennai city. The study period was between November 2000 to October 2001. Initial survey was carried out to select the
Plate 2

a - Cistern
b - Dipper
suitable cisterns with mosquito breeding for the study. Three cisterns with mosquito breeding were selected for the study. These cisterns were found outside the houses and were unused (Plate 2a).

A dipper (Plate 2b) with 8 cm diameter and 240 ml water holding capacity was used for sampling. Samplings were taken daily and the sample size varied. Larval and pupal counts for each dip were recorded and the larvae were returned to the respective cisterns before the next sample was taken. Pupae were collected and brought to the laboratory for emergence. Peak noon water temperature was recorded daily.

The dipper is the most commonly used tool for collecting mosquito larvae and pupae that occur in large and small collections of ground water, in rock pools and a variety of large container type habitats (Service, 1976).

**CONCRETE CEMENT TANKS (NON-POTABLE)**

Rapid urban expansion has resulted in a considerable stress on already meager drinking water supplies. Due to this water scarcity people tend to store water in different types of containers. Cement tanks are commonly used for this purpose. The mosquitoes prefer to breed in water storage tanks (cement tanks), for both potable and non-potable purpose. The breeding pattern of mosquito in non-potable constructed cement
Plate 3

a - Outdoor Cement Tank
b - Indoor Cement Tank
tanks was carried out at Perambur, Chennai city. The study period was between November 2000 to October 2001. Initially a survey to select the suitable tank with mosquito breeding was carried out. Three tanks both of indoor and outdoor sources of mosquitoes were selected (Plate 3a & 3b).

A dipper with 12 cm diameter and 350 ml water holding capacity was used for sampling. Samplings were taken thrice a week. Sample size varied. Larval and pupal counts for each dip were recorded and the larvae returned to the respective tanks before the next sample was taken. Pupae were collected and brought to the laboratory for emergence. Peak noon water temperature was recorded.

CONSTRUCTION SITE

The building construction in the city due to rapid urbanization also serves as a source for the proliferation of mosquitoes. The water in construction sites were normally stored in construction pits and plastic barrels. The study was carried out from February 2001 to June 2001 at Nungambakkam, Chennai. Initial survey to select suitable breeding sites was carried out. Two curing pits and three drums (plastic barrels) with immature positivity were selected for the study (Plate 4a & 4b).
Plate 4

a - Curing Pit
b - Plastic Barrel
A dipper with 12 cm diameter and 350 ml water holding capacity was used for sampling. Samplings were taken on alternate days. Sample size varied. Larval and pupal counts for each dip were recorded and the larvae returned to the respective tanks before the next sample was taken. Pupae were collected and brought to the laboratory for emergence. Peak noon water temperature was recorded daily. Pupae were taken to laboratory and identified upon adult emergence.

LAB STUDIES

The present study was conducted in the P.G. & Research Department of Zoology, Loyola College, Chennai. The vector mosquitoes selected for this study were Anopheles stephensi (Malaria), Culex quinquefasciatus (Filaria) and Aedes aegypti (Dengue).

A substantial number of mosquito larvae and pupae for this study were obtained from a stock (Anopheles stephensi, Culex quinquefasciatus & Aedes aegypti) which was maintained in laboratory. The synthetic chemical insecticide (Baytex 83%EC & Abate 50%EC) and botanical insecticide (Neemazal T/S 1% EC) were used for their toxic effect against these larvae. The larvae were exposed to different concentrations, prepared in parts per million (ppm) basis, through serial dilutions based on the proportion of the insecticide used.
COMPOUNDS USED FOR THE STUDY

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Commercial Name</th>
<th>Formulation</th>
<th>Active ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant product (Neem)</td>
<td>Neemazal - T/S 1% EC</td>
<td>Emulsifiable Concentrate</td>
<td>Azadirachtin A</td>
</tr>
<tr>
<td>Organophosphorus compound</td>
<td>Baytex (Fenthion 83% EC)</td>
<td>Emulsifiable Concentrate</td>
<td>Fenthion</td>
</tr>
<tr>
<td></td>
<td>Abate (Temephos 50% EC)</td>
<td>Emulsifiable Concentrate</td>
<td>Temephos</td>
</tr>
</tbody>
</table>

REARING AND COLONIZATION OF MOSQUITOES

Preliminary field studies were conducted in and around Loyola College campus, Chennai, to observe the presence and abundance of larval mosquito population. The larvae and pupae were collected from the field using a dipper 12 cms. in diameter and 350 ml capacity and were transferred into plastic containers, which were provided with holes on the top to provide free movement of air for larvae. The larvae were taken to the laboratory in these containers and transferred to trays with water from the natural breeding sources. The water will remain unchanged till the emergence of the adult, as change in water may increase mortality in larval population due to change in the water parameters.
Plate 5

a - Mosquito Cages for rearing adults
b - Adult mosquitoes in the Cage
Plate 6

a - III instar larvae
b - IV instar larvae
c - Pupae
EMERGENCE AND REARING OF ADULTS

The pupae were collected in a cup and were kept inside the cage of size 30 x 30 x 30 cm (Plate 5a). An average of about 100 - 150 pupae were kept in a single cage for emergence. The pupae developed into adults in one or two days and both the male and female imago were fed with 10% sucrose solution. The female mosquito requires blood meal for the development of ovary to lay eggs. After two to three days of emergence the females were fed with blood meal, this helps the female mosquitoes to lay eggs. The oviposition traps were placed inside the cage into which they are forced to lay eggs (Plate 5b).

LARVAL COLLECTION

The ovipositional cups were kept undisturbed for 1-2 days to see the hatching of first instar larvae. Then the larvae were transferred gently into the trays and were maintained till they become pupae. The larval developmental stages include I instar, II instar, III instar (Plate 6a), IV instar (Plate 6b) larvae and Pupae (Plate 6c). The larvae were fed with larval feed - dog biscuits and yeast powder in the ratio of 3:1. The early third and fourth instar larvae were used for the study.
Plate 7

a - Larval Bioassay
b - Pupal Bioassay
LARVAL BIOASSAY TEST

The early third and fourth instar larvae of all the three vector species were separately tested with different concentrations of insecticide (WHO, 1960). Batches of 20 larvae were exposed to 250 ml of a particular concentration of test solution. Five or more concentration of the insecticide giving between zero and 100% mortality of larvae in 24 hours of exposure were recorded. Triplicates were done at each concentration. Parallel controls were maintained (Plate 7a).

PUPAL BIOASSAY TEST

The pupae of each mosquito species were separately tested with different concentrations of insecticide. Batches of 10 pupae were exposed to 250 ml of a particular concentration of test solution. Five or more concentration of the insecticide giving between zero and 100% mortality of larvae in 24 hours of exposure were recorded. Two replicates were done at each concentration. Parallel controls were maintained (Plate 7b).

AWARENESS AMONG STUDENTS

Mosquito control can be divided into two areas of responsibility: individual and public. Control of mosquito and mosquito borne diseases would be successful only if the community cooperates with the modern research and government implements. So a questionnaire was circulated
among the student community and their level of awareness was also recorded. Five top colleges in Chennai City were selected for the study. Thousand samples were collected from each college, with a total of 5000 samples. The questionnaire was evaluated and grouped into 4 groups (bad, average, good and excellent).

**STATISTICAL ANALYSIS**

The results were analysed statistically in a computer using the software package "SPSS", version 10.1 (September 9, 2000; copyright SPSS INC., 1989-2000). For computing $L_{C_{50}}$, the data were subjected to Finney's (1971) method of Probit analysis as detailed by Regupathy and Dhamu (1990).