INTRODUCTION
The findings of Larveran, the French physician on 
**Plasmodium** - a protozoan parasite as causative agent for 
**Malaria** and findings of Sir Ronald Ross on mosquito as 
carrier of Plasmodium, placed mosquito at the centre of the 
stage in transmission of Malaria to Mankind. In fact, findings 
of Ross initiated man's efforts to protect himself from 
mosquito bites and disease transmission. Since then the war 
between man and mosquito has been continuing indecisively.

1.0 MOSQUITO IN RELATION TO PUBLIC HEALTH:

Mosquitoes are the most important group of **Arthropod** 
(Insects) vectors of tropical diseases. They are remarkably 
adapted and are fully cosmopolitan with over 3000 species 
distributed throughout the world. It is fortunate that only 
a few species are known to be natural vectors of disease. 
Throughout human history, mosquitoes have been a constant 
impediment to man's progress causing nuisance, agony and 
ill-health with their painful bitings for blood and ability 
to transmit dreadful diseases like malaria, dengue, yellow 
fever etc. Though man is putting forth his maximum efforts 
deploying new strategies against this mosquito with latest 
scientific spirit, till today no promising approach has been 
made.

1.1 MOSQUITO TRANSMITTED DISEASES:

(A) **Malaria** is one of the major tropical diseases, which 
drew the attention of health ministry of several nations.
Till today, malaria control is a big challenge to medical research. Though malaria is, today primarily a disease of the tropics, it may become common in temperate zone by the turn of this century. Despite the outstanding progress of W.H.O. programme to eradicate, malaria started in 1955, no substantial progress has been achieved in disease control. Malariologists can no longer predict the complete eradication of this disease in the foreseeable future, besides their considerable net gains in their field. Efforts in malaria control can be fruitful if any break through is made in malaria 'vaccination' or 'vector control' through genetic engineering or biotechnology.

(B) *Filaria* is another disease transmitted by mosquito. It is estimated that nearly three hundred million people have been affected by this disease. Among eight important species of Wuchereria - filarial parasite, *W. bancrofti* and *W. burgia* are the most common parasites transmitted by *Culex* and *Mansonina* species of mosquitoes respectively.

(C) *Yellow fever*, is transmitted by an urban mosquito - *Aedes aegypti*. This disease is usually transmitted from infected people of seacoast community.

(D) *Dengue* is also confined to tropical region. It is not so for registered in Asia. Dengue does not have any animal
reservoir and it is endemic only in areas where *Aedes aegypti* is prevalent. Control of dengue is possible by the control of *Aedes aegypti* since this mosquito is closely associated with man and his urban environment.

(E) **Encephalitis** is caused by viruses which are transmitted by both *Aedes* and *Culex* species. The viruses transmitted by mosquitoes attack central nervous system.

Mosquito thus plays important role in spreading the deadly tropical diseases. So far no definite promising curative agents is formulated for these diseases. In this crisis, prevention is the only remedy. Vector control (mosquito) is thus important.

2.0 GENERAL MOSQUITO CONTROL MEASURES IN PRACTICE:

2.1 ADULT MOSQUITO CONTROL METHODS:

(A) Mosquito nets:

Though scientists have been struggling to develop an everlasting approach in mosquito abatement, mosquito net still proved to be perpetual and economical device. Some improvements have taken place by introducing impregnated nets with some cidal agents. Permethrin impregnated nets are being used increasingly in many parts of tropics to control malaria (W.H.O., 1989; Rozendaal, 1989, Lindsay et al.,
1989). Permethrin-impregnated bednets are capable of killing mosquitoes when they come in contact with those nets (Darriet et al., 1984; Snow et al., 1987; Lindsay and adiamah, 1992) and some may become physically irritated and fly away (Miller, 1990). China and Tanzania have successfully tried dipping mosquito nets in pyrethroid solution.

(B) Fumigation (Fogging):

Mosquitoes can be driven out from human habitats or killed by fumigation. The smoke of garlic, sulphur, pyrethrum, cresol and other derivatives of naphthal are considered to be effective in driving out mosquito. Recently two new pyrethroids with cidal action on adult - Culex tarsalis, have been developed (Schaffer, et al., 1990).

(C) Repellents:

It is no wonder to say 'the market for mosquito repellents is expanding as fast as the rate at which mosquito breeds'. Now a variety of mosquito repellents are available in the market - from creams and coils to mats. The active ingredient in both coils and mats is 'synthetic pyrethroid' because of its rapid knock-down effect. Birley (1987) reported that esbiothrin containing mosquito coils gave rapid knock down and 94% inhibition of biting in
laboratory and gave 84% protection in fields. Recently, Mihara and Toshioshono (1991) suggested Allethrin fumigation against pyrethroid resistant *Culex tritaeniorhynchus* adults. However, Anaso et al., (1990) suggested Citrus sinensis and Citrus paradise orange peels as a good substitutes for some brands of mosquito coils which contain unacceptable high levels of toxic insecticide dicophone. It has been reported repeated use of these mosquito repellents may cause systemic allergic reactions like nausea, vomiting, headaches and disturbances in central nervous systems. It is reported that there is tangible evidence of presence of nitroso compound a known carcinogen in smoke of mosquito coils (Indian Express, 1991).

(D) Insecticides:

Though certain commonly used insecticides like D.D.T. (Dichloro-diphenyl-trichloroethane) pyrethrum, Benzene hexachloride (BHC) are of considerable effect on adult mosquitoes, these mosquitoes are developing resistance to these insecticides. Each new insecticide makes yet another gene resistant. Because of flight gift, mosquitoes are disappointing the purpose of insecticide usage. Nearly 50 per cent of insecticide fails to reach the target and in turn creates environmental problems.
Mosquitoes to-day are bolder hardier than ever before. After some years, each insecticide works as a sieve, saving the stronger ones. Each new insecticide makes yet another gene resistant. W.H.O. expert committee on insecticides reported (7th report) that 102 species of arthropods of medical and veterinary importance became resistant by the end of 1968. By turn of this century another 1500 species of insects will become resistant to insecticides (Bhargva and Venkataramani 1988). Mosquito resistance to pesticides and environment quality for better living demand alternative strategies.

(E) Adult traps:

A variety of traps have been designed for emerging mosquitoes. These are conical or box type traps which float upon water surface (Service, 1970, 1976; Pritchard, 1980). Girikumar and Venkateswara Rao (1984) designed a lowcost trap for collection of mosquitoes emerging from open pit-latrines and ditches beside human dwellings. The results with 15-d-cyphenothrin insecticide treated sound trap revealed 80.9% females, and 75.6% male population of Aedes were trapped (Ikeshoji and Hanhen gyap, 1990). Kusukabe and Toshiki (1990) reported that by trapping of both sexes of mosquito, more efficient control is achieved than by manipulating the male population alone. Several hand-held
aspirators or collapsible cages are being designed for collection mosquitoes in entomological field work (Collins, 1990).

(F) Genetic control:

The aim of genetic control is to bring about reproductive failure in mosquito. In India male population of *Culex fatigans* sterilized by gamma radiation resulted in inviability of eggs in females. (Krishnamurthy et al., 1961). According to Laven (1967) deployment of sterilized males reduce mosquito population in confined areas only.

2.2 LARVAL MOSQUITO CONTROL:

The reports and experience of researchers and Entomologists revealed that control of larvae is a more convenient and effective approach than other approaches in mosquito control.

(A) Elimination of breeding grounds:

As mosquito larvae and pupae are aquatic, elimination of breeding grounds is of primary importance. Swampy, marshy grounds, cesspools, pits, and ditches must be drained. Water must be prevented from storing in tree-holes, house hold containers etc.

(B) Chemical control:

Of these practices, the oldest practice is usage of petroleum oil or kerosene. It has been reported that amines have pupicidal and larvicidal action (Clarke et al.,
1978). Coming to latest practices several immature mosquitoes are being killed through applied surface films. (Velentuk and Turchenko 1976; Levy et al., 1980). Several mosquito species have been tested against monomolecular organic surface film (Mulla et al., 1983). An oily material called 'monoxic' is the latest approach in the fight against mosquito. This 'monoxic' forms oily film just one molecule thick spread across the surface of ponds and drowns mosquito larvae when they come to the surface to breath. (New Scientist, 1987). Combined organophosphorus compound with oil has a larvicidal and fumigating effect on the population of Culex pipiens (Sichinava 1987). The spraying operation of Bendiocarb (Ficamr) is also effective in keeping low mosquito density (Amalaraj, 1987). The Alpha-terthiienyl which is photo-toxic, showed LC$_{50}$ value for first instar larvae of Aedes at 0.002 ppm. As rice fields are one of the important mosquito breeding centres, a lot of work is being carried out to evaluate pesticide toxicity on mosquito larvae and predators beneficial in mosquito reduction in rice fields. It is reported that pesticides are more effective on Culex than Anopheles (Shim, 1987). The recent studies on cidal action of insect growth regulators assumes importance in mosquito control research. Laboratory studies of cyromazine produced 97% and 99% inhibition in adult emergence at 1.5 ppm and 1 ppm (Nelson, 1986). Penbluran and
furyltriazine (IGR) have also potent larvicidal and pupicidal action against Anopheles stephenei (Saxena, 1986).

(C) Biological control:

The oldest form of biological control of mosquito is usage of the fish - Gambusia affinis. Recently an indigenous species - Aplochellus blochii, has been recommended for mosquito larval control. This fish is advantageous over Gambusia as it has higher survival and predating rates (Rajagopalan, 1984). Various aquatic bugs, notably Ranatra, Nepa, Notonecta and Zaitha play a significant role in mosquito reduction. Gold fish and perch can often be advantageously used in garden ponds, fountains etc. The world health organization has sponsored promising research with Poecilia reticulata, the guppy, in Indonesian rice fields and with Oreochromis spilurus in underground reservoirs in Somalia.

By introducing pathogens and parasites we can check the larval population. Larval mosquito population can be reduced by releasing nematode parasite Reesimermis nielseni (Peterson, 1975). Another promising nematode parasite is Romanomermis culicivorax (Mitchell and Mitchell, 1982; Laird et al., 1982). Effective knockdown of mosquito larvae is achieved by cercarial infection Venkateswara Rao et al., 1985).
The accepted truth is that a mosquitocide among laboratory chemicals is no more reliable; but mosquitocides from the environment like bacteria, fungi, biocides etc., may provide a hopeful remedy. Development of microbes for mosquito control is being programmed rapidly during the last decade. Bio-technology may play major role in this field in future.

Bti (H-14) and other strains of *Bacillus thuringiensis* produce protein endotoxin that causes larval death. Strains of *Bacillus* have proved to be effective larvicide with several advantages. They can be mass-produced by conventional fermentation techniques and harmless to environment. In addition, they are effective against chemically resistant mosquitoes. But the disadvantage is that it does not persist long in the environment and so it must be reapplied frequently. *Bacillus sphaericus* offers hope for overcoming this drawback. The application of biotechnology in developing improved *Bacillus* strains with broad spectrum of action, more persistance, greater recycling potential is the latest trend in this field.

Entomopathogenic fungi seem to be promising agents against larval mosquitoes (YuHYO - SOK, 1986). *Metarhizium anisopliae* destroys a broad variety of target insects, including every species of mosquito that has been tested
(Riba et al., 1987). *Agenidium giganteum* seems to be promising in temporary pools such as rice fields. It has many advantages over *Bti* in that, it has a spore and can be mass produced in fermentation tanks, at a cost lower than *Bti*. One more advantage is that once it is released into environment, it persists long and need not be reapplied.

(D) Plant products as larvicides:

The studies on effects of crude extracts of plant materials on mosquito larvae as larvicides or growth inhibitors is another interesting aspect in mosquito control strategy. The extracts of *Ervatamia* and *Cassia holosericea* inhibited mosquito population. These two plants seem to have juvenomimetic activity (Qureshi, 1987). The extract of grain sorghum seedling has significant larvicidal action on *Culex pipiens* (Jackson, et al., 1990). Hasspieler et al., (1990) described the mode of action of plant derived phototoxin terthienyl in mosquito larvae.

2.3 INTEGRATED MOSQUITO CONTROL METHODS:

At this alarming state of mosquito menace, we are forced to accept the fact of "no universal and definite approach is available for mosquito abatement". However, we must concentrate on integrated control method(s), which comprise physical, chemical, biological and environmental
procedures. So, new strategies have to be formulated against mosquito, which are fully acceptable from health and environmental stand points, depending on our socioeconomic and climatic conditions. In rice fields of Korea periodic water drain, selective/reduced use of pesticides/herbicides, using microbiological agents and reduced insecticide treatment, helped in bringing down the population of *Culex* and *Anopheles* (W.H.O. 1986).

2.4 MOSQUITO MENACE IN INDIA:

India as one of the tropical countries, with a warm climate and other optimal conditions, provides a favourable climate for mosquito thriving. It has been estimated that nearly 40% of health budget of Government of India is being allocated for malaria control programme. Till 1965, malaria was under control, but later it was slowly raising and became alarming by end of 1976, with 6.74 million malaria cases registered. Every year, malaria continues to hit not less than a million Indians and killing hundreds. Development of resistance to chloroquine by *Plasmodium* and to insecticides by mosquito may be the major cause for this situation. Major malaria vectors in India became resistant to DDT, BHC and Malathion.
Socio economic factors, rapid urbanization, with increasing population, poor maintenance in health and sanitary conditions, and man's indifference at the knowledge of vector control are encouraging unopposed rapid mosquito thriving. It is well known fact that no public health programme is successful unless the community is actively involved besides Government and semi-Government institutions.

2.5 WORK IN OUR DEPARTMENT:

From the experience of entomologists in pest management, one is convinced that larval control is easier, convenient and truly more effective. Measures directed against larval mosquitoes effectively reduce the incidence of mosquito born diseases. (Kitron and Spielman, 1989). As larvae are aquatic, we can repeat the cidal exposure, which is not possible in case of adults, as they are air borne. As adulticide spraying or fumigation covers all human habitations, there is a possibility of their effect on non-target organisms including man. Such possibility is less in case of larvicides especially when they spray in drainages, sewages etc.

Convinced about this, attempts are being made in our department for last several years to screen as many of the
house-hold items which are environment friendly as possible for their anti-larval properties.

Among biological products of animals, bile salts gave higher larval mortality (Damodaram, 1983). Soaps and detergents which are of common use also have larvicidal action. (Khaleel Sahib, 1986). As part of this exercise drugs of common uses have also been screened.

2.6 DRUGS AS TOXIC COMPOUNDS:

At present day, no introduction is necessary about drug usage in disease control (Chemotherapy). A drug is defined as any substance used for the purpose of diagnosis, prevention, relief or cure of disease in man or animal. Drugs relieve man from pain and suffering.

To provide a safe basis for application in man, acute and subacute toxicity of drugs have been tested in several animals. In general, experiments with animal models, are performed to elucidate altered physiological or pathological condition and to investigate the effect of drugs in pathological or normal organisms. The toxicity tests of drugs include tests for acute toxicity, chronic toxicity, teratogenicity and carcinogenicity and mutagenecity. Now several toxicologists show keen interest to investigate drug effects on animal metabolism, which comes under the field of "Pharmacological Toxicology".
2.7 DRUG STRUCTURE-ACTIVITY RELATIONSHIP:

It is well known that the toxicity as well as the therapeutic activity of a drug depend on chemical structure of the drug. Knowledge about drug chemical structure is useful for (i) synthesis of new compounds with more specific actions (ii) synthesis of competitive antagonist and understanding the mechanism of drug action. Several examples in the literature illustrate the importance of drug structure. For instance, of three furocoumarins, chalipin, oxypeucodamine and imperatorin, that are characterized by a psorlene nucleus with a furan ring attached to the coumarin moiety, only chalipin shows significant hepatotoxic effects when administered to rats (Emerole et al., 1981). Hepatotoxicity is thought to be due to the presence of -dimethyl alkyl group in the 3-position of the coumarin nucleus in chalipin which is absent in the other two compounds. When the toxicity of the drug is so very specific its hepato toxicity must manifest not only in rats, but in all other animals with metabolism and biochemical composition similar to that of rat.

2.8 ROLE OF DRUGS IN PEST CONTROL:

The drugs which are synthetic in origin generally have organic chemical composition. Majority of insecticides are
also made from organic chemical compounds. There is thus possibility for some relationship in chemical composition of drugs and insecticides. But both are differentiated by their chemical structure and toxicity. In case of drugs much care is taken about their toxicity to minimize side effects in non-target organism. But in case of insecticides such degree of safety measures are not being followed so strictly. As drugs are used as microbicides (antibiotics) and vermicides (anti helmintics), why don't they act as pesticides? or at least as pesticide synergists? As every pesticide makes a new gene resistant, in order to avert environmental imbalance, some of the drugs are being used as synergists on the basis of their action on microsomal mixed function oxidase, the detoxicating enzymes of lipid soluble xenobiotics and their effect on the pest mortality. In this approach several antibiotics were tested for their effect on the action of some insecticides. Some of the antibiotics like streptomycin, terramycin and tetracyclin were used as synergists (Datta and Baral, 1980).

2.9 CAN WE USE PHARMACOLOGICAL AGENTS AS MOSQUITOCIDES?

In the animal kingdom the metabolic pathways and their related catalysing enzymes are more or less same. When it is a question of Embden Meyerhof Pathway (EMP) or Krebs cycle, it is more or less similar from Protozoa to Mammalia,
of course, with minor variations. When it is a question of Ribonucleic acid, it is more or less the same with Ribose and the bases Adenine (A), Guanine (G), Cytocine (C) and Uracil (U) from protozoa to man, of course, with minor differences in the base sequence. Therefore the question of how much a drug that is hepatotoxic to rat, is also hepatotoxic to another animal, depends on how much similar the animal is to the rat physiologically and metabolically. Thus this concept can even form the basis for establishing the phylogenetic similarity among various species of animals. Extending this line of thinking, it may be asked – what are the drugs in the mammalian context, that can even be tried on lower organisms without hesitation?

Many drugs used for curing diseases in man and his domestic animals are directed against the pathogens – may be virus, bacterium, fungus, protozoan or metazoan. When these drugs work against these lower organisms when administered to man or domestic animal, will not they work against these pathogens in vitro? Some at least should work, if not all. Can any of these drugs working against these lower organisms in vitro, work also against mosquito larvae?

Klein et al., (1991) studied Anopheles darlingi fed on vivax malaria patients before and after treatment with chloroquine disulphate. Their only concern was to see
whether these mosquitoes become infective inspite of the drug having been taken by them, along with the blood of the patient. They reported that the frequency of infected mosquitoes and the mean number of oocysts were lower in the mosquitoes that fed on patients 2 to 4 hours after the initial of chloroquine than in mosquitoes fed before treatment. They concluded that chloroquin affects either gametocytes, fertilization, zygotes and/or ookinetes of Plasmodium vivax, but not subsequent stages of development. They never thought of evaluating the effect of chloroquine on the mosquito itself and what way this effect of chloroquine on the mosquito would have affected - the development of Plasmodium vivax in side this drugged mosquito.

Tesh and Guzman (1990) reported that the widespread use of ivermectin - a semi-synthetic macrolide antibiotic in veterinary and human medicine (Campbell et al., 1983; Taylor and Geene, 1989; Ottesen et al., 1990) have an un-recognized effect on mosquito population. Mosquitoes after ingestion of ivermectin developed signs of acute toxicity including paralysis, lethargy, incoordination and difficulty in movement. Death usually occurred in 48-72 hours.

The author, therefore felt that screening of different drugs for their larvicidal action on mosquitoes will be
quite interesting in the field of Integrated Mosquito Control Programme. The author screened some of the pharmacological agents (drugs) available in local market, for their larvicidal action. These results formed the basis for the award of M.Phil degree to the author.

2.10 BRIEF REVIEW OF WORK UNDER M.PHIL DEGREE PROGRAMME

As part of an integrated mosquito control programme being evolved in our division of pathobiology, a total of 16 drugs from four groups viz., Antibacterial, Antihelmintics, Antimalarials, Antihyperlipidaemia drugs were screened for larvicidal action. Among these 16 drugs only 7 drugs seemed promising. These seven drugs were once again screened more carefully, at lower concentration ranges for longer durations.

The first stage of IV instar larvae were selected as these larvae are more susceptible (Van Emden et al., 1974). There is also scope for studying the rate of pupation, pupal mortality and rate of metamorphosis. The larvae were treated with different concentrations of drug solution made with ordinary tap water. Ten samples were set for each concentration. 10 samples in ordinary tap water were the controls. Both treated and control were not fed for the following reasons. (1) To study the mode of drug entry into
animal. (2) if food is accepted, those larvae which take more food along with drug get affected easily when compared to larvae which consume less food or no food. (3) while drug is added to water containing which also contains the food, drug may be rendered non-toxic or there may be formation of new compounds which may produce toxicity to larvae due to drugfeed interaction. So drug effect can't be clearly delimitated.

Each sample contained 20 larvae in 160 ml of test solution or tap water, in glass tumblers of same size and shape. Abbott's formula was used to correct the rate of mortality of treated animals for mortality in the controls. Larval mortality, rate of pupation and emergence were recorded. For every twenty four hours until the control mortality reached 50 per cent. A concentration duration product (CDP) was calculated as suggested by WHO (WHO, 1965).

Data collected clearly indicated that all the pharmacological agents screened were not having similar effect on mosquito larvae. There is a wide difference in the concentrations which are toxic to larvae. The lowest concentration of 2.21 ppm is for chloroquine and this causes 50 per cent mortality in a period of 48 hours (Table - 1, Fig. 1). But, for 50 per cent mortality within 24 hours
duration, the concentration of 13.0 ppm is necessary. To decide which concentration is more toxic the concentration duration product (CDP) is computed. The lower the concentration duration product, the more toxic or more efficient it is. Thus chloroquine with the lowest concentration duration product of 100.8 is most toxic at a concentration of 2.21 ppm with the exposure period of 48 hours (Table - 1). Of all the drugs tried chloroquine was thus found to be most toxic.

In case of pupation rate, there was considerable inhibition in the rate of pupation when larvae were exposed to chloroquine. No pupal mortality was noticed during experimentation. It was therefore concluded from these results that chloroquine was quite effective against larval mosquitoes. The natural question that follows is how it affects larval mosquitoes?

Effects on individual organisms may range from rapid death through sublethal effects to no effects at all. Paracelsus observed over four centuries ago that "All things are poisons, for there is nothing without poisonous qualities. It is only the dose which makes a thing a poison" (Goldstein et al., 1974). The most that one might be able to say about the difference between poison and other substances is that relatively small amount of a poison is usually sufficient to produce adverse effects.
The present investigation is carried out, as an extension of earlier work, in order to find out to what extent chloroquine is responsible for the observed mortality in treated larvae. The question of whether chloroquine is entering the larvae from the ambient medium and producing any adverse effects is to be answered.

One approach is to discover the primary lesion and to explore the ramifications in terms of very specific physiological and biochemical processes, that ensure from these lesions. However, this is not an easy approach. The alternative approach is to concentrate on the health of the individual exposed and find out how well it is functioning (Bayne et al., 1979; McIntyre and Pearce, 1980). Growth is one parameter that can be studied in order to assess the individual's well being. Lysosomes, the intracellular sacs first described by de Duve (1963) serve as another interesting indicator of well being in animals. There are many such indicators of well being of an animal which can be conveniently studied. Based on results of such studies on these parameters, how well the individual is functioning under the given stress can be assessed.

In the present study the above said alternative approach is adopted. How well the mosquito larvae function under chloroquine stress has been assessed by choosing
parameters like survival capacity, revival capacity, pupating capacity, metamorphosing capacity, flight capacity, lysosomal enzyme activity and biochemical composition.

MATERIAL AND GENERAL METHODS

For the present study, Fourth instar mosquito larvae of species *Culex quinquefasciatus* are selected which is dominant in Tirupati and its surroundings. A mosquito colony is being maintained in our division for the last several years.

A. Description of insectorium:

The main interest of establishing insectorium is to get larvae of same age, more or less isogenic which are maintained under uniform conditions with balanced diet. The entire mosquito colony is maintained at $25 \pm 2^\circ C$ temperature throughout the year with help of air conditioners. The relative humidity (RH) of 70-80 per cent is maintained with help of humidifier (Electrically operated Humistat with automatic control - Doctaire E-type). In summer moistened sponges are kept additionally inside mosquito cages to maintain humidity. Photoperiod is maintained with help of flourescent tube lights which are on between 9 A.M. - 5 P.M. daily. Temperature and humidity is constantly recorded with help of thermo hygrograph. In this well furnished
insectorium a mosquito colony of *Culex quinquefasciatus* is being maintained since 1981.

(B) Maintenance of mosquito colony:

(i) Collection of egg-rafts: The egg-rafts of *Culex quinquefasciatus* are collected with spoons from bowls of water inside mosquito cages and transferred into plastic containers (size 6 cm x 6 cm diameter) filled with fresh water. The eggs hatch after 24 hours and actively moving larvae can be seen in water.

(ii) Maintenance of larvae: The larvae are then transferred into circular enamel trays of 24 cm diameter which are filled with stored tap water to half of their capacity (Plate - I; Fig. A). These larvae are fed *ad. libitum* with powdered groundnut cake. The water in the tray is changed every 24 hours to remove debris due to left over food, nitrogenous waste, faecal matter, excuvia etc.

(iii) Adult colony: After larvae developed into pupae they are collected into transparent plastic containers (6 cm x 6 cm) with lids. The pupae are separated into males and females and are kept in the containers in the ratio of 1 : 1. These containers are left in mosquito cages (of size 30 cm x 30 cm). After metamorphosis, the emerging adults enter the cage (Plate - I; Fig. B). The adult mosquitoes are fed on 5 per cent sucrose or glucose solution through a piece of
sponge. Pigeons are used for providing blood meal which is necessary for females to lay eggs and so supplied in vivo ad. libitum (Plate - I; Fig. C). Finger bowls with water are placed in the cage for the purpose of egg-laying.

Well fed, agile larvae of the same age and size belonging to first stage of IV instar are used for our experimental purpose. The methods employed for different experiments are described in detail in the respective chapters.
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* Average of 10 independent observations.
PLATE - I

Insectorium

Fig. A : Thermohygrometer for recording temperature relative humidity.

Fig. B : Humidifier for maintaining relative humidity.
PLATE - II

Mosquito colony maintenance

Fig. A : Larval mosquito colony.

Fig. AB : Adult mosquito colony.

Fig. C : Blood meal for mosquitoes.