

BIO-ECOLOGY OF CULEX QUINQUEFASCIATUS
PRINCIPAL VECTOR OF LYMPHATIC FILARIASIS IN
PANAJI, GOA, INDIA.

THESIS SUBMITTED TO THE
GOA UNIVERSITY

FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

IN

ZOOLOGY

BY

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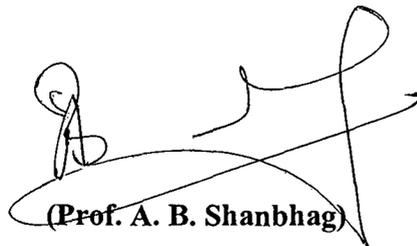
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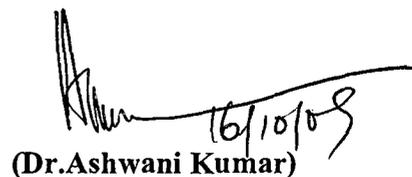
Certificate

This is to certify that the thesis entitled '**Bio-Ecology of *Culex quinquefasciatus* Principal Vector of Lymphatic Filariasis in Panaji, Goa, India**' submitted by **Mr. Mahesh B. Kaliwal** for the award of the Doctor of Philosophy in Zoology is based on the results of the investigations carried out by the candidate under our supervision. The thesis or any part thereof has not been previously submitted for any other degree or diploma of any University or Institute. The material obtained from other sources has been duly acknowledged in the thesis.



(Prof. A. B. Shanbhag)

Co-Guide



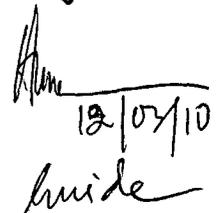
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(S. L. Hote)



12/07/10
guide

Statement

I hereby state that this thesis for the Ph.D. degree on '**Bio-Ecology of *Culex quinquefasciatus* Principal Vector of Lymphatic Filariasis in Panaji, Goa, India**' is my original contribution and any part thereof has not been previously submitted for the award of any degree/diploma of any University or Institute. To the best of my knowledge, the present study is the first comprehensive study of this kind from the area. The literature pertaining to the problem investigated has been duly cited. Facilities availed from other sources are duly acknowledged.



(Mahesh B. Kaliwal)

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In Loving Memory of

My Parents

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Abbreviations

<i>Ae.</i>	<i>Aedes</i>
<i>An.</i>	<i>Anopheles</i>
cm	centimeter
<i>Cx.</i>	<i>Culex</i>
DDT	Dichloro diphenyl trichloro ethane
EDTA	Ethylene Diamine Tetra Acetic Acid
ft	foot
gm	gram
h	hour
IGR	Insect Growth Regulator
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
l	litre
LC	Lethal Concentration
M	Mansonia
mg	milligram
ml	millilitre
Na_2HPO_4	Disodium hydrogen phosphate

NaCl	Sodium chloride
NVBDCP	National Vector Borne Diseases Control Programme
OC	Organochlorine
°C	Degree Centigrade
OP	Organophosphate
PMHD	Per man hour density
S. No.	Serial Number
SP	Synthetic pyrethroide
TBE	Tris-Borate EDTA Buffer
TE	Tris Buffer
WHO	World Health Organisation
wt.	weight

Chapter 1: INTRODUCTION

Human health is affected by several diseases. The magnitude of these diseases is further influenced by several factors like needs of routine life, socio-economic status, human habits/life style, place of living and its geographic location and the local ecology which is influenced by meteorological variables such as rainfall, temperature, humidity etc. There are plethora of communicable diseases prevalent in the tropics and sub-tropics between 40° North and 40° South of Equator. Among communicable diseases, the vector borne rickettsial, viral, protozoan or helminthic diseases are of high public health concern and cause significant economic loss.

These diseases are transmitted either from man to man or animals to man (zoonosis). Mosquitoes, sandflies, houseflies, tsetseflies, blackflies, lice, ratfleas, reduviid bugs, ticks, mites and cyclops are the arthropod vectors involved in the transmission of several vector borne diseases in humans. Among these vectors, mosquitoes are the most important insects of medical importance.

Mosquito borne diseases are now resurgent as global health problem (Gubler, 1998). Malaria, lymphatic filariasis, Japanese encephalitis, dengue fever / dengue haemorrhagic fever and chikungunya fever are the most important mosquito borne diseases prevalent in India. The steep decline of malaria in almost all the countries during the early years of eradication and the collateral benefits achieved in the control/total disappearance of plague and kala azar raised great hope for elimination of many of the vector borne diseases. Unfortunately, these hopes were belied and soon there was wide spread resurgence of malaria (Sharma, 1995). This was followed by large scale resurgence of Kala azar in Bihar with the cessation of DDT indoor residual spraying in the areas cleared of malaria (Rehman, 1989). These were the pointers to the

potential of vectors which have the inherent capacity to build up rapidly and strike in the absence of insecticidal cover.

Presently, Southeast Asia contributes 2.5 million cases to the global burden of malaria. Of this, India alone contributed 76% of the cases (Kumar et al., 2007). In addition to this problem, there has been an increasing trend of filariasis during the last three decades and the disease has become the major public health problem in the country (Sharma et al., 1987; Das et al., 2006). The rapid spread of Japanese encephalitis which is often a fatal zoonotic infection of children, to newer area is also of serious concern (Danda et al., 1996; Victor et al., 2000).

Dengue fever is endemic in many parts of India and the epidemics have been reported from different states of the country (Parida et al., 2002). Dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) are the serious manifestations of dengue fever and have emerged as important public health problem in Southeast Asia and Western Pacific regions (WHO, 1985). Chikungunya virus which was assumed to have disappeared from India and South East Asia (Pavri, 1986), has re-emerged in many states of India. Microbiologists have postulated that the re-emergence and spread of chikungunya is due to a variety of social, environmental, behavioral and biological changes and their combinations (Ravi, 2006; Laharia and Pradhan, 2006).

The re-emergence and spread of vector borne diseases to newer areas, ecological changes, vector resistance to insecticides coupled with behavioral changes among vectors due to sustained insecticidal pressure and community awareness about environmental pollution caused by large scale use of chemicals, have made vector

control a challenging task. The different diseases, their causative agent/s and the type of mosquito vector genera involved in the transmission are presented in Table 1.

Lymphatic filariasis (LF) is ranked as the second most common cause of physical disability next only to malaria among the debilitating tropical vector borne diseases (WHO, 1995). The unabated population growth, particularly in the developing countries of Asia, Africa and Latin America and the consequent ecological changes having adverse impact on all round deterioration in ecology and environment has also exacerbated the magnitude of Lymphatic Filariasis and other vector borne diseases (Danda, 1995).

Lymphatic filariasis is the common term for a group of diseases that are caused by *Wuchereria bancrofti* Cobbold, *Brugia malayi* Brug and *Brugia timori* Partono. Since, these parasites primarily affect the lymphatic system of man, the disease is commonly termed as Lymphatic filariasis. The disease though not fatal, is associated with social stigma due to deformities, causing human misery and sorrow (Figs.1 & 2). Many recent studies have illustrated the devastating social, psychological, economical and sexual issues due to massive swelling of limbs, groins and breasts. The disease is debilitating as it interferes with day to day activities resulting in severe functional impairment and physical disability thereby reducing the working man hours and earning capacity of the individual. There is strong feeling of shame and embarrassment in patient with hydrocoele associated with sexual disability and dysfunction. The disease also hampers the marriage prospects of young, specially the females.

Table 1: Mosquito borne Diseases, their Causative agent and Vector genera responsible for transmission

Sr.No.	Name of Disease	Causative organism	Vector genera
1.	Malaria	Protozoa <i>Plasmodium vivax, P.falciparum</i> <i>P.malariae & P.ovale</i>	<i>Anopheles</i> spp. (About 50 species)
2.	Lymphatic Filariasis	Nematodes <i>Wuchereria bancrofti, Brugia malayi</i> & <i>Brugia timori</i>	<i>Culex, Mansonia,</i> <i>Anopheles</i> & <i>Aedes</i> spp.
3.	Dengue Fever	Viruses DEN Group B, serotypes 1,2,3 & 4 (Family- <i>Flaviviridae</i>)	<i>Aedes</i> spp.
4.	Chikungunya Fever	CHIKV- Group A (Family- <i>Togaviridae</i>)	
5.	Yellow Fever	Yellow Fever virus – Group B (Family- <i>Flaviviridae</i>)	
6.	Japanese encephalitis	Japanese encephalitis virus-Group A (Family- <i>Flaviviridae</i>)	<i>Culex, Anopheles</i> & <i>Mansonia</i> spp.
7.	California encephalitis	California virus- Group C (Family- <i>Bonyaviridae</i>)	<i>Aedes</i> & <i>Culex</i> spp.
8.	Eastern equine encephalitis	Alphavirus- Group A (Family- <i>Togoviridae</i>)	
9.	Western equine encephalitis	Alphavirus-WEE virus (Family- <i>Bonyaviridae</i>)	
10.	West Nile encephalitis	West Nile virus-Group B (Family- <i>Flaviviridae</i>)	
11.	St. Luis encephalitis	St. Luis virus- Group B (Family- <i>Flaviviridae</i>)	

A



B

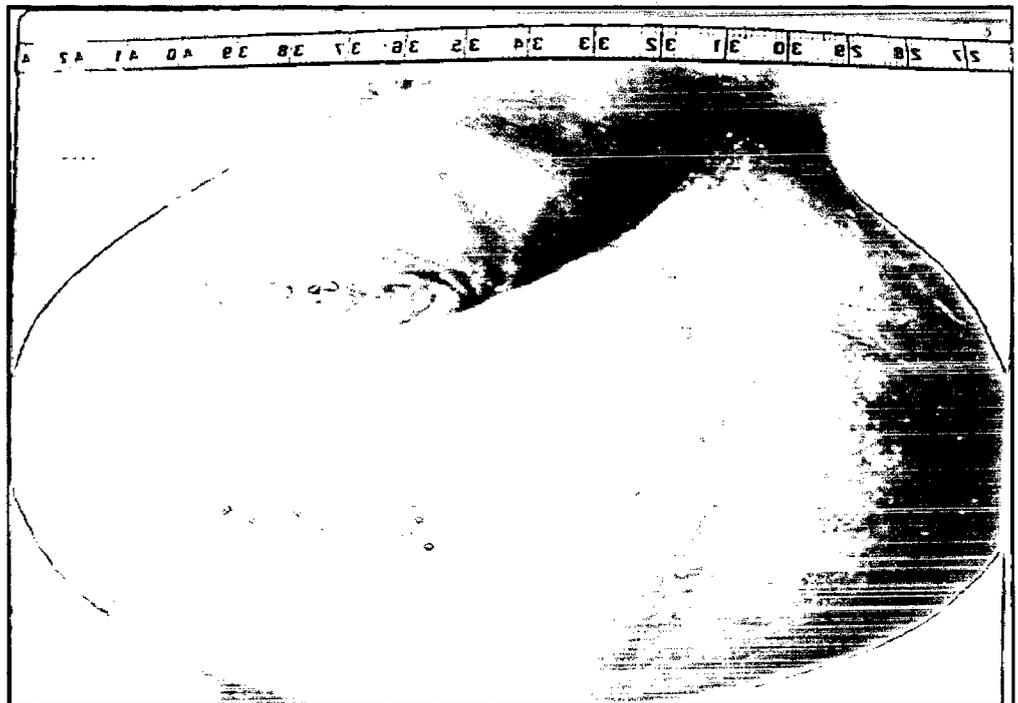
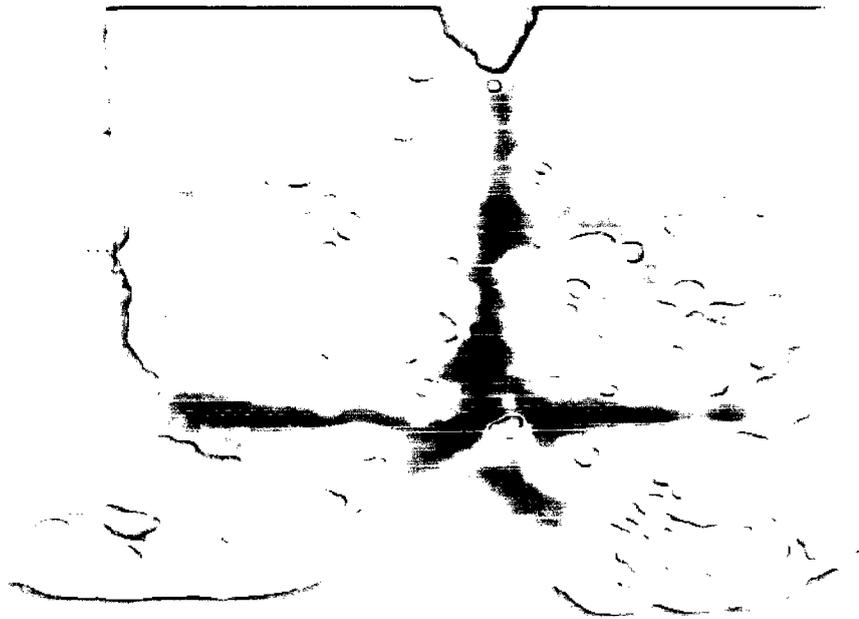
Fig. 1(A&B) : Persons suffering with Lymphatic Filariasis

Besides disfigurement with lymphoedema, elephantiasis, hydrocoele, etc., the chronic cases of LF are unable to care for self and suffer isolation from the community. Loss of social support, family stress, shame and stigma due to sexual disability- all together complicate the matters. Overall, Lymphatic filariasis is a disease of the poor and it is prevalent in urban, periurban and rural areas.

Life Cycle of *Wuchereria Bancrofti* Cobbold

Man is the definitive host for filarial worms wherein adult male and female matured filarial parasites mate and produce microfilariae. Mosquito is the intermediate host. The adult parasites are usually found in lymphatic system of man. They produce as many as 50,000 microfilariae per day, which find their way into the blood circulation. The life span of microfilariae is not exactly known which may survive up to a couple of months. The parasite cycle (Fig. 3) inside the mosquito body begins when the microfilariae are picked up by the vector mosquitoes during their blood feeding from an infected person. Once inside the mosquito host, the development of microfilariae begins, they undergo two moults transforming to I and II stage (L1 & L2) and finally growing into infective third stage larvae (L3). Under optimum conditions of temperature and humidity, the duration of the parasite cycle inside mosquito (extrinsic incubation period) is about 10-14 days. When the infective mosquito harbouring L-3 stage larvae feeds on a healthy human host, the infective larvae are deposited at the site of mosquito bite, from where they gain entry into the lymphatic system through the wounds on the skin. A large number of infective bites may be necessary for patent microfilaraemia. In the human host, the infective larvae develop into adult male and female worms and lodge themselves in lymphatics.

A



B

Fig. 2(A&B) : Persons suffering with Lymphatic Filariasis

Filariasis

(*Wuchereria bancrofti*)

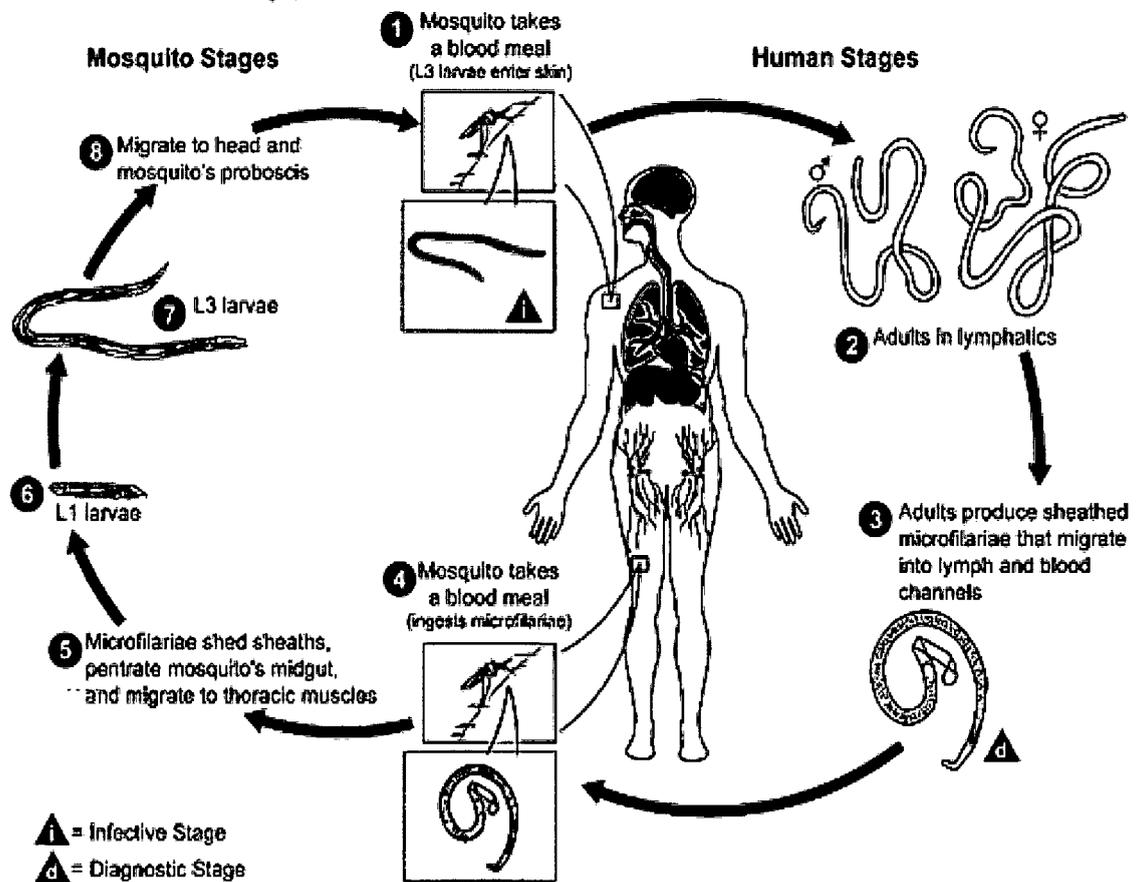


Fig. 3: Life Cycle of *Wuchereria bancrofti* in Mosquito and Man.

The adult worm survives for about 5-8 years or sometimes up to 15 years or even more. Adult males live for a short period compared to females which can survive for longer periods up to 40-50 years. The duration between the infective bite and production of microfilariae is about one and half year for *W. bancrofti* and nine months to one year for *B. malayi* (VCRC, 1988).

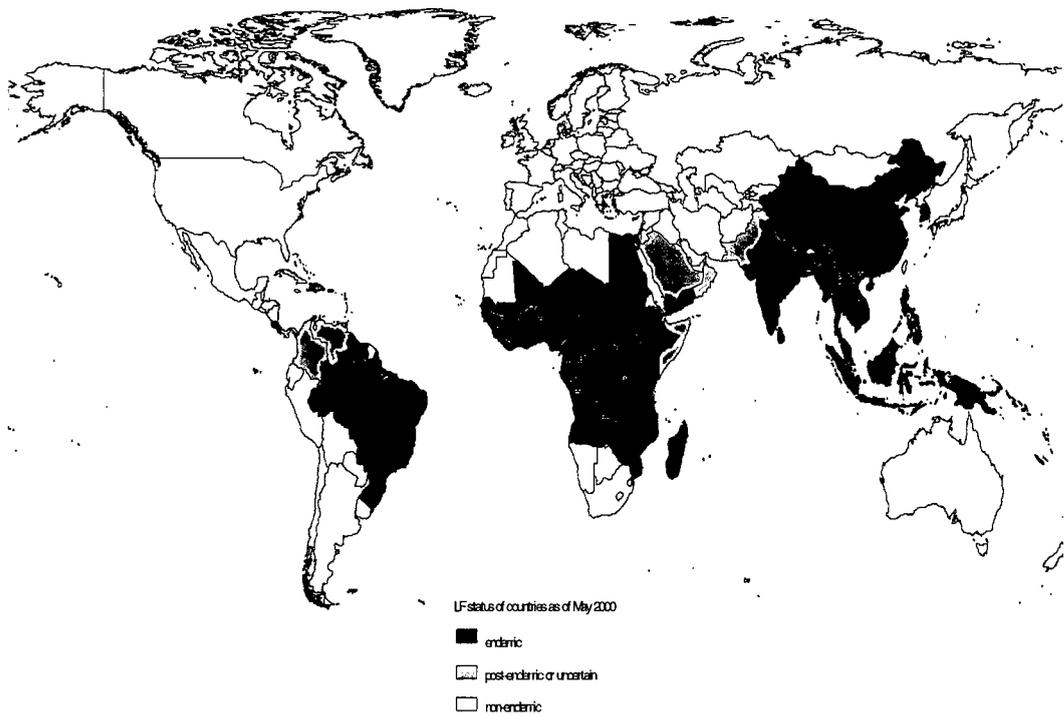
Epidemiological Situation of Lymphatic Filariasis

At present, world wide 1.3 billion people are at the risk of lymphatic filariasis infection and about 120 million people are affected in 83 countries (WHO, 2006). Of the estimated 128 million lymphatic filariasis cases, 91% are caused by *Wuchereria bancrofti* (Michael and Bundy, 1997). The magnitude of the lymphatic filariasis problem in the world (Fig. 4) is presented below (CD Alert, 2001).

Total Population afflicted.....	1.2 billion
People with Lymphoedema/Elephantiasis.....	15 million
People with Hydrocoele.....	25 million
People with Acute Inflammatory Attacks.....	15 million
People with Chyluria.....	2 million
People with other conditions (often hidden)....	63 million

In India, the disease was recorded in as early as 6th century B.C. by the famous Indian Physician 'Susruta' in his book, 'Susruta Samhita.' In 7th century A.D. Madhavkara described the signs and symptoms of the disease in his treatise, 'Madhavnidhan' which hold good even today. In 1709, Clarke called elephantoid legs as 'Malabar legs' in Cochin.

Countries with lymphatic filariasis



 World Health Organization
Global Programme for Elimination of Lymphatic Filariasis

Fig. 4: Countries including India with the problem of lymphatic filariasis.

The discovery of microfilaria (mf) in the peripheral blood was first made by Levis in 1872 in Kolkata and the developmental forms of filarial parasites of man in *Culex quinquefasciatus* were discovered by Mansion in 1878 (Anonymous, 2004).

Forty percent of world's filariasis disease burden is contributed by India alone wherein 450 million people are exposed to the risk of this infection with 31.26 million people with microfilaremia, 7.44 million people with lymphoedema (elephantiasis) and 12.88 million people with hydrocoele and the estimates of health burden due to filariasis disease suggests that 2.06 million disability adjusted life years (DALYs) are lost in India and annual wage loss at current prices is estimated at 811 million US dollars (Shenoy, 2002).

Indigenous LF cases are reported from 20 states/UTs namely, Andhra Pradesh, Assam, Bihar, Chhattisgarh, Goa, Gujarat, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu, Uttar Pradesh, West Bengal, Pondichery, Andaman Nicobar Islands, Daman & Div, Lakshwadeep and Dadra & Nagar Haveli (Anonymous, 2004).

In main land of India, the bancroftion filariasis caused by *Wuchereria bancrofti* and transmitted by the ubiquitous vector *Culex quinquefasciatus* has been the most predominant infection contributing to 99.4% of the filariasis disease burden of the country and malayan filariasis caused by *Brugia malayi* and transmitted by *Mansonia* mosquitoes is mainly restricted to rural pockets and contributes the remaining 0.6% of filariasis disease burden (Anonymous, 2004). The largest endemic tract of malayan filariasis presently exists along the central part of Kerala and other localized foci are in Assam, Orissa, Madhya Pradesh and West Bengal. Both *W. bancrofti* and *B. malayi*

infections in main land India exhibit nocturnal periodicity of microfilariae coinciding with vector feeding behaviour.

In 1974, diurnal sub-periodic *W. bancrofti* infection was discovered among aborigines inhabiting Nicobar group of Andaman & Nicobar Islands (Kalra, 1974). Diurnal *Ochlerotatus (Finlaya) niveus* group of mosquitoes were incriminated as the vectors of this infection, which were formerly classified as *Aedes (Finlaya) niveus* (Shriram et. al., 2005).

WHO in its World Health Organization Assembly in 1997 has targeted the elimination of LF by 2020, through annual mass drug administration to all the people at risk. The mass drug administration strategy was based on the hypothesis that if majority of the people in a community consume single dose of DEC annually once, it will reduce the parasite load and if continued for sufficiently long period, may eliminate filariasis. Elimination efforts primarily rely on reduction in lymphatic filariasis transmission till elimination is achieved through annual mass drug administration, appropriate management of individual patients both in acute and chronic stages to prevent disability and improving quality of life, community participation, IEC (Information, Education, Communication), manpower development, monitoring and evaluation (CD *Alert*, 2001).

Achieving the target of elimination by 2020 would however need a strong political commitment, concerted efforts by the public health administrators, public health professionals, clinicians and community at large from all the member countries. Government of India being the signatory to the resolution, envisages eliminating the disease by 2015 (CD *Alert*, 2001).

The Global Programme to eliminate lymphatic filariasis (GPELF) was launched in the year 2000 (Sunish et al., 2007). Although, significant progress in initiating MDA programmes in endemic countries has been made, the emerging challenges to this approach have raised questions regarding the effectiveness of MDA alone to eliminate LF without the inclusion of supplementary vector control (Bockarie et al., 2009).

Lymphatic Filariasis Problem in Goa

Goa is one of the filariasis endemic states of the country. The endemicity of Goa for bancroftian filariasis has been known since many years (Wagh, 1976). In a recent survey conducted in 2008, 191 disease cases of lymphatic filariasis have been reported from all the Health Centres (PHCs/UHCs/CHCs) from Goa. Out of 191 cases, 107 cases are reported from North Goa district and 84 cases from South Goa district (Source: NVBDCP- Goa). The number of female persons affected is 113 and males- 78. The number of persons whose legs were affected was 145 and in 46 patients the other organs such as scrotal sacs in males, breasts in females and upper arm were affected. More cases of filariasis are reported from the coastal plains of Goa, having humid and warm climate without drastic variations in temperature throughout the year, favoring density build up and increasing the longevity of the vector mosquitoes in the areas. Mass drug administration with single dose of 6 mg/Kg body weight Diethyl carbamazine tablets annually is being carried out since 2004 in Goa. During 2005 to 2008, 22 new microfilariae carriers have been detected through limited sentinel night time parasitic surveillance in Goa.

Reservoir or source of infection is the person with circulating microfilariae in his peripheral blood. mf carriers are usually without any recognizable symptoms or

illness. A person may continue to be a mf carrier without any disease manifestation for a prolonged period. The period for which the person will be microfilaraemic depends on the fecundic life span (the period for which the adult *Wuchereria* female produces microfilariae) of the adult worm which is about 5.4 years for *W. bancrofti* and 3.4 years for *B. malayi*. (VCRC, 1997). The individuals with chronic disease on the other hand are usually negative for mf. In chronic lymphoedema, the night blood examination to detect microfilariae, ICT Card Test for filarial antigenaemia and ultrasonography for locating the adult worms are usually negative (Weil et al., 1996).

All age groups of people are susceptible to infection in endemic areas. Filarial infection has been recorded even in infants of six month age, but the infection has been found to rise with age upto 20-30 years and not consistently thereafter (Anonymous, 2004). There is plenty of evidence now, which suggests that LF infection is first acquired in childhood in several instances, even though the clinical manifestations start appearing much later, mostly in adult life (Shenoy, 2006).

The methods currently in use to detect the filarial parasites include- 1. Thick blood smear by wet film or stained blood smear examination; 2. Membrane filtration; 3. Detection of adult worms by ultrasound 4. Immunodiagnosics. Of all the methods, the wet film examination can be easily done. For this, a sixty cubic millimeter thick blood smear is taken after 8 P.M. The wet smear can be examined directly under microscope for microfilariae or can be dried overnight, dehaemoglobinised next day, stained with JSB stain and examined under microscope. This is the standard method adopted to detect the microfilariae.

Clinical Manifestations of Lymphatic Filariasis

Clinical manifestations of LF depend upon the different stages in the course of infection in the human host and the load of the adult worms. The following manifestations may be encountered.

- A. **Stage of Invasion:** The infective larva gains its entry into the human host and starts undergoing further development. Diagnosis at this stage rests on the triad of eosinophilia, lymphadenopathy and a positive intradermal test with the supporting evidence of history of residence in the endemic area.
- B. **Asymptomatic or Carrier Stage:** This stage is usually with no clinical manifestation. The carriers are usually detected by night blood examination.
- C. **Stage of Acute Manifestation:** These cover filarial fever, lymphangitis, lymphadenitis and lymphoedema of various parts of the body and epididymo-orchitis in the male.
- D. **Stage of Chronic Manifestation:** The clinical manifestations comprise of elephantiasis of genitals, legs or arms, hydrocoele, chyluria etc. Hydrocoele is the commonest manifestation of bancroftion filariasis in the male population.

The adult worms cause the dilation of lymphatic vessels resulting in their damage and dysfunction. This leads to slow flow of lymph which may cause lymphoedema, kidney damage and chyluria from rupture of dilated lymphatics into urinary system. Typical acute inflammatory attacks of LF occur due to the entry of bacteria through breaks in lymphoedemous skin. Stasis of lymph provides conditions for rapid growth of these bacteria. Damage to small lymphatic vessels results in fibrosis and progression of elephantiasis.

Mosquitoes and Vectors of Lymphatic Filariasis

Mosquitoes are small and delicate insects with three segmented body consisting of head, thorax and abdomen. They have three pairs of long and slender legs and a pair of membranous wings for flight. The second pair of wings are reduced to small knob like structures called halteres which are used for equilibrium. The mosquitoes may be distinguished by their long and slender proboscis extending forward with the palps. Mosquito life cycle includes four distinct stages viz., egg, larva, pupa and adults. The females excepting to those belonging to genus *Toxorhynchites* are haemotophagous and require a blood meal for the development and maturation of eggs. The first stage of larva hatch out from the egg and grow to fourth stage larva and further to pupa. Finally, adult female and male mosquitoes emerge out from the pupae (Fig. 5).

In addition to disease transmission, the mosquitoes often cause much discomfort and possess great nuisance potential. Though, the amount of trauma produced by the bite is negligible, the injection of saliva in the body may produce a reaction. The pruritus with which it is associated, often results into scratching and may be followed by secondary infection (Gordon and Lavoipierre, 1962).

Mosquitoes are found all over the world. They are found at a height of 14000 feet in Kashmir and as low as 3760 feet below sea level in gold mines in South India (Russel et al., 1943). About 3200 species of mosquitoes are reported worldwide with several sub-species (Dixit et al., 2002).

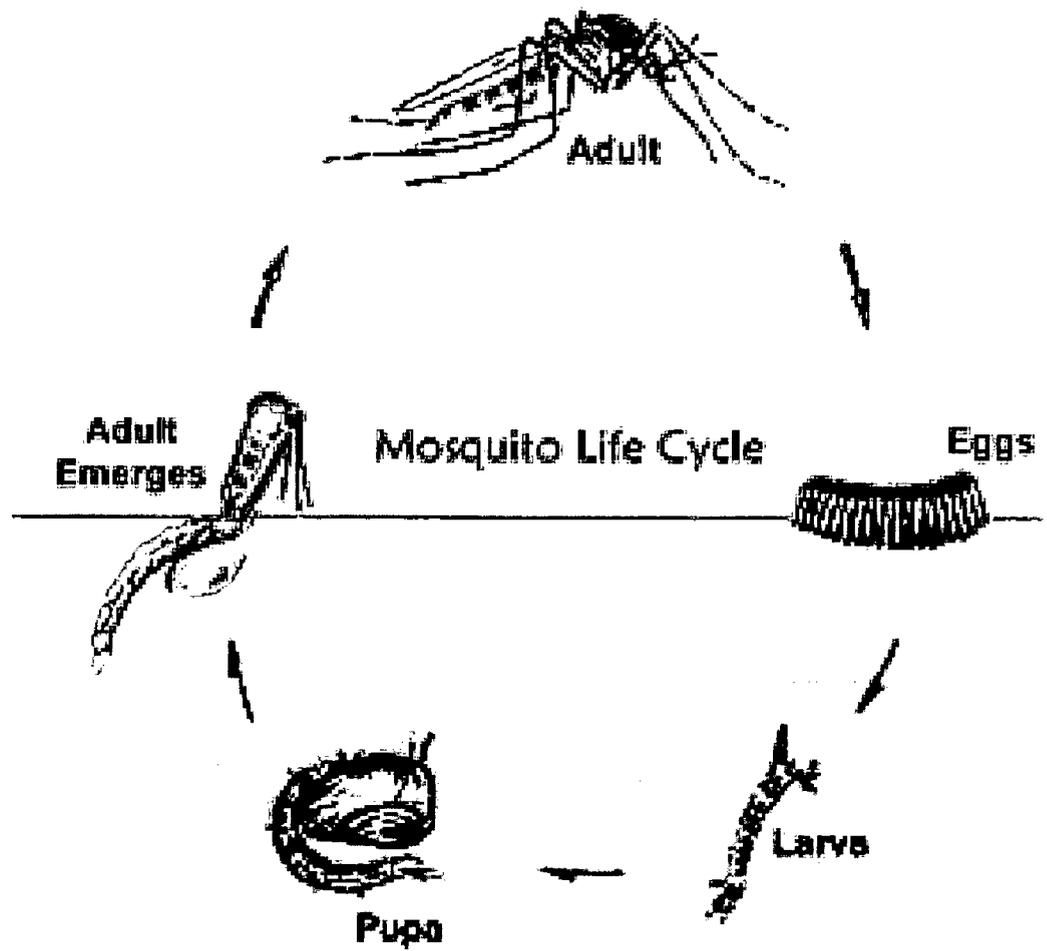


Fig. 5 : Life cycle of *Culex quinquefasciatus*

The systematic position of mosquitoes in the classification of animal kingdom is as follows.

Phylum : Arthropoda
Class : Insecta
Sub-Class : Pterygota
Division : Endopterygota
Order : Diptera (two winged insects)
Subdivision ; Nematocera
Superfamily : Culicoidea
Family : Culicidae (Mosquitoes)

Family- Culicidae is further classified into Subfamily- Anophelini and Subfamily- Culicinae. The important genera under Culicinae are *Culex*, *Aedes* and *Mansonia*.

Each group of mosquitoes differ in their breeding, feeding, biting and resting habits from one region to the other as a function of climatic change. The knowledge of mosquito habits, their distribution and abundance is essential from the point of view of proper understanding the role they play in disease transmission as well as for controlling the mosquito vectors. A number of mosquito species are known to be the vectors of parasitic and viral diseases. Nine species of mosquitoes belonging to genus *Anopheles* act as vectors of malaria in different geographical regions of India (Kumar et al., 2007). Two species of genus *Aedes* act as the vectors of dengue fever/DHF and chikungunya fever. Japanese encephalitis virus has been isolated from six species of genus *Culex*, three species of genus *Anopheles* and one species of genus *Mansonia* (Banarjee, 1987).

Different species of mosquitoes prevalent in an ecological set up differ in their susceptibility to disease pathogens. The same species may exhibit significant difference in vectorial efficiency in different ecological conditions. There is ample evidence of this phenomenon in malaria and certain other mosquito borne infections including filariasis (Das, 1976). *Mansonia* mosquitoes are generally refractory to *W. bancrofti* infection, but *Mansonia (Mansonioides) uniformis* is found to naturally transmit *W. bancrofti* in New Guinea (Rook, 1957). *Culex quinquefasciatus* an efficient vector of periodic *W. bancrofti*, has been reported to be the poor vector of *W. bancrofti* in tropical Africa (Hammon et al., 1967).

In case of lymphatic filariasis, there are many reports on human filarial infections detected in mosquitoes in different endemic areas of the world. In India, natural and experimental infections of the periodic *W. bancrofti* have been detected in 17 species of mosquitoes, of which 5 are culicines and 12 are anophelines (Das, 1976). To incriminate a species of mosquito as a vector of LF, it is necessary to obtain the infective larvae (L-3 stage) which can be identified with certainty. The infective larvae of LF have been detected in ten species of mosquitoes and of which, 9 are anophelines and one is *Culex*. Owing to its abundance, anthropophilism and feeding activity, *Culex quinquefasciatus* is the principal vector of bancroftion filariasis in India (Das, 1976; Samuel et al., 2004)). Globally, the majority of the lymphatic filariasis caused by *W. bancrofti* is transmitted by *Cx. quinquefasciatus* (Sunish et al., 2007). Similarly, *Mansonia (Mansonioides) annulifera* is the principal vector of brugian filariasis, while *M.(M). uniformis* is the secondary vector of this infection. The vectorial role of *M. (M). indiana* is very limited due to its very low density.

At the time when the third expert committee on filariasis met in 1973, the tropical urban mosquito was designated as *Culex quinquefasciatus* in North America and as *Culex pipiens fatigans* in the rest of the world. Subsequently, the work done by two groups of authors led the international scientific committee to adopt *Culex quinquefasciatus* and this change simplified the former situation where a same species was designated under two different names (Subra, 1983).

Cx. quinquefasciatus is also the vector of West Nile virus (Godsey et al., 2005), Japanese encephalitis virus (Nitapattana et al., 2005), Saint Louis encephalitis virus (Jones et al., 2002) and secondary vector of Western equine encephalitis (Aviles et al., 1990). Chikangunya virus also has been isolated from the adults of *Cx. quinquefasciatus* collected from field in Southeast Asia (Halstead et al., 1969).

Cx. quinquefasciatus is the predominant species in the urban areas, especially in those areas having inadequate or faulty drainage system. The pace at which the unplanned urbanization is observed, increased industrialization, consequent increase in human population and movement and also the involvement of various segments of the society in the creation of man-made mosquitogenic conditions, all of which are responsible for increased prevalence of *Cx. quinquefasciatus*.

The density of vector population is subjected to seasonal prevalence and also on its reproductive potential and survival/mortality rate at different stages of development from egg laying to adult emergence due to different biotic or abiotic factors. The study on life table of the vector species can elucidate the survival/mortality rate of the species. Resting habits (endophily or exophily) and resting habitats may also vary in different areas. Biting activity of vector species is an important parameter in understanding the

vectorial potency and transmission dynamics of filariasis. Feeding behavior (endophagic or exophagic) of the mosquito species and the feeding preference for human blood (anthrophilic) or animal blood (zoophilic) may vary subject to the availability and accessibility of host in the immediate environment of the vectors (Lee et al., 1954; Kaul and Wattal, 1968a; Samuel et al., 2004).

The completion of extrinsic incubation period of the disease pathogen to complete its growth inside the mosquito body is dependent on the longevity of the vector species. Therefore, the longevity of the vector species interalia determines the disease transmission. The potential risk of transmission also depends upon infection/infectivity rate of vector in time and space, the reservoir of infection and environmental factors. Temperature and humidity play an important role not only in the survival of the vector species and development of filarial parasites in the mosquito host, but also in the survival of infective larvae deposited on the skin of the vertebrate host (Das, 1976). As such, the climatic conditions in different endemic areas determine the active transmission period.

The longitudinal observations of the density pattern, breeding habitats, biting activity, longevity, infection/infectivity rate are essential to understand variations in the transmission potential during different periods of the year which could help to undertake the vector management/disease management operations to reduce the potential risk of lymphatic filariasis transmission.

The antivector measures are undertaken both against immature stages and the adult mosquitoes. The antilarval measures comprise of physical, biological and chemical methods. Insecticides belonging to organochlorine, organophosphate,

carbamate and synthetic pyrethroid are being used in health and agriculture sectors and indiscriminate use of these chemicals has resulted in the resistance to several insecticides by the vector mosquitoes (WHO, 1992; Sunaiyana et al., 2006; Mukhopadhyay et al., 2007). The relevant information on various potential breeding habitats, helps to selectively apply suitable antilarval measures to prevent/control mosquito breeding and to judiciously use the chemical methods. This reduces the chances of development of resistance against insecticides being sprayed and also limit the health hazard posed by the toxic chemicals. The anti-adult measures are done mainly by the spraying of insecticides. As the chemicals are used/being used both against the larval and adult stages, it is imperative to generate information on the susceptibility/resistance status of vector species to the sprayed insecticide/s prior to and during the use. This information is vital for effective vector management.

Although, mass drug administration(MDA) is being carried out with the annual dose of Diethyl carbamazine tablets- a drug effective against the circulating microfilariae in the blood of infected persons, the effect of the drug is doubtful against adult filarial worms since in many filariasis cases, microfilaria reappear after certain period (VCRC, 1988). A high coverage of population and compliance of drug intake at mass scale in the endemic areas is essential under MDA.

It seems unlikely that MDA alone would be able to interrupt LF transmission in area of *Culex* transmission of LF due to their high vectorial efficiency (Jayasekera et al., 1991). The implementation of mass chemotherapy with annual single dose of DEC (6 mg/ Kg body weight) combined with vector control may yield better results in reducing the mf rate in the population (Manoharan et al., 1997).

Therefore, vector control is essential for sustained interruption of LF transmission (Burkot et al., 2006). Accordingly, incorporation of vector control in the global LF elimination programme has been advocated as it potentially decreases the time required for elimination of LF (Sunish et al., 2007). It has also been reported that at lower level of community microfilaria load (CMFL) and higher level of vector density, vector control would be more cost effective (Das and Vanamail, 2008). In such a situation, dual strategy of vector control and treatment of mf carriers is being followed by National Filaria Control Programme in the country.

Therefore, besides treating patients, achieving reduction in the vector population with appropriate and timely vector control measures and prevention of mosquito bites through personal protection are of great significance to reduce the risk of LF transmission. For formulating an effective vector management strategy, sound knowledge on bio-ecology of the principal vector *Cx. quinquefasciatus* is essential. In Goa, such information is scarce, fragmentary and outdated (Bounsulo, 1968; Thavaselvam et al., 1993). Goa has undergone significant ecological changes due to increased urbanization and industrialization over the years, coupled with increase in human population and large inflow of migrant population from different filarial endemic states of the country, leading to the creation of increased number of man-made mosquitogenic conditions and also making available the reservoir of infection from different areas. However, *Culex quinquefasciatus*, the principal vector of lymphatic filariasis has never been subjected to systematic and thorough scientific investigation in Goa.

Transmission of infection through vectors is considered to be a density dependant phenomenon. The density pattern depicted by the vector species in any area is influenced by gross ecology of the terrain and meteorological variables (Kaul and Wattal, 1968b). The weather has been considered as a predominant cause of variations encountered in insect population and to a great extent sets the stage for the process of population regulation. As such, findings on vector populations of one geographic region cannot be fully applied to the other. Therefore, the present study with the following aims and objectives has been undertaken in Panaji, Goa which is the known filarial endemic area in the state of Goa.

Aims and Objectives of the Study

1. To carry out literature search and collect the information on vector species *Cx. quinquefasciatus*, pertaining to various entomological and epidemiological studies, meteorological / weather data, physical features and developmental activities .
2. Detection and collection of immatures (eggs, larvae and pupae) from different habitats in the field to determine the larval and pupal indices viz. per dip density and container index of *Cx. quinquefasciatus* immature stages.
3. To study the development from egg to adult emergence to assess the survival rate under life table of *Cx. quinquefasciatus* in the laboratory at ambient temperature and relative humidity.
4. Collection of adult mosquitoes from the field to study adult density of *Cx. quinquefasciatus*, resting habits and habitats covering all the seasons of the

year to know the seasonal prevalence/variations in population density of vector species.

5. Whole night hourly collection of mosquitoes landing on man to find out man-biting activity, man-mosquito contact rates, preferential human body parts for vector biting and seasonal variations in biting rate of *Cx. quinquefasciatus*.
6. Blood meal analysis of field population of *Cx. quinquefasciatus* to find out the feeding preference of vectors to determine the anthropophilic index.
7. Dissection of field collected female *Cx. quinquefasciatus* mosquitoes to find out longevity of mosquitoes (Parity rate) and filarial parasites in different regions of the body for determining vector infection and infectivity rates.
8. To carry out tests to find out current susceptibility status/resistance level of both larval and adult populations of *Cx. quinquefasciatus* to different insecticides.

Chapter 2: REVIEW OF LITERATURE

2.1 Breeding Behaviour of *Cx. quinquefasciatus*

Mosquito species exhibit considerable plasticity in their selection of their breeding places while others are more restricted in their choice (Service, 1976). The specific cues that trigger oviposition behavior in mosquitoes are largely unknown (Muturi 2008). Many species of mosquitoes are very specific in their requirement of physico-chemical characteristic of their breeding waters (Sehgal and Pillai, 1970; Sinha, 1976). Blaustein and Kotler (1993) stated that mosquitoes use chemical and biological cues to detect the presence of larval competitors and avoid ovipositing in such habitats. According to Shilulu et al. (2003) and Piyaratnea et al. (2005), mosquito species differ in the type of aquatic habitats they prefer for oviposition based on location, physico-chemical condition of the water body and the presence of potential predators.

Cx. quinquefasciatus is known to breed in diverse ecological niches (Barraud 1934; Chow and Thevasagayam 1957; Fernando 1963; Mattingly 1969, Kaul et al. 1977; Muturi et al. 2007 a,b). De Alwis and Munasinghe (1971) have indicated that pH of the medium is important in controlling the breeding activity of *Cx. quinquefasciatus* and the species preferred alkaline media with pH ranging between 7 and 8.2 and pH above 8.2 inhibited its breeding.

Kaul et al. (1977), analyzing the chemical characteristics of breeding waters of *Cx. quinquefasciatus* found that the species was mainly alkalinophilic, breeding within the optimum pH range of 7 to 9 with heavy breeding occurring up to 8.5 pH and showed wide tolerance to total alkalinity, total hardness and chlorides and the breeding waters

were always characterized by either absence or very low contents of dissolved oxygen and nitrites but higher contents of biochemical oxygen demand, nitrates and free ammonia. They also pointed out that besides the chemical factors, there may be other physical and biological factors viz. fauna and flora which would influence the breeding of this mosquito species.

In the areas around Delhi, Kaul et al. (1977) encountered *Cx. quinquefasciatus* breeding in artificial and natural water bodies, such as catch-pits, septic tanks, stagnant drains, ground pools and ditches, which were invariably made or influenced by man. However, heavy breeding was found to be associated with high pollution conditions and water temperature in the range of 14°C and 30°C and the temperature below and above this range seemed to act as limiting factor.

Sarkar et al. (1978) studied the seasonal breeding of mosquitoes in drains in Tejpur town of Assam and found that *Cx. quinquefasciatus* was the predominant species with its incidence throughout the year and had a fairly wide range of adaptability to physico-chemical characteristics viz., turbidity (30 to 500 units), pH (7.9 to 8.6), total alkalinity (204 to 1156 mg/l), chloride 10.4 to 1358.8 mg/l, total nitrogen 12.3 to 196.2 mg/l and oxygen absorbed from KMnO₄ in 4 hours at 37°C (3.1 to 89.4 mg/l). They also observed that the shade in the larval habitat did not appear to have any effect on the breeding and distribution of *Culex quinquefasciatus* larvae as reported by Njogu and Kinoti (1971).

Menon and Rajagopalan (1980) studied the relative importance of different breeding places contributing to the breeding of *Cx. quinquefasciatus* in Pondichery. Drains, cesspits, wells, pools and cisterns were checked for the breeding. According to

them, the drains, cesspits and cisterns continued to breed throughout the year and drains and cesspits together found to form the most important breeding habitats both in terms of surface area and daily emergence. Wells supported low to moderate breeding. In dry season, drains were more important while during monsoon the cesspits were equally responsible for adult emergence in Pondicherry.

Yasuno (1974 and 1977) had studied breeding of *Cx. quinquefasciatus* in Delhi rural areas and Rajagopalan et al. (1977) had studied breeding in Delhi urban areas. Rajagopalan (1980) compared the breeding data of Pondicherry with Delhi rural (Yasuno, 1974 and 1977) and Delhi urban (Rajagopalan et al. 1977) areas where generation time and net reproductive rates varied widely with seasons. Pondicherry has a moderate climate without any extremes both during summer and winter and being a coastal area, the climate is highly humid. Under meteorological variables, temperature did not influence the survival or growth of larvae but, the rainfall influenced the survival of immature and breeding in Pondicherry.

Delhi rural and urban areas have extreme climatic conditions with a very hot summer (many days exceeding the thermal death points) and very cold winter (prolonging the immature duration considerably and the survival rates were very low). But, rural and urban areas differed from each other in the breeding habitats. In Delhi rural area, innumerable irrigation wells were the main breeding habitats in dry months when water conditions are ideal for breeding and during rainy season there was a shift in breeding places when domestic receptacles were important (Yasuno et al. 1977). In Delhi urban area, drains were the major breeding places throughout the year

(Rajagopalan et al. 1977). In Delhi, temperature was the influencing factors to regulate the survival and growth of the mosquito immatures.

Narayanan and Maruthanayagam (1985) encountered mosquito breeding in water meter chambers in Pondicherry and the most predominant species was *Aedes aegypti* followed by *Cx. quinquefasciatus*, *Armegeges subalbatus* and *Aedes albopictus*. Malhotra et al. (1987) carried out mosquito breeding survey in Tirap and Subansiri district of Arunachal Pradesh and detected the breeding of 18 species belonging to 5 genera. In the survey, *Cx. quinquefasciatus* was found in very large numbers and it formed 65.47% of the total larval collection. The larvae/pupae of *Cx. quinquefasciatus* were encountered in metallic drums, cut bamboos, cemented tanks, water pits, ditches, ponds, drains, tyres, etc. and their predominance may be due to wide range of larval adaptability to different physico-chemical characteristics of water sources. Ishil and Sohn (1987) found that the larvae of *Cx. quinquefasciatus* complex (*Cx. quinquefasciatus* and *Cx. torrentium*) were abundant in highly polluted pools. Bang (1989) stated that urbanization has led to ecological degradation favoring the breeding of *Cx. quinquefasciatus* in many cities throughout the tropical and subtropical areas of Southeast Asia region.

Srivastava (1989) found the *Cx. quinquefasciatus* larvae in tree holes in the villages of Nadiad of Kheda district in Gujarat. Kulkarni and Naik (1989) conducted the mosquito breeding survey in several localities of Goa and reported that *Cx. quinquefasciatus* was breeding in ground pools, cement tanks, rock pools, paddy fields, stream beds, fallen coconut shells and glass containers. Kumar and Chand (1990) while studying the filariasis problem in coastal and sub-coastal villages of Ganjam, Orissa

observed that ditches and pits were the main sources for the breeding of *Cx. quinquefasciatus* in the sub-coastal villages which were usually absent in extreme coastal villages.

In the breeding surveys carried out by Kaliwal (1991) and Kumar and Thavaselvam (1992) in Panaji, *Cx. quinquefasciatus* was found breeding in cement tanks, wells, iron/plastic barrels, curing water collections inside the buildings under construction and ground pools. Raina et al. (1992) observed prolific breeding of *Cx. quinquefasciatus* in the waste waters from the slums as well as the wet latrines getting collected in adjacent cesspools, pits and the low lying land in Yamuna Pusht and Timarpur slums of Delhi because of total absence of the underground disposal of sewage and sullage. Batra et al. (1995) also observed extensive breeding of *Cx. quinquefasciatus* in stagnant and slow moving polluted waters in underground sewerage system in different areas in Delhi.

Gupta et al. (1992) carried out the survey to detect the breeding in intra-domestic breeding sources in Nadiad taluka of Kheda district, Gujarat and found that *Cx. quinquefasciatus* was one of the predominant species breeding in underground tanks, overhead tanks, water tanks kept both inside and outside, earthen pots and other miscellaneous containers.

Hassan et al. (1993) studied the physico-chemical factors of the breeding habitats of *Cx. quinquefasciatus* in the towns of North Western Peninsular Malaysia and stated that in most areas of its distribution, *Cx. quinquefasciatus* prefer habitats rich in dissolved matter and such habitats tend to have high total dissolved substance (TDS), which is the sum of all organic, inorganic and suspended solids in water. They found

that the larvae of *Cx. quinquefasciatus* were most abundant in polluted drains containing 1.0 to 2.0 g/litre of dissolved oxygen, 1.0 to 2.4 g/litre of soluble reactive phosphate and 0.1 to 0.9 g/litre of ammoniacal nitrogen.

Kanhekar et al. (1994) in their breeding survey during 1991 and 1992 in Rajahmundry town of Andhra Pradesh, recorded *Cx. quinquefasciatus* per dip larval density in the range of 1.1 to 25.3 and pupal density in the range of 1.3 to 8.4 during 1991 and 3.5 to 16.4 larval density and 1.2 to 7.8 pupal density during 1992. Cesspits were the most preferred breeding sites for *Cx. quinquefasciatus* followed by cesspools, unlined drains and brick lined drains. The wide range of fluctuations in larval/pupal densities observed in the study were attributed probably to ecological factors and the antilarval operations by the local National Filaria Control Programme unit.

Eapen and Chandrahas (1994) reported the breeding pattern in Cochin, Kerala and found that the drains were one of the important breeding habitats of *Cx. quinquefasciatus*. Cochin city received around 2000-2500 mm rainfall between June and August which washed away the mosquito larvae resulting in sharp decline in the adult density. After the rains, ground level water bodies were created which became favourable breeding sites of *Cx. quinquefasciatus* and the drains were gradually colonized with this species. By February, ground level water bodies dried up and the breeding of *Cx. quinquefasciatus* shifted to the drains, resulting in the increase of density.

In Dibrugarh town of Assam, intense breeding of *Cx. quinquefasciatus* was noticed by Bhattacharya et al. (1996) in choked drains containing the polluted waters. They found that 99.6% of polluted drains, 23.6% of unused tyres and 1.4% of unused

battery cases were with *Cx. quinquefasciatus* breeding. Urmila et al. (1999) encountered *Cx. quinquefasciatus* breeding in septic tanks in the University campus of Mysore, Karnataka.

Singh et al. (2000) detected the breeding of *Cx. quinquefasciatus* in dirty water collections like drains in and outside the houses, cesspools and water collections near the river in Pathankot town of Panjab. Murty et al. (2002) reported heavy breeding of *Cx. quinquefasciatus* in the irrigational channels, cesspits and cesspools in the rural areas of East and West Godavari districts of Andhra Pradesh.

Studies by Muturi et al. (2007 a, b) revealed that *Cx. quinquefasciatus* thrives in a variety of aquatic habitats including rice fields, canals, seepage areas, ditches, marshes, pits and temporary pools in Mwea, Kenya. Muturi et al. (2007 b), while evaluating the impact of rice cropping cycle on the prevalence and abundance of mosquito species in the rice fields in Mwea, Kenya, found that *Cx. quinquefasciatus* was one of the predominant species breeding in the rice field and dissolved oxygen, number of tillers and height of the rice crop were the significant predictors of *An. arabiensis* and *Cx. quinquefasciatus*. In addition, *Cx. quinquefasciatus* was also negatively associated to water depth and positively with turbidity.

Umar and Don Pedro (2008) studied the effect of pH on the larvae of *Aedes aegypti* and *Cx. quinquefasciatus*. In their bioassays, fourth instar larvae of field and laboratory strains of both the species were exposed to varying pH regimes and quantal mortalities were assessed after 24 hours. The results indicated that maximum survival of both field and laboratory strains occurred between the pH values of 6.5 and 8.0 and outside these range the mosquito larvae suffered high mortalities in 24 hours of

exposure. The responses of both the strains of *Aedes aegypti* and *Cx. quinquefasciatus* were similar, indicating that pH was not exerting any selection pressure on the field strain in the local environment.

Studies were conducted by Muturi et al. (2008) to investigate the environmental factors affecting the distribution of *Cx. quinquefasciatus* and *An. arabiensis* in Mwea, Kenya. The sampling unit comprised all non-paddy aquatic habitats (pools and marshes) and ten randomly selected paddies and canals. The collection of 1,974 mosquito larvae yielded four species dominated by *Cx. quinquefasciatus* (73.2%) and *An. arabiensis* (25.0%). Both the species were encountered in all four types of habitats. Pools were associated with significantly higher *Cx. quinquefasciatus* larval abundance. *Cx. quinquefasciatus* larvae were positively associated with dissolved oxygen, total dissolved solids, Chironomidae larvae and Microvelidae adults and negatively associated with emergent vegetation.

Cx. quinquefasciatus has shown its versatility in sharing the breeding habitats and high degree of interspecific association with other mosquito species viz., *Culex gelidus*, *Cx. vishnui*, *Cx. fuscans*, *Armigeres subalbatus*, *Aedes aegypti*, *Anopheles stephensi*, *An. vagus*, *An. gigas*, *An. kushingensis*, and *An. arabiensis* (Malhotra et al. 1987; Kaliwal, 1991; Kumar and Thavaselvam 1992; Muturi et al. 2008).

2.2 Oviposition and Development from egg to adult

Culex mosquitoes lay the eggs in rafts. The number of eggs laid by the individual female (fecundity) differs and the net emergence of adults also varies. Total number of eggs in the raft, hatchability of eggs (viable eggs), mortality at different stages of larval and pupal development collectively determine the net adult emergence

of the mosquito species. There are very few studies on the fecundity of wild mosquito populations, even for some medically important species (Yang et al. 2005).

Das et al. (1967) conducted laboratory experiments to study the influence of mating on blood feeding, oviposition and viability of eggs in *Cx. quinquefasciatus*. The results indicated that the blood feeding increased with insemination but was not dependant on it. However, a positive correlation was found to exist between insemination and oviposition rates and viability of eggs. A few egg rafts were laid by virgin females would indicate that some other type of stimulus is also necessary for oviposition. Mating was observed to have direct bearing on the viability of eggs laid. 13 egg rafts laid by virgin females were found to be non-viable.

De' Meillon et al. (1967) studied the development of *Cx. quinquefasciatus* in the laboratory and found that the period taken for the development from eggs to pupa was 5-7 days in Rangoon, Burma (Myanmar). They observed the biphasic pattern of oviposition in *Cx. quinquefasciatus*. In West Africa, the highest oviposition activity of *Cx. quinquefasciatus* was observed at sunset by Subra (1971).

Rajagopalan et al. (1975) studied the development and survival of immature stages of *Cx. quinquefasciatus* in the environs of Delhi. The average time period observed for the development from egg to pupae was 21 days in cesspools and 37 days in wells during cold season and 11 to 19 days in wells during hot season. The survival rate from egg to adult emergence observed by them was 27.8% in cesspools and 2.3% in wells during cold season and the survival rate ranged between 4 and 40% in wells during hot season. High mortalities were observed at I and IV instar larvae in all habitats both in cold and hot seasons and at the pupal stage only in the wells in the cold

season. The observed larval mortality pattern was similar to the results obtained in *Aedes aegypti* by Southwood et al. (1972) in Bangkok, Thailand. Zharov (1980) while studying the method for determining the actual fertility of blood sucking female mosquitoes, found that *A. vexans* Meigen laid a range of 156-198 eggs per female.

Panicker et al. (1981) studied the oviposition rhythm of nine species of mosquitoes in the laboratory. Though oviposition pattern of *Cx. quinquefasciatus* was monophasic and primarily nocturnal, a few egg rafts were obtained throughout the day. Soon after dusk, the oviposition activity intensified and reached its peak after midnight and then declined. The study indicated that different mosquito species exhibit different internal or endogenous rhythm (biological clock) in their oviposition behavior.

Menon and Sharma (1981) carried out the experiments to study the life table attributes of *Anopheles stephensi* type form and *Anopheles stephensi* var. *mysorensis* and the results did not show significant variation with respect to reproductive and survivorship characteristic from other population of *A. stephensi* studied.

Kaul et al. (1984) studied the influence of temperature and relative humidity on the gonotrophic cycle of *Cx. quinquefasciatus* under ambient laboratory conditions. They observed minimum length of gonotrophic cycle (LGC) of 2-3 days (May) and maximum of 81 days (November) in Delhi. In cooler months (October, 1969 to March, 1970) LGC was much greater (Median: 20.64 days) and in warmer months of April through September, 1969 (Median: 4.69 days). The study demonstrated the significant correlation between the LGC (length of gonotrophic cycle) and temperature, relative humidity. The two meteorological factors and their joint effect showed an

inverse association with LGC indicating that this cycle increases with the decrease in the temperature, relative humidity and their joint effect.

Panicker and Rajagopalan (1984) studied the biology of *Anopheles subpictus* in the laboratory. The number of eggs laid by individual female ranged from 38 to 286. The mean hatchability of eggs was 64.7 percent. The rate of pupation ranged from 15.8 to 80.7% with an average of 50.2%. The females slightly outnumbered males and the average male to female sex ratio ranged between 1 : 1.04. Majority of mosquitoes followed the oviposition pattern of 7-2-2 days during hot season and 7-3-3 days in the rest of the year. The observed mean range of duration of various immature stages in the study were : eggs- 40.8 ± 0.75 h, I instar- 33 ± 1.02 h, II instar- 35.5 ± 2.23 h, III instar- 43.6 ± 1.65 h, IV instar- 77.9 ± 3.96 h, Pupae- 27.3 ± 1.32 h. The duration of the immature stages increased with increase in the density. Survival of males and females ranged between 2 and 24 days and 2 and 22 days respectively. In an earlier study by Mehta (1934), *Anopheles subpictus* females survived for a short duration of 5-11 days and 50% of females died when they were 5 days old under controlled conditions of temperature and humidity in the laboratory.

Chadee and Haeger (1986), while studying on the eggs of mosquitoes, reported that *Cx. quinquefasciatus* from a wild population laid 30-350 eggs per raft. Salazar and Moncada (2004) carried out the experiment with *Cx. quinquefasciatus* during January-February and September-October of 2001 at ambient environmental conditions in Bogota, Colombia. They observed that the oviposition occurred 5-8 days after blood ingestion. The number of eggs per raft ranged between 152 and 203. The hatch rate was 62.5%. The asynchronous egg hatch, the short duration of the pupal state (11% of the

total developmental time) and high efficacy of adult emergence from the pupal stage (98.6%) were noted. The observed high percentages of hatch (83.6%), pupation (86.6%) and emergence (98.6%) under the average temperature conditions of 14.5°C and 15.1°C and average relative humidity of 72.5% and 74.1%, demonstrated the adaptation of *Cx. quinquefasciatus* to Bogota's cool, high altitude environment.

Yang et al (2005) studied the gravid rate and the number of eggs in the gravid females in the wild populations of *Cx. quinquefasciatus* on the island of Kauai, Hawaii. *Cx. quinquefasciatus* had much higher gravid rate (0.56 – 0.98) than *Aedes nocturnus* (0-0.24). The monthly average number of undeposited eggs per gravid females significantly differed in both the species. The range of undeposited eggs per gravid female was from 80.9 to 163.1 for *Cx. quinquefasciatus* and 29.8 to 71.7 for *Aedes nocturnes*. For both the species, no significant peak was found in monthly gravid rate and average number of undeposited eggs per gravid female.

2.3 Prevalence and Seasonal Distribution of Adults

Prevalence of the vector species, its high density and seasonal distribution influence the rate of disease transmission in time and space. *Cx. quinquefasciatus* is the predominant species in the urban areas, especially in those areas having inadequate or faulty drainage system (Das, 1976). Rapid urbanization and industrialization without proper drainage facilities are said to be responsible for the proliferation of the vector species (Mattingly 1962; Kaul 1964; Singh,1967; Chandra 2001). Singh (1967), encountered higher densities of *Cx quinquefasciatus* in urban areas as compared to rural areas.

Nagpal et al. (1983) studied the mosquito fauna of Nainital Terai (Uttar Pradesh) and recorded 29 species belonging to 8 genera. In this, *Cx. quinquefasciatus* was the most predominant species and it contributed 87.25% to the total number of culicines collected. In another study by Nagpal and Sharma (1983) in Andaman Islands (India), recorded 24 mosquito species belonging to 5 genera and *Cx. quinquefasciatus* contributed 44.96% among all the culicines.

Raina et al. (1990) reported *Cx. quinquefasciatus* 10 man hour density of 163.20 from the area of Sillberia PHC under Midnapur district of West Bengal. Prasad et al. (1992) carried out the mosquito collections in the villages of 5 PHCs of Shahjahanpur district of Uttar Pradesh and they encountered *Cx. quinquefasciatus* average man hour density of 25.8. Dutta et al. (1995) in their collection of adult mosquitoes from a tea estate in upper Assam, encountered *Cx. quinquefasciatus* as the most prevalent species in human dwellings, showing man hour density of 68.5.

Adhikari and Haldar (1995) studied the entomological aspects of lymphatic filariasis and microfilaria density in Colliery and Non-Colliery areas in Burdwan district, West Bengal. *Cx. quinquefasciatus* was found to be the predominant species in indoor collection and significantly high number of *Cx. quinquefasciatus* was collected from Colliery area. A total of 1746 and 849 females of *Cx. quinquefasciatus* were collected from Colliery and Non-Colliery areas respectively. The higher density of vector species in Colliery area was one of the major reasons for higher prevalence of filariasis in that area.

In Brazil, *Cx. quinquefasciatus* is widely distributed throughout the country and is often abundant in and around human habitations (Brito et al. 1997). Rajendran et al.

(1997) carried out entomological survey in 18 administrative areas of Chavakad taluka (Kerala) in relation to filariasis and encountered 14 species of mosquitoes with *Cx. quinquefasciatus* as the predominant species contributing 84.85% to the total number of indoor resting mosquitoes collected. *Cx. quinquefasciatus* adults were encountered in all the 18 areas surveyed and relative density of the resting population ranged between 0.83 to 8.0 per man hour.

Singh et al. (2000) conducted the entomological studies in filariasis non-endemic areas of Pathankot (Punjab) and found that 10 man hour density of *Cx. quinquefasciatus* ranged between 40 and 343 in different localities surveyed and authors stated that the temperature in this town was unfavourable for survival of the mosquitoes for most part of the year. In another study by Singh et al. (2002) in Bagdogra town in Darjeeling district of West Bengal, 10 man hour density of *Cx. quinquefasciatus* ranged from 30.0 to 65.0 in different localities of the town. 49 *Cx. quinquefasciatus* females were dissected and none was found positive in the study. Bagdogra town is known to be endemic for filariasis.

Murty et al. (2002) studied the prevalence of *Cx. quinquefasciatus* in the filaria endemic rural and urban areas of the East and the West Godavari districts (EGDT and WGDT) of Andhra Pradesh, India. In the rural areas of EGDT and WGDT, the highest mean per man hour densities (PMHDs) were 47.7 and 34.1 and the lowest densities were 21.3 and 32.3 respectively. In the urban areas of EGDT and WGDT, the highest mean per man hour densities (PMHDs) were 5.5 and 6.5 and the lowest densities were 2.4 and 5.2 respectively. The study showed high prevalence of *Cx. quinquefasciatus* in rural areas.

Studies were conducted by Muturi (2007) to determine the ecology of the rice-land mosquitoes in Mwea rice agro-ecosystem, Kenya. In this study, adult mosquitoes were collected indoors and outdoors to determine mosquito species diversity and abundance in relation to land use. 25 mosquito species, dominated by *An. arabiensis*, *Cx. quinquefasciatus* and *An. pharoensis* were collected and mosquito densities were highest in planned rice agro-ecosystem and lowest in non-irrigated agro-ecosystem, demonstrating the impact of land use on the mosquito species diversity and abundance.

The abundance of the vector species varies in different months and seasons of the year. The influence of the climatic and the environmental conditions on the density pattern of *Cx. quinquefasciatus* have been studied by different workers. Seniorwhite (1926, 1934) who had intensively studied the bionomics of *Cx. quinquefasciatus* in India, stated that this species was not a hardy species and is very susceptible to climatic conditions. However, under natural conditions in the field, the interpretation of response of the mosquito species to the changes in climate alone is difficult because of other variables in the environment (Rajagopalan, 1980).

Kaul and Wattal (1968b) studied the density pattern of *Cx. quinquefasciatus* in Arthala near Delhi and recorded that April was the month of peak prevalence and the vector species was scarce in January. The population crisis encountered in January was attributed by them to the direct influence of temperature as the low temperature probably prolonging the development from egg to adult as well as could be inducing mortality in larval and adult stages. The ratio between males and females observed by them in Arthala ranged between 1 : 1.5 to 1 : 6.5 in different months. The ratio was nearer at high density level and it was greater at low adult density level when the

conditions were unfavourable, revealing the fact that under unfavorable conditions, the females represent the residual long lived survived population. The males encountered were generally lower in number than the females and this was conceivable in view of the lower-life expectancy of the males (Wattal et al., 1961).

Dhar et al. (1968) in their study on *Cx. quinquefasciatus* in Rajahmundry town of Andhra Pradesh, observed the prevalence of the adults in all months of the year with a definite upward trend in density which started from July-August in monsoon period and reached the peak in November-December in post-monsoon period. They reported that the temperature and rainfall played vital roles in regulating the population density of the species in Rajahmundry. In their study, the ratio between males and females of *Cx. quinquefasciatus* varied between 1 : 1.5 and 1 : 5.8 in different months, the female population was nearly equal to the male population in May (probably due to more heat) but, in other months the number of females collected was higher than males indicating the outdoor resting behavior of males.

Subra (1973) conducted the ecological studies on *Cx. quinquefasciatus* in an urban zone of west African Sudan and Savanna and reported that the adults were scarce during hot and dry seasons and the density increased throughout the rainy season. In another study by Yasuno et al. (1977) in Delhi rural areas, there was a marked increase in the population density of *Cx. quinquefasciatus* during April-May, thereafter with further increase in temperature the density declined, maintained at a low plateau during monsoon and further decreased in winter. Rajagopalan et al. (1977) also observed the peak density of *Cx. quinquefasciatus* during March–April months when the maximum temperature was 30.5 °C and relative humidity was 57% in Faridabad.

Rajagopalan (1980) correlated the adult density build up pattern with the breeding of *Cx. quinquefasciatus* in Pondichery. After February, due to gradual drying up of breeding places the density declined. With the first rains of south west monsoon in June-July, the breeding surface area tremendously increased resulting in the increase of density. During September the increasing trend was reversed due to overflowing of cesspits and flushing away of drains by heavy rains which was marked in the November month. The temperature did not play any role in population growth as both immature and adult survival rates as well as net reproductive rates were high during dry and hot months. But, the heavy rains in November could reduce the reproductive potential of this species and the environmental condition such as reduction in breeding surface could cause the decrease in the population growth rate in Pondicherry.

Rath et al. (1984) studied the prevalence of *Cx. quinquefasciatus* in two rural localities (Haldiapada complex and Gopalpur complex) of Puri district (Orissa). In Haldiapada complex, per man hour density of *Cx. quinquefasciatus* ranged between 5.1 (January) and 19.2 (July). In Gopalpur complex, the per man hour density of *Cx. quinquefasciatus* ranged between 6.8 (November-December) and 11.5 (February). The variation in the vector density of both places was highly significant ($t=5.05$, $p<0.01$). They also noticed that the variation in climatic conditions of both areas was insignificant to have any differential influence on mosquito breeding.

De and Chandra (1994) studied the prevalence and seasonal distribution of *Cx. quinquefasciatus* at Kanchrapara in West Bengal. They found that *Cx. quinquefasciatus* was the most predominant species and contributed 91.24% to the total number of mosquitoes collected. Average man hour density was quite high ranging from 12.00 to

61.75. The species was most prevalent during summer (45.75%) and the density gradually decreased in winter (31.23%) and rainy season (23.02%). According to them, the prevalence of *Cx. quinquefasciatus* was at low level during the monsoon possibly because of the flooding of the breeding sites due to heavy rainfall. During winter and summer, the breeding sites were found to be in normal conditions, though variations in temperature and rainfall did exist. Chandra et al. (1993) and Chandra (1997) in two different studies, observed high densities in winter and rainy seasons in urban Calcutta. Dash et al (1998) recorded peak density in January and a trough in May-June months in Khurda district of Orissa. Dixit et al. (2002) in Raipur, Chattisgarh recorded the prevalence of the species in all months of the year with the high densities during February-March months and no significant relationship was observed between the variations in density of the vector and the variations in temperature and/or humidity in the study area.

In some areas, two peaks in density build up pattern of *Cx. quinquefasciatus* were observed during different periods. Sinha (1969) observed the first peak during February-March and the second peak during October-November in Bhagalpur. In Delhi urban area, two peaks of high density (post rainy months and post winter months) and two troughs of low density (hottest months and coldest months) were observed, thus the high temperature in summer and low temperature in winter mainly regulate population growth in the area (Rajagopalan et al. 1977). In two other studies in Gurgaon city, the highest density of *Cx. quinquefasciatus* was recorded during May and July months (Singh et al. 1984; Singh and Yadav, 1984).

Jain et al. (1989) conducted a community based longitudinal study on the epidemiological aspects of bancroftian filariasis in a semi-urban community of Kerala state during October, 1983 and September, 1984. *Cx. quinquefasciatus* was prevalent in all the months of the study with the highest per man hour density (29.0) in September and the lowest density (5.9) in November month. In the same study, two peaks in the adult density were observed- first peak in February (13.8) and the second peak in September.

Gakhar and Vandana (1996) studied the seasonal variations of culicine mosquitoes in Rohtak district of Haryana and observed that *Cx. quinquefasciatus* was the most dominant species and it was present throughout the year in high densities with two adult density peaks- the first peak in the month of December (88.9 PMHD) and the second peak in April (151.5 PMHD). The maximum and minimum temperatures, relative humidity and rainfall during the period of first and second peaks were: 22.9⁰C, 10⁰C, 67.3%, 0.44 mm and 35.8⁰C, 17.7⁰C, 35.2% and 0.75 mm respectively. The species was present in low densities in August and September months. According to them, the density of this species seemed to be governed by many factors which may act synergistically, low relative humidity and rainfall from April to June and December favoured the population and the species tolerated the extreme temperatures of these months.

Gakhar and Vandana (1996) also reported that female and male mosquitoes contributed about 64% and 36% respectively to the total culicine collection. They observed further that the highest female: male ratio was in *Cx. vishnui* (7:1) and the

lowest ratio in *Cx. quinquefasciatus* (1.1:1). This suggested the different population structure as also the life expectancy of the two sexes for different species.

Laporta et al. (2006) studied the ecological aspects of *Cx. quinquefasciatus* adult population in outdoor shelters in Sao Paulo-SP, Brazil. The mosquitoes were collected using the mechanical aspirator and *Cx. quinquefasciatus* adults were encountered in all the months during September, 03 to August, 04. The number of males and females collected in different months ranged between 54 (September) and 1198 (January) and between 6 (September) and 379 (June) respectively. The total number of males and females collected were 6313 (76%) and 1985 (24%) respectively. 90.1% of females had empty abdomen, 7.4% had blood and 2.5% had mature eggs. The high proportion of males was attributed to the tendency of males staying in outdoor shelters. They also observed that the correlation between number of mosquitoes encountered versus temperature and rainfall was weak.

Indoor Resting Behavior of *Cx. quinquefasciatus*

Mosquitoes differ in their resting behavior and they may prefer resting indoor (endophilic) or outdoor (exophilic). *Cx. quinquefasciatus* is one of the most common mosquitoes found in human habitations in the tropics and subtropics of the world (Selvi et al., 2007). *Cx. quinquefasciatus* is regarded mainly an endophilic mosquito (Barraud, 1934; Horsfall 1955). While studying the distribution, seasonal prevalence and biology of mosquitoes in Pune district of Maharashtra (India), Rao and Rajagopalan (1957) observed that the species is predominantly endophilic and its outdoor resting density was only one fourth of the indoor resting density.

Diwan Chand et al. (1961) in Uttar Pradesh observed that the species was mainly indoor. Singh et al (1963) who studied the house frequenting behavior of *Cx. quinquefasciatus* in Uttar Pradesh (India), recorded a high percentage of (77.8%) staying in the same dwelling once they had occupied it. Chandra et al. (1988) recorded the high densities of *Cx. quinquefasciatus* in the temporary human habitations than those in brick built houses and cattle-sheds.

Kulkarni and Rajput (1988) studied the preponderance and day time resting habitats of culicine mosquitoes in Bastar district of Madhya Pradesh and found that *Cx. quinquefasciatus* showed high preference to rest indoors in human dwellings and accounted for 34.9% of the total collection. Ningegouda and Vijayan (1992) collected 46.9% fed, 25% semigravid and only 15.6% unfed *Cx. quinquefasciatus* females from indoor resting sites, indicating the endophilic nature of the species in Mysore city, Karnataka. On the contrary, Laporta et al. (2006) reported that 90.1% were unfed, 7.4% fed and 2.5% gravid *Cx. quinquefasciatus* females in the outdoor collections made in the shelters in Sao Paulo, SP.

Indoor resting habitats of *Cx. quinquefasciatus* may be variable in different places. Chow and Thevasagayam (1957) in Karunegala, Ceylon found that about 60% of *Cx. quinquefasciatus* rested on hanging objects and clothes inside the human dwellings. Wattal & Kalra (1960) studied the resting behavior of *Cx. quinquefasciatus* near Ghaziabad in Uttar Pradesh and found that walls and corners were preferred resting places both during sprayed and unsprayed periods. They found that 50, 18%, 13%, 11% and 7% of *Cx. quinquefasciatus* rested on wall and corners, ceiling on cobwebs, objects on floor, hanging objects and shelves respectively.

Pal et al. (1960) in Ernakulam, Kerala found more *Cx. quinquefasciatus* resting on the hanging objects than walls and roofs and this resting habit remained unchanged even when the catching stations were sprayed with residual insecticide. 52%, 34% and 14% of *Cx. quinquefasciatus* rested on hanging objects, walls and ceilings respectively.

Abdulcader (1967) reported that *Cx. quinquefasciatus* in Ceylon also showed the highest preference to rest on the hanging objects. About 78%, 11%, 8% and 2% of *Cx. quinquefasciatus* females rested on hanging objects, walls, household articles and underneath of roofs respectively. The *Cx. quinquefasciatus* male mosquitoes also showed similar order of resting preference in the study. Dhar et al. (1968) in Rajamundry (Andhra Pradesh) found that 43.2%, 30.1%, 19.9% , 3.5% and 3.2% of *Cx. quinquefasciatus* rested on hanging objects, walls, ceilings, horizontal surfaces and objects on floor respectively. They also observed that 71.6% of total *Cx. quinquefasciatus* population preferred to rest above four feet from the ground. The preferences for the objects and the height for resting were found to be of similar order for both the sexes of the vector species.

2.4 Biting Activity of *Cx. quinquefasciatus* on Human Host

The host seeking behavior is an innate behavior of the female mosquitoes to secure a blood meal which is essential for the completion of gonotrophic cycle and oviposition, to continue their species propagation. Biting activity is one of the important aspects of the biology of vectors to which disease transmission is depended. The intensity of transmission of filarial infection depends upon the high biting density, anthropophily and high survival rates of vector (Wattal, 1976). Many factors influence the number and periodicity of mosquitoes biting on man. Haddow (1954) commenting

on the biting activity of African mosquitoes stated that there are different groups within the same species which bite in successive waves and that the factors like wind, 'impulse to bite' and the proximity of the breeding places influence the number of mosquitoes biting at a given time. Subra (1980) stated that the biting activity of *Cx. quinquefasciatus* is not uniform in different geographical areas.

Reid (1961) studied the number of *Cx. quinquefasciatus* mosquitoes attracted to man, calf and goat in the baited nets in Malaysia and found that man : calf ratio was 1.7 : 1, whereas man : goat ratio was 2.8 : 1, indicating the preference for man. Studies on the biting activity by Sasa et al. (1965) in Bangkok and De Meillon and Sebastian (1967) in Rangoon (Burma/Myanmar) reported that the mid night hours were the peak biting hours of *Cx. quinquefasciatus*. De Meillon et al. (1967), while evaluating the *Wuchereria bancrofti* infection in *Cx. quinquefasciatus* in Rangoon (Burma), estimated that 15,500 cumulative infective bites are required to produce the microfilaraemia in man. De Meillon et al. (1967) observed in Rangoon that the risk of infection was directly proportional to the biting density of the vector. Samarawikrema (1967) encountered infected and infective mosquitoes all through the night in hourly human bait collections in Sri Lanka and according to him the variations in the biting rhythm of different age groups of the mosquito vectors also influence the transmission of filariasis.

Rajagopalan et al. (1977) investigated the biting frequency of *Cx. quinquefasciatus* both indoor and outdoor during March to October months in Faridabad town (Uttar Pradesh). *Cx. quinquefasciatus* was the dominant species in the biting collection and the number biting outdoor was more. They observed peak biting of the species after the midnight hours in April and May and the peak was shifted to pre

midnight hours during June to October. In March no clearly defined biting peak was discernible. Biting pattern was similar both indoor and outdoor. Biting was highest during hot months (March to June) and it was reduced during monsoon with least biting in July. They opined that the biting behavior and the number of mosquitoes biting on human were influenced by many factors, such as physical impact of rains restraining the biting activity, lower density of vector during monsoon and the impact of larvicidal/adulticidal measure.

Self et al. (1978) from Jakarta reported that *Cx. quinquefasciatus* was the dominant species in biting collections and the biting peak occurred soon after the midnight. Rajagopalan et al. (1981) reported that the biting density was higher in the rainy season than in winter and summer season in Pondichery.

Rao et al. (1981) in their study in East Godavary district of Andhra Pradesh collected the mosquitoes biting on human bait in March, June, September and December months from September, 1975 to March, 1978. The second quarter of the night (2100 h – 2400 h) showed the maximum biting activity as more than 40% of the whole night mosquitoes were found feeding on human bait during this part of the night. This pattern was observed in March, June, September but, the maximum biting activity was shifted to the third quarter (0100 h – 0300 h) of the night in December. The highest (49.8%) and the lowest number (4.7%) of biting mosquitoes were encountered in March and June months respectively. They also estimated from the biting cycle that about 8200 vector mosquitoes would bite a man in a year in the study area, comprising about 940 infected mosquitoes and 32 infective mosquitoes which was lower than what was observed in Howrah by Roseboom et al. (1968). They encountered 14.6%, 17.8%,

16.2% and 51.4% of *Cx. quinquefasciatus* on face, hands, body and legs respectively, indicating the highest preference for the legs.

A case control study was undertaken by Chakravarty et al. (1991) to study man-vector contact in microfilaraemic and amicrofilaraemic human population in five villages of Varanasi district (Uttar Pradesh). The mosquitoes which landed between 2100 h to 2400 h were collected and analysed. Only *Cx. quinquefasciatus* in the collection were recorded. The number of vector mosquitoes landed on microfilaraemic and amicrofilaraemic individuals was not statistically significant ($p>0.05$) in the endemic area. When, the vector landings were analysed on the basis of different microfilaria (mf) density in the mf carriers, then also no significant difference was found ($p>0.05$).

Vanamail and Ramaiah (1991) carried out whole night (1800 h – 0600 h) collection of mosquitoes on human bait in Pondichery and found that the mid night hours were the peak biting hours of *Cx. quinquefasciatus* with the maximum activity around 0100 h. Similar analysis of microfilarial periodicity also showed that the density of microfilariae in the peripheral blood of humans was maximum at about 0100 h (Vector Control Research Centre, Pondichery- Unpublished data). The coincidence of peak biting hour of vector population and the appearance of microfilaria in the peripheral blood at its maximum density will facilitate the optimum infection of vector population which is to the advantage of filarial worms.

Attraction of mosquitoes towards human or other hosts depends on the host seeking behavior of the mosquitoes which perceive visual, thermal and olfactory stimuli that enable them to detect light source, odour and several other volatile chemicals

emanating from the skin, breath and waste products of their hosts (Takken, 1991; Davis and Bowen, 1994).

Several other reports have established the involvement of host odour, carbon-dioxide, lactic acid, sebum and sweat as attractants for these mosquitoes (Schreck et al., 1990; Knols et al., 1994; Maijerink and Van Loon, 1999). According to Gibson (1996), the evidence from behavioral studies suggest that the differences in host preferences may reflect differences in the relative responsiveness to carbon dioxide and other more specific host odors.

Host cues or volatile chemicals emanating from the human host are not the only factors that determine mosquito attraction or preference, other factors such as : environmental (odours from other sources, prohibitive wind speeds); physiological conditions (circadian phase, gonotrophic stage and nutritional status; the mosquito genotype; olfactory proteins involved in response to external stimuli-all these factors have been reported to affect the responsiveness of mosquitoes (Costantini et al.1998).

Ramaiah et al. (1992) reported the biting rate of *Cx. quinquefasciatus* in Pondicherry prior to, during and post implementation of integrated vector management (IVM) strategy. The annual biting rate (ABR) of *Cx. quinquefasciatus* was 26,562 during the pre-IVM period of 1979-80 and showed gradual decline from 1981 onwards reaching the lowest level of 1,662 in 1984. It increased many folds during the post-IVM years with a peak level of 31,807 in 1988. The average ABR during IVM period was 4003 ± 1195 against $21,911 \pm 4413$ during post- IVM period.

Eapen and Chandrahas (1994) carried out the study on biting rate of culicines by collecting the mosquitoes landing on indoor human baits between 1800 h to 0600 h

in three localities of Cochin city (Kerala, India) covering pre-monsoon (March-May), Monsoon (June-November) and Post-monsoon (December-February). Cochin city is endemic to bancroftian filariasis. Out of total 8 species of mosquitoes encountered in the study, *Cx. quinquefasciatus* was the predominant species and contributed 95.6% to the total number of mosquitoes collected. *Cx. quinquefasciatus* exhibited man-mosquito contact from dusk to dawn. Hourly peak biting occurred at 2200-2300 h both in pre and post-monsoon seasons and the shift in biting peak to around 0100-0200 h was conspicuous during the monsoon season. Seasonally, the man biting rate was highest in post-monsoon being 896 bites/man/night followed by pre-monsoon season with 328.5 bites/man/night and the least in monsoon season being only 10.5 bites/man/night. They attributed highest biting rate in the post-monsoon season to high adult density of the species due to profuse breeding in the open drains and the least biting rate during the rainy season is attributed to washing away of breeding in drains and other breeding sites due to heavy rains between June and August months.

Pipitgool et al. (1998) studied the biting density and biting cycle of *Cx. quinquefasciatus* in Khon Kaen city in Thailand and found that the peak biting activity of the vector species was between 2200 h and 2400 h. Dekker and Takken (1998) conducted the study with the traps baited with human, calf and carbon dioxide and found that *Cx. quinquefasciatus* entered human baited traps significantly more than carbon dioxide or calf baited traps.

Mboera and Takken (1999) also conducted field experiments using man baited, calf baited and goat baited tents to determine host preference of *Cx. quinquefasciatus* in Muheza, north-east Tanzania. A total of 2565 mosquitoes were collected and *Cx.*

quinquefasciatus contributed 96.6% to the total collection. Significantly larger number of unfed host seeking *Cx. quinquefasciatus* were caught in the man-baited tent than in the calf-baited or goat-baited tents ($p < 0.05$). The human baited tent caught a larger number than a calf baited or a goat baited tent ($p < 0.05$). Man:calf ratio was 7.8 : 1 while man : goat ratio was 10 : 1. The results indicated that given the equal access to a human, calf and goat host, *Cx. quinquefasciatus* selected the human host in preference to other two vertebrates.

Chandra (2001) presented a comprehensive account of biting activities of *Cx. quinquefasciatus* in urban (Culcutta) and rural area (Memari village) of West Bengal. In his study, mosquitoes which landed on human baits both indoor and outdoor in Culcutta and Memari village were collected for 24 hours (0600 h to 0600h). Out of total number of *Cx. quinquefasciatus* collected for 24 hours in urban and rural areas, 98.0% and 94.2% respectively were collected during 1800 h to 0600 h only, indicating exclusively night biting activity of the species. *Cx. quinquefasciatus* was the dominant species and contributed 66% to total collection in urban and 70% in rural areas. Outdoor biting tendency was more pronounced in rural area than in urban area. Interestingly, combining both indoor and outdoor, the biting peak occurred at 0100-0200 h.

In urban areas, Chandra (2001) recorded hourly biting peak of *Cx. quinquefasciatus* earlier in the indoors (1200-0100 h) than in the outdoors (0100-0200 h). Seasonally, the peak varied between 1200 h and 0200 h in all the seasons. The species had one distinct peak during May at indoor and two peaks at outdoor during May and August. Biting density was higher in the rainy season than in winter and summer. Average biting rates per man per night were 142.25 indoors and 154.58

outdoor. In rural area, hourly peak (0100-0200 h) coincided both in the indoor and outdoor. The peak was during 0100-0200 h in all the seasons. Monthly peak biting was noticed in July both at indoor and outdoor and there was no seasonal variations in biting density. Average biting rates per man per night were 219.37 indoors and 316.33 outdoors.

Shriram et al. (2005) studied the diurnal pattern of human biting activity of *Ochlerotatus (Finlaya) niveus* which is the vector of diurnally subperiodic *Wuchereria bancrofti* in five villages in Nicobar. In this study, the biting activity was seen throughout the day (day biter) with a major peak at dusk (1700-1800 h) and a minor peak at dawn (0400-0600 h). The biting periodicity remained qualitatively similar in all the seasons. However, in the summer months, the peak during dusk hours was more pronounced. Peak biting hours of *Oc. niveus* coincided with the peak appearance of microfilariae in the peripheral blood which facilitates the optimum infection of the vector population.

Oduola and Awe (2006) studied behavioural biting preference of *Cx. quinquefasciatus* in human host in Lagos Metropolis, Nigeria. Their results showed that *Cx. quinquefasciatus* was the predominant species (90%) over other species present and the numbers preferring the foot region (298) for biting was significantly higher ($p < 0.05$) when compared with other different parts of human body such as the ankles, calf and thigh. The study indicated that the foot region of the human host has a stronger influence in orienting the mosquitoes towards the human host. The observed peak biting period of *Cx. quinquefasciatus* was 2200 – 0200 h in Lagos Metropolis Nigeria.

Though, the study of Odoula and Awe was restricted to the lower limb, the other studies have made significant contributions on the role of different components of human sweat from other parts of the human host in the attraction of mosquitoes (Schreck et al., 1990; Geier et al., 1996), for example, in an experiment involving 8 species of mosquitoes, five species preferred to receive their blood meals in the head, facial region and the upper torso of the human host (De Jong and Knols, 1996).

Muturi (2007b) while evaluating the sampling device for their efficiency in outdoor collection of *Cx. quinquefasciatus* and other rice-land mosquitoes in Mwea, Kenya found that CO₂-baited CDC light traps were superior to non-baited CDC light traps for the collection of *Cx. quinquefasciatus* and *Cx. annulioris*.

2.5 Preference of *Cx. quinquefasciatus* for Blood meal

The host preference pattern of vector mosquitoes influences greatly the dynamics of transmission of infection in the community. Mosquitoes feed on a range of different host vertebrates such as human, monkeys, horses, dogs, pigs, camels, other ruminants, birds, etc. Some species have developed a characteristic host preference, feeding preferentially on humans (anthropophagic), mammals other than man (zoophagic) or birds (ornithophagic). Some vector mosquitoes exhibit distinct preference in this regard while others are facultative (Rao, 1984).

It is likely that differences in feeding patterns reflect differences in responsiveness to stimuli present throughout the host-location process (Costantini et al. 1998). Although, in many mosquito species there is a genetically determined host preference, the preference shown by a particular species of mosquito for one vertebrate host or another is likely to be influenced by environmental conditions (Takken, 1991) as

well as the ecology and the behavior of both host and vector (Gillies, 1988). Host selection by vectors is thus a result of a combination of intrinsic preferences modulated by extrinsic factors (Mboera and Takken, 1999). This means that a mosquito may still feed on a non-preferred host if this is the only one present because the opportunity to feed is more crucial for reproduction than the source of blood meal. Several studies are made on blood meal analysis from India and abroad.

Service (1963), in northern Nigeria found that 68.6% of blood meals of *Cx. quinquefasciatus* were positive for human blood, 25.7% positive for avian blood and 5.7% on other animals. Reisen and Boreham (1976) in their study in Pakistan found nearly 37.6% of *Cx. quinquefasciatus* fed on human blood. A study by Kay et al. (1979) from northern Queensland, Australia found dogs accounted for 54% of blood meals, humans 8.9% and birds 29.7%.

Arunachalam (1987) recorded very high anthropophilic index (98.7%) in the *Cx. quinquefasciatus* females collected from the human dwellings. Ningegouda and Vijayan (1992) studied the host preference of indoor resting populations of *Cx. quinquefasciatus* and seasonal variations in Mysore, Karnataka. The results of the study revealed that 94% of the blood meals were positive for human blood, 1.3% for bovine blood and 3.0% for avian blood and 1.7% blood meals were negative to all three. The vector species showed very high host preference for human and seasonally, there was no much variations in feeding preference as the anthropophilic index ranged between 91.9% (monsoon) and 96.5% (pre-monsoon). The endophilic nature of the species, dense human population and very low cattle population were said to be the factors for high anthropophilic index.

Mahapatra et al. (1995) studied the seasonal trends in the feeding behavior of *Cx. quinquefasciatus* in Puri district of Orissa, however, they did not find much variations in the anthropophilic index which was found to be 88.07%, 93.7% and 90.4% in summer, rainy and winter seasons respectively.

Ifteara et al. (1994) in their study from three sites in Bangladesh, encountered very high feeding preference of *Cx. quinquefasciatus* for human blood and found that 78%, 97% and 72% of the identified blood meals were of human. Bogh et al. (1998) also found the anthropophilic index as high as 95.5% for *Cx. quinquefasciatus* collected indoors on the Kenyan coast.

Michael et al. (2001) quantified the mosquito biting patterns on humans by DNA fingerprinting of blood meals of *Cx. quinquefasciatus*. Out of 276 blood meal PCR finger prints from an urban focus endemic for bancroftian filariasis in South India, they found that on an average 27% of the mosquitoes caught resting within individual households, had fed on people outside the household. Additionally, 13% of mosquitoes biting within households contained blood from at least two people, with the rate of multiple feeding depending on the density of humans in the household. They concluded that these complex vector feeding behaviors can be accurately assessed by this method and they underlined the potential of this tool for investigating the transmission dynamics of infection.

Dixit et al. (2002) studied the feeding behavior of *Cx. quinquefasciatus* collected from different biotopes in Raipur town in Chattisgarh. The vector species in Raipur exhibited high anthropophilic index of 89.65%, 60% and 63% for the collections made in human dwellings, cattlesheds and mixed dwellings respectively. The results indicated

the feeding preference of *Cx. quinquefasciatus* for human host irrespective of the biotopes and the vector given the choice, is preferentially anthropophilic in nature but, in the absence of human host in the vicinity, it can feed on other available hosts.

On the contrary, *Culex quinquefasciatus* collected from Delhi and nearby villages by Kaushal Kumar et al. (2002) were found to be zoophilic in nature and fed on human beings when they are in close contact with them. Anthropophilic index for *Cx. quinquefasciatus* was 41.4% from human dwelling collection, 19.8% from cattle sheds and 23.8% from mixed dwellings. Monthly analysis of feeding data showed the minimum anthropophilic index (18.19%) in winter months and the highest anthropophilic index (51.25%) in summer month of June. According to them, the feeding behavior was highly influenced by the type of biotope, village and areas, seasonal climate, sleeping behavior of human population and the availability of alternative host. During summer, due to hot and humid climate, human beings slept in open with minimum clothing, thus increasing the man-mosquito contact. On the contrary, during winter they covered their bodies with full clothing and slept indoor, minimizing the contact rate with the mosquitoes.

Samuel et al. (2004) analysed the blood meal of *Cx. quinquefasciatus* and *Ma. annulifera* collected from Kuttanadu, Kerala where Bancroftian and Malayan filariasis are co-existing. The results showed highly anthropophilic nature of both the species. 74% of 1148 blood meals of *Cx. quinquefasciatus* and 66% of 1180 blood meals of *M. annulifera* were positive for human blood.

Several reports in US (Bohart and Washino, 1978; Irby and Apperson, 1988; Reisen et al, 1990) on the feeding behavior of *Cx. quinquefasciatus* suggest that the

species fed minimally on humans. Irby and Apperson (1988) found no human feeding and 91% bird feeding in North Carolina. But, the results of the blood meal analysis obtained by Niebylski and Meek (1992) and Zinser et al. (2004) indicated substantial human feeding.

Niebylski and Meek (1992) analysed the blood meal sources of *Cx. quinquefasciatus* in two urban sites and one wooded site in Louisiana and the results suggested that the mosquitoes are opportunistic feeders that feed readily on humans or birds, *Cx. quinquefasciatus* collected from more typical residential areas fed 65-70% on dogs, 9-15% on human and 6-30% on bird blood and *Cx. quinquefasciatus* collected from a wooded area fed 23-33% on dog, 13-23% on human and 43-53% on bird blood.

Zinser et al. 2004 analysed the blood meals of *Cx. quinquefasciatus* mosquitoes collected from a residential area in Tucson. In their study, out of 84 blood meals tested, approximately 50% of blood meals were of human, 32% of bird, 12% of dog, less than 3% of cat and the remaining had mixed blood.

Molaei et al.(2007) tested 672 blood meals of *Cx. quinquefasciatus* collected in Harris County, Texas revealed that 39.1% had acquired blood from birds, 52.5% from mammals other than human and 8.3% were mixed mammalian and avian blood meals. The authors conclude that *Cx. quinquefasciatus* is an opportunistic feeder and principal mosquito vector of WNV in this metropolitan area.

Muturi et al.2008 conducted the blood meal analysis to determine the host feeding pattern of culicine mosquitoes collected indoors and outdoors in Mwea Rice Scheme, Kenya. Out of 1714 blood meals tested, the samples of *Cx. quinquefasciatus* alone contributed 96.1% and the remaining five culicines contributed only 3.9%

samples. *Cx. quinquefasciatus* was the only species which fed on human, indoor collected populations of *Cx. quinquefasciatus* had significantly higher frequency of human blood meals (9.8%) as compared to outdoor populations (3.0%). Mosquitoes mainly fed on bovines and goats. 73.5% of mixed blood feeding was observed in *Cx. quinquefasciatus*.

2.6 Longevity - Parity Rate

The approximate age of the mosquitoes is determined based on the number of ovipositions made by the females. The product of the number of serial dilations and intervals between successive gonotrophic cycles can provide an estimate of the age of females (Samarawikrema, 1962; Gillies & Wilkes, 1965). The number of ovipositions by a female depends on a multitude of factors, including the availability of hosts and success in getting the blood meals, intrinsic longevity of the female and survival rates, climatic conditions and intervals between re-feeding (Service, 1976). The parity rate and age grading are the important parameters which throw light on the changes in the population structure of vector mosquitoes (De Meillon & Khan, 1967). The potential for transmission comes from the female mosquitoes that survive a minimum of two gonotrophic cycles (Shriram et al., 2005).

This method not only distinguishes between nulliparous and parous females but also indicate the number of ovipositions taken place. This is referred as Polovodova's method (Polovodova, 1949) and is based on the dilations formed in a follicular tube of the ovariole after a mature egg has passed down through into the oviduct. At first, a large sac-like dilation is left, but this eventually contracts to leave a small but distinct bead-like swelling, now termed the dilation or follicular relic (formerly the corpus

luteum). In some species distinct dilations are recognized in the ovaries after each oviposition.

The period between successive ovipositions which may be longer than the duration of the gonotrophic cycle may vary in the field conditions. The delays between the repetitive gonotrophic cycles can invalidate the estimation of survival rates based on the parity and the percentage of parous mosquitoes will be greatly reduced by large increases in emergence, and this will result in low estimates of survival. Conversely in an ageing population, i.e., where there are fewer births than deaths, the survival rate will be too high. (Service, 1976).

Brady (1963) found in both *Anopheles gambiae* and *Anopheles funestus* that the proportion parous varied according to the stage of blood digestion and ovarian development. For these reasons he thought it prudent not to try to estimate their daily survival rates. Gillies & Wilkes (1965) found that the first gonotrophic cycle in *Anopheles gambiae* lasted 3 to 4 days and later cycles 3 days. Mark-recapture experiments some time provide valuable information on the frequency of feeding in nature and the duration of the gonotrophic cycle. In Tanzania, for example, Gillies & Wilkes (1965) established from marking and releasing that the gonotrophic cycle in parous *Anopheles gambiae* was 3 days. Similarly in Bangkok by marking and releasing *Aedes aegypti* Sheppard et al. (1969) postulated that the gonotrophic cycle was probably 3 days. In the Central African Republic, In Florida, Lowe et al. (1973) has reported a gonotrophic cycle of 3-4 days duration in *Culex pipiens fatigans*.

Different parous rates may be found in mosquitoes caught at bait and those resting in natural or artificial shelters. It may be necessary to sample biting populations

over their entire biting cycle to avoid any bias in parity associated with biting times (Gillies & Wilkes, 1963). DeMeillon et al. (1967b) found a lower parous rate in *Culex pipiens fatigans* collected in a variety of outdoor shelters than in females collected inside houses. They suggested that after emergence adults rested outside for some time before flying into houses to seek a blood meal. In another study, DeMeillon et al. (1967e) found that when adults of *Culex pipiens fatigans* fed before 2400 hr some two-thirds oviposited 2 days later, that is on the third night, but practically all females that fed after midnight oviposited on fourth night. They also found that late feeding and sugar feeding also delayed oviposition. In the Republic of the Congo, the parous rate of *Anopheles gambiae* caught in huts with CDC light traps was 44.6%, compared with 77.4% in adults caught biting whereas the parous rate of *Anopheles nili* was higher in adults caught in light traps inside huts than those caught at bait (Service, 1976).

The proportion of *Culex tritaeniorhynchus summosus* was greater in pig-baited traps than in those from dry ice traps. Moreover, a higher parous rate occurred in adults caught during the latter half of the night in pig-baited traps (Yajima et al., 1971).

Self et al. (1978) recorded the parity rate of 23% in Jakarta with the values ranging from 11.07% to 43.0%. Nathan (1981) found average parity rate of 28.1% in Trinidad with the monthly values ranging between 16.6% to 37.5%. In all these places, no seasonal variations in parity rates were evident and it was likely that low parity rate was partially responsible for the low infectivity rates which have been observed in these areas. High proportions of nulliparous females in these studies was attributed to high number of breeding sites available in big towns and the mosquito catching stations located in the vicinity of these breeding places.

The parity rate in Andhra Pradesh (India) was 29.4% with the monthly values ranging between 20.5% and 35.7% and further it was found that the seasonal variations in the climatic conditions did not show marked effect on the daily survival of vector mosquitoes (Rao et al., 1981). Similar phenomenon of climatic conditions on the vector mosquitoes of *Brugia malayi* has been observed by Chandrasekharan et al. (1976) in Kerala.

The higher rates of parity in *Cx. quinquefasciatus* have also been reported. In three villages of Tanzania, average parity rates were ranging from 46.2% to 57.9% (MacMohan et al., 1981). In Mambui (Kenya), the average parity rate was 63.4% with the highest parity rate of 82.7% in *Cx. quinquefasciatus* occurred during the rainy season which corresponded with the transmission peak (Wijers & Kiilu, 1977). In the same village, considerable differences were observed between the parity rates recorded in three different parts of the village indicating the importance of sampling site selection to get the accurate figures of this parameter. In Mysore, India the observed average parity rate was 42.8% with the monthly values ranging from 36.0% (October) to 67.6% (January), triparous and quadriparous females were encountered during cold season (November and January) indicating the survival of the vector species up to the completion of four gonotrophic cycles (Ninge Gowda & Vijayan, 1992).

Tellez et al. (1995) while studying the blood feeding frequency and life expectancy of *Aedes aegypti* in an urban area of Merida city, State of Yucatan, Mexico dissected 267 females, out of which 43.0% were parous, 13.3% were nulliparous and 43.7% semigravid or gravid. The estimated length of the gonotrophic cycle by them was 7 days.

Mboera et al. (1998) determined the best position for Centres for Disease Control (Atlanta, GA) light traps, in relation to human occupied bed nets for trapping of host-seeking *An. gambiae* and *Culex quinquefasciatus* mosquitoes in Tanzania. Significantly higher catches were recorded for both the species when the trap was positioned at the foot end of the bed, near the top of the net. Parity rates were significantly higher near the top of the net than at the level of the host. They concluded that catch size and parity rates of host seeking females for both the species differ according to the trap position in relation to the host occupying the bed net, but the factors causing this phenomenon are not yet understood.

Laporta & Sallum (2008) estimated the parity rate, length of gonotrophic cycle and density of *Cx. quinquefasciatus* at the Parque Ecologico do Tiete (PET), Sao Paulo, Brazil. Adult of *Cx. quinquefasciatus* were collected from the vegetation along the edges of a polluted drainage canal with the use of a battery-powered backpack aspirator. They examined the ovaries of 255 females and established the parity rate of 0.22 and determined the length of the gonotrophic cycle under laboratory conditions to be 3-4 days.

2.7 Infection and Infectivity Rates in *Cx. quinquefasciatus*

Vector competence with regard to *W. bancrofti* transmission can vary in *Cx. quinquefasciatus* populations from different geographical areas (Janousek and Lowrie, 1989). This is probably explained by genetic differences since McDonald (1967) found that mosquito susceptibility to filariae is governed by inheritable factors. It appears that the expression of the gene controlling the susceptibility of the mosquitoes to parasitic infection differs from species to species (Das, 1976). For instance, *Cx. quinquefasciatus*

is highly susceptible to periodic *W. bancrofti*, but it is not an efficient host to sub-periodic *W. bancrofti*.

A decrease in the transmission potentiality of *Cx. quinquefasciatus* was observed when mosquitoes were fed on the high microfilaria count carriers (>100 mf/20 cmm) and the mean number of infective larvae per infective mosquito in such cases was significantly lower than the expected ingestion of parasites (Das, 1976). In contrast, the mean number of infective larvae per infective mosquito compared well with expected intake of microfilaria when the mosquitoes were fed on low microfilaria count carriers (Krishnaswamy et al., 1959).

The infective microfilaria larvae when present in large number may cause fatal functional disorders in the mosquito (Omori, 1966). The mortality of infected mosquitoes has been found to be high during the first few days of infective blood meal and during the infective larval stage (Menon & Ramamurty, 1941; Kershaw et al., 1953).

The infection rates and infectivity rates of variable intensity in *Cx. quinquefasciatus* have been reported from different areas endemic for filariasis. Bounsulo (1968) had reported the infection rate of 0.7% and infectivity rate of 0.2% from Panaji, Goa during 1966. Azeez and Chakravorti (1980) studied the seasonal prevalence of filarial infection in *Cx. quinquefasciatus* from Dhanbad, Bihar. The infection rate was recorded in all months of the year. The highest infection rate was 10.7% in July and the lowest was 1.4% in February. The infectivity rate was just over 1.5% in hot and humid months May-October (with a casual slip in August) and thereafter, it was practically below 0.5%. Both infection and infectivity rates were of

higher order during hot and humid months, when people use minimum clothing and sleep outdoors, resulting in more exposure to mosquito bites.

Rao et al. (1981) conducted the detailed entomological investigations in East Godavari district of Andhra Pradesh and recorded that the infection rate ranged from 6.1% (April) to 11% (November), infectivity rate ranged from 0.1% (March) to 2.6% (September) and average number of infective larvae per infective mosquito ranged from 1.0 to 3.4%. The monsoon period from July to September contributed the highest transmission representing >40% of infective mosquitoes and >60% of infective larvae of *W. bancrofti* detected all through the year. The moderate climate and high relative humidity could have contributed for maximum transmission during monsoon period. They did not find any definite correlation between vector density and vector infection and infectivity rates. Earlier, the studies by Subrahmanian and Tampi (1958) in Mangalore, Karnataka and Nanda et al. (1962) in Barabanki, Uttar Pradesh also did not show any correlation between the adult density of *Cx. quinquefasciatus* and infection and infectivity rates. De Meillon et al. (1967) also found similar phenomenon in Rangoon, Burma.

Rath et al. (1984) studied the prevalence of bancroftial filariasis in two rural communities from Haldiapada complex and Gopalpur complex in Puri district of Orissa. In Haldiapada complex, the infection rate and infectivity rate varied between 2.7% and 10.5% and 1.4% and 4.1% respectively. Infective mosquitoes were not found in December, January and March months. In Gopalpur complex The infection rate and infectivity rate varied between 3.2% and 10.2% and 1% and 3.7% respectively. Infective mosquitoes were not found in December, January and July months. There was

no association of adult density of *Cx. quinquefasciatus* with infection and infectivity rates in both areas.

Jain et al. (1989) in their study related to epidemiological aspects of bancroftian filariasis in a semi-urban area in Kerala, encountered both infection rate and infectivity rate in *Cx. quinquefasciatus* in different months. Vector infection rate recorded was between 0 and 4.4 and infectivity rate between 0 and 2.2. The infected mosquitoes were encountered for 10 months, while the infective mosquitoes were observed only for three months (February, November and September).

Kumar and Chand (1990) studied the prevalence of *W. bancrofti* in coastal and sub-coastal villages of Ganzam, Orissa. In their study, out of 686 *Cx. quinquefasciatus* dissected from the villages close to the coast, only 4 were found positive with *W. bancrofti* larvae indicating the low infection rate of 0.58 and the infectivity rate of 0.29. On the other hand, 152 females out of 1040 dissected from sub-coastal villages were found positive showing an infection rate of 12.26 and infectivity rate of 4.43. The low endemicity of filariasis in extreme coastal villages also coincided with the very low vector infection and infectivity rates.

Raina et al. (1992) studied the status of lymphatic filariasis in some slum clusters namey, Hari Nagar, Yamuna Pusht near Vijay Ghat along the Ring Road and Timarpur in Delhi. Though, the mf rate of 6.3, 2.2 and 3.7 and disease rate of 1.4, 0.5 and 0.1 was found in the three areas, the dissection of *Cx. quinquefasciatus* did not reveal any human filarial infection except in Yamuna Pusht where out of 139 dissected, only one *Cx. quinquefasciatus* was found positive for infective stage of filariasis larva. Prasad et al. (1993) while assessing the filariasis situation in rural areas of Shahjahanpur

district (UP) found that the filariasis is more endemic in rural areas than in urban areas and in their preliminary entomological survey, they encountered the human filarial larvae in *Cx. quinquefasciatus* indicating the active transmission of the disease.

Vanamail et al. (1993) proposed the method for estimating the risk of infection (RI) with *W. bancrofti* from a resting population of *Cx. quinquefasciatus* based on a longitudinal study in seventeen sites in Pondicherry. Risk of infection calculated considering the density of parous mosquitoes collected per man hour and mean number of infective stage larvae per parous mosquito significantly correlated with mf prevalence in humans both during early and late in the control operation. RI varied significantly between early and late period and on average, RI reduced significantly during control operation.

De and Chandra (1994) detected the infection in 50 *Cx. quinquefasciatus* mosquitoes (infection rate=2.3%) at Kanchrapara in West Bengal. Number of infected mosquitoes was higher in the rainy season 20 (5.42%) in comparison to summer 16 (1.31%) and winter 14 (2.43%). Overall infectivity rate of *Cx. quinquefasciatus* was 0.28% (6 out of 2167 mosquitoes). The numbers of infective mosquitoes in the winter, summer and rainy season were 1 (0.17%), 1 (0.08%) and 4 (1.08%) respectively. The low infectivity during summer may be due to low survival and higher mortality of mosquitoes in general and infected mosquitoes in particular as reported from Vellore, India (Laurence, 1963) and Ceylon (Samarawikrema, 1967) where filarial transmission was interrupted during dry hot months of the year.

Kanhekar et al. (1994), while studying the aspects of bionomics of *Cx. quinquefasciatus* in Rajahmundry town in Andhra Pradesh during 1991 and 1992,

recorded the infected mosquitoes for 8 months. The infection rate varied between 0.8 (September, 1992) and 8.6 (May, 1991) during two years. The infective mosquitoes were encountered only once (1.1 in November, 1991 and 0.6 in August, 1992) in both the years.

Dutta et al. (1995) studied the status of filariasis in the labour population of a tea estate in upper Assam and revealed more than 8% positivity for microfilariae of *W. bancrofti*. A total of 186 live mosquitoes of *Cx. quinquefasciatus* were dissected and different stages of filarial larvae were detected from the thoracic region of 14 mosquitoes, the infectivity rate being 3.8. Detection of microfilariae in younger age groups and L3 (infective stage) of filarial larvae in the vector in several specimens, indicated indigenous transmission in the area.

Adhikari and Haldar (1995) while studying the comparative prevalence of filariasis in Colliery and Non-Colliery areas in Burdwan district of West Bengal, encountered more number of infected and infective *Cx. quinquefasciatus* mosquitoes in Colliery area than in Non-Colliery area. In Colliery area, 164 females were positive for *W. bancrofti* and 54 (32.9%) mosquitoes were with L3 stage larvae. In Non-Colliery area, 7 females were positive for *W. bancrofti* and 2 (28.6%) mosquitoes were with L3 stage larvae. The number of infected vectors and their infectivity were significantly higher ($p < 0.001$) in Colliery area as compared to Non-Colliery area.

Brito et al. (1997) undertook the experiment to compare the vectorial competence of *Cx. quinquefasciatus* strains from filariasis endemic and non endemic areas located wide apart at around 2000 kilometers in Brazil. The results showed that the survival rates and the number of infective larvae that developed did not differ in

both the strains indicating virtually the similar susceptibility to infection with *W. bancrofti* strain of filariasis endemic area. The observations have also been made by Magayuka and White (1972) in Kenya and Tanzania that *Cx. quinquefasciatus* strains from the areas free from lymphatic filariasis, can transmit the disease as efficiently as that occurring in endemic areas.

Rajendran et al. (1997) conducted a sample survey to assess the filariasis situation in all 18 administrative units of Chavakad taluka of Trichur district in Kerala. Microfilaria carriers with *W. bancrofti* were detected in 8 areas and this infection constituted 87.88% to total 33 mf carriers detected, while *B. malayi* infection was found in only one area. Infection with filarial parasites was found only in *Cx. quinquefasciatus*. Of the total 1978 *Cx. quinquefasciatus* dissected, infection was found in 9 (0.45%) females and infective stages in 3 (0.15%) mosquitoes. The infection rate ranged between 0.45% and 1.94% and infectivity rate between 0.45% and 0.97% in different areas. The mean number of developing larvae and infective larvae per positive mosquito was 2.88 and 2.33 respectively.

Subramanian et al. (1998) studied the uptake of *W. bancrofti* microfilariae by *Cx. quinquefasciatus* and their development in relation to human mf density by allowing a total of 1096 wild mosquitoes to feed on 13 volunteers sleeping under partially open bed-nets. The mosquitoes which harbored significantly high number of larvae died during 12 days indicating excess mortality among heavily infected mosquitoes. The L3 yield was related to mf intake indicating that *W. bancrofti*-*Cx. quinquefasciatus* complex showed 'limitation', i.e. a decreasing yield for an increasing uptake. Both the number of mf ingested and the number of L3 larvae developing per

mosquito were found to be highly aggregated, with the level of aggregation decreasing in a non-linear way with human mf density.

Singh et al. (2000) studied the filaria transmission in a non endemic area of Pathankot (Punjab) to find out any indigenous transmission of the disease and also to assess the filariasis situation. A total of 339 female *Cx. quinquefasciatus* were dissected and none was found positive for filarial parasite indicating the absence of active transmission in the area. As observed in the present study by Singh et al. (2000), extreme climatic conditions with low humidity during most part of the year, probably act as a natural check against the transmission of filariasis round the year in these places. As such, non endemic areas continue to be non endemic for filariasis despite considerable increase in mf rate among the migratory population and also increase in the vector density.

Hoti et al. (2001) conducted the study to evaluate the specificity and sensitivity of PCR assay in the detection of infection with *W. bancrofti* in *Cx. quinquefasciatus*. The results of the study revealed that Ssp 1 PCR was highly species specific and the assay detected as little as 0.04 pg of *W. bancrofti* DNA. Minimum number of parasite detectable in pools of mosquitoes was 1 mf. Therefore, it was concluded that Ssp 1 PCR assay has potential application in rapid assessment of transmission of filariasis.

Singh et al. (2002) in another study from Bagdogra town endemic for bancroftian filariasis in Darjeeling district of West Bengal, detected 35 microfilaria carriers (mf rate 2.31%) among the local residents of Bagdogra. Out of 49 *Cx. quinquefasciatus* females dissected none was found positive for *W. bancrofti* infection. Murty et al. (2002) reported considerable difference in the infection and infectivity rates

in *Cx. quinquefasciatus* between the rural and the urban areas in East and West Godavari district of Andhra Pradesh. The highest infection rate (43.6%) and infectivity rate (13.2%) were found in the rural areas. In urban areas, the highest infection rate of 7.5% and infectivity rate of 3.6% were encountered.

In Thailand, *Aedes spp.* and *Mansonia sp.* are the important vectors of lymphatic filariasis. Pumidonming et al. (2005) conducted an experiment to study whether *Cx. quinquefasciatus* mosquitoes collected from Phitsanulok city in Thailand can be a vector to *W. bancrofti* larvae development and onward transmission. The mosquitoes were exposed to infected blood meals by artificial feeding method and dissections of all infected mosquitoes on day 14 showed L3 stage microfilaria larvae, indicating the capacity to transmit the disease. Interestingly, more infective rates were found in mosquitoes that were fed with blood meals contained a high density of microfilaria.

Both vector density and community microfilaria load (CMFL) influence the intensity of transmission. Das and Vanamail (2008), using a logistic regression approach, have established a relationship between the risk of infection index (RII), vector density and CMFL. The analysis indicated that there is no risk of transmission as long as CMFL is maintained below 5 microfilaria (mf)/60 mm³ and the vector per man hour density (MHD) is <25. However, the transmission may continue if the vector density is >25 and CMFL is < 5 mf/60 mm³. According to Das and Vanamail (2008), in situations where the CMFL is very high, parasitic control by mass administration may be cost effective in interrupting transmission. But, at lower level of CMFL (<4 mf) and higher level of vector density it might be more cost effective to use vector control methods.

2.8 Susceptibility Status of Larvae and Adults of *Cx. quinquefasciatus* to Insecticides

Integrated vector control approach has been universally accepted as an effective tool for vector management. However, the primary approach used for mosquito control has mainly relied on pesticides (Pridgeon et al. 2008). The indiscriminate application of these insecticides in public health and agriculture has posed serious problems, the important being the development of resistance by the vectors. The wide spread development of resistance to insecticides in mosquitoes is one of the factors responsible for hampering vector control programmes (Das and Rajagopalan, 1981). As per the documented list of insecticide resistant vector species, 56 anopheline and 39 culicine mosquito species have developed resistance to the insecticides (WHO, 1992). Since insecticides, particularly organophosphate, pyrethroid and carbamate are still an integral part of vector management strategies, evaluation of vector management programs must regularly be carried out to determine the rate at which they are contributing or enhancing resistance development (Selvi et al., 2007).

A choice has to be made among the available insecticides based on the present susceptibility status of the vectors both in space and time. As such, it is necessary to conduct the periodic studies on the susceptibility status of both larval and adult populations of the vector species. Several reports have been published on this aspect of vector mosquitoes of malaria, lymphatic filariasis, Japanese encephalitis and dengue/chikungunya fever. Some of the reports related to resistance, with particular reference to *Cx. quinquefasciatus* are reviewed here.

The chemicals are applied against both larvae and adults to control the mosquito vectors, as such, the resistance is developed at both the stages. Brown and Pal (1971) reported that the application of larvicides is more liable to induce the resistance than the adulticides in the field. According to Beard (1952), this may be due to the fact that the larvae are exposed to the chemicals for a longer period resulting in the elimination of all susceptible individuals and permitting the survival of those which have already developed resistance in the field.

In India, the first evidence of DDT resistance in *Cx. quinquefasciatus* was observed in a village near Delhi in 1952 (Brown and Pal 1971). Rajagopalan et al. (1954) recorded the resistance to BHC in *Culex quinquefasciatus* in India. Later, resistance to DDT and dieldrin in *Cx. quinquefasciatus* was reported from some other areas like Nagpur, Pune, Patna in India (Mukhopadhyay et al. 1993).

Different strains of mosquitoes may react differently to selection for resistance. A study on Pondyichery strain of *Cx. quinquefasciatus* by Das and Rajagopalan (1980) revealed highly susceptible status of the species to fenthion, although the same chemical was under use for antilarval operation for two years, probably because the frequency of fenthion resistance gene is so low that it could not be selected for resistance.

In Rajahmundry (Andhra Pradesh, India), Rao et al. (1989) found that the larvae of *Cx. quinquefasciatus* were susceptible to malathion, fenitrothion, fenthion and Temephos larvicides. They also noted that *Cx. quinquefasciatus* adults were highly resistant to DDT (3.5% mortality) and dieldrin (12.2% mortality). In the same study, there was 98% mortality against fenitrothion, 94.4% mortality against malathion, 97.2% mortality against propoxur and fully susceptible to Deltamethrin. Recent study by

Mukhopadhyay et al. (2006) in the same area has recorded very high degree of resistance to malathion and fenitrothion and susceptible to temephos and fenthion in the larval population and zero mortality against DDT and Malathion in the adult populations of *Cx. quinquefasciatus*.

The susceptibility tests carried out by Thavaselvam et al. (1993) in Goa had recorded 76% mortality against 3.125 mg/l Malathion and 91% mortality against 0.125 mg/l Fenitrothion in the larval population of *Cx. quinquefasciatus*, showing low degree of resistance to these larvicides, but, the larvae were fully susceptible to Temephos. In the same study, they have reported resistance to DDT and Malathion in the adult populations of *Cx. quinquefasciatus*.

The resistance to temephos and fenthion in *Cx. quinquefasciatus* and other mosquitoes in India have been documented (WHO, 1992; Pillai, 1996). Patnaik et al. (1997) in a field trial recorded that temephos 50% EC was not effective for culicine larvae control even in the dose four times higher (0.05%) than the recommended doses and suggested urgent monitoring of susceptibility status of culicine larvae. The larvae of *Cx. quinquefasciatus* collected from different areas in Delhi showed considerable variations in its susceptibility to Fenthion indicating the possible resistance to the insecticide in Delhi (Mittal et al. 1999).

Mouches et al. (1987) and Pillai (1996) reported that the resistance to organophosphorus compounds in *Cx. quinquefasciatus* was mainly due to the over production of detoxifying esterases. It is well known that a very high resistance to one insecticide confers certain degree of cross resistance to other insecticides which are widely dissimilar in nature (Babers and Pratt, 1951). In a study conducted by Tadano

and Brown (1965), when DDT resistant strain and normal strain of *Cx. quinquefasciatus* were subjected to fenthion selection for 22 generations, a ten fold increase in tolerance to fenthion was noticed in resistant strain whereas only four fold increase in tolerance was noticed in normal strain. Pieris and Hemingway (1990) reported from Sri Lanka that the positive cross resistance to organophosphates and carbamates and negative resistance to permethrin was noticed in a temephos selected *Cx. quinquefasciatus*.

The study by Gonzalez et al. (1999) determined the levels and main mechanisms of insecticide resistance in a *Cx. quinquefasciatus* population from Rio de Janeiro, Brazil. Malathion, chlorpyrifos, primiphos-methyl, propoxur, cypermethrin, deltamethrin, lambda-cyhalothrin and DDT were tested. The results showed that Benfica strain was susceptible to none of the tested insecticides, resistant to DDT and highly resistant to chlorpyrifos. The results also corroborated the statement of Roush (1993) that enhanced oxidative mechanism appears to be the major resistance mechanism for all insecticide classes, except cyclodienes in mosquitoes, esterases are important in resistance to OP insecticides and occasionally pyrethroids.

The susceptibility status of mosquito vectors of malaria has been reported from different areas. Vittal and Deshpande (1983) reported the resistance in the adult population of *An. culicifacies* important rural malaria vector to Malathion, which were already resistant to DDT and HCH insecticides from Maharashtra (India). Singh and Bansal (1996) tested the susceptibility of adult *An. stephensi* important urban malaria vector to six insecticides in three desert districts- Barmer, Jodhpur and Pali of Rajasthan (India) and reported resistance to DDT, dieldrin ; partial resistance to malathion and

susceptible to fenitrothion, propoxure and permethrin insecticides. Sharma et al. (1999) have reported resistance to DDT in adults of both *An. fluviatilis* and *An. culicifacies* and fully susceptible to deltamethrin and lambda-cyhalothrin from Nainital district of Uttar Pradesh. Baruah and Shiv Lal (2004) tested the susceptibility status of *Anopheles minimus* adults to DDT and deltamethrin in three districts of Assam (India) and recorded 96.3% to 98.3% mortalities to DDT and complete susceptibility to deltamethrin. Hanafi-Bojd et al. (2006) evaluated the larval susceptibility status of *An. fluviatilis* and *An. dthali* to the diagnostic doses of temephos, Chlorpyrifos-methyl, fenitrothion, Methoprene and *Bacillus thuriangiensis* and found that both the species were susceptible to all larvicides from Hormozgan province in Southern Iran.

The susceptibility status of mosquito vectors of Japanese encephalitis has been reported from some areas. Sundaram et al. (1989) tested the adults of *Culex vishnui* and *Anopheles hyrcanus* group against the diagnostic doses of DDT and dieldrin in the villages of South Arcot and Tirunelveli districts of Tamil Nadu and found that both the species were resistant to DDT and dieldrin, but, susceptible to malathion. Kulkarni and Naik (1991) reported that *Cx. tritaeniorhynchus* adults were susceptible to dieldrin, malathion, fenitrothion and propoxur, but, the results with DDT were equivocal and require verification. Vijayan et al. (1993) conducted larval susceptibility tests with *Culex tritaeniorhynchus* and *Cx. fuscocephala* in Mysore city (Karnataka) against fenitrothion, fenitrothion, malathion, temephos, cypermethrin and deltamethrin and found that the efficacy of pyrethroids was very high as compared to OP compounds and the larvae of *Culex tritaeniorhynchus* were highly tolerant to all the chemicals tested than *Cx. fuscocephala*. A study by Bansal and Singh (1995) from Thar desert of Rajasthan,

reported that *Cx. pseudovishnui* adults were susceptible to permethrin and resistant to dieldrin and propoxur while *Culex tritaeniorhynchus* adults were susceptible to permethrin and resistant to DDT, dieldrin, fenitrothion and propoxur. However, a verification was required with other insecticides for both the species.

There are reports on the susceptibility status of dengue/chikungunya vectors. In view of wide dispersal of the vector *Aedes aegypti* and dengue virus (DEN) in the rural areas of Maharashtra state, a study was undertaken by Mourya et al. (1993) to conduct the insecticide bio-assays and the results showed resistance to DDT at larval and adult stages of *Aedes aegypti* populations. Biochemical analysis by them showed that resistance to DDT was probably due to increase in the kinetics of glutathione S-transferase. Thavaselvam et al. (1993) from Goa, have reported high degree of resistance to DDT and dieldrin and susceptible to malathion and fenitrothion in the adults of *Ae. aegypti* and on the other hand the larvae were fairly susceptible to DDT and fully susceptible to malathion, fenitrothion and temephos. Entomological investigations by Mourya et al. (1994), during the outbreak of dengue in 1992 in Shahjahanpur city (Uttar Pradesh), showed that *Ae. aegypti* mosquitoes were resistant to DDT and had some tolerance to malathion in the adults and larvae. Biochemical analysis done by them, suggested that DDT resistance was related to elevated glutathione s-transferase and tolerance to malathion was due to a little increase in esterase activity.

Andrade (2003) conducted the bioassays with the larvae of *Cx. quinquefasciatus* collected from an area not subjected to any chemical control and another area subjected to such treatment from Brazil. The results allowed suspicion of resistance to

cypermethrin and gave evidence of resistance to cyfluthrin and temephos from the area not subjected to any chemical control and resistance to temephos from the treated area.

Liu et al. (2004) have reported that two strains of *Cx. quinquefasciatus* collected from Alabama, USA showed high levels of resistance to Permethrin, deltamethrin, resmethrin (all pyrethroids) and cross-resistance to chlorpyrifor (OP-insecticide). The study by Qiang Xu et al. (2005) from Alabama, USA indicated that the high levels of permethrin resistance in two strains of *Cx. quinquefasciatus* are conferred by multiple mechanisms. The P450 monooxygenase-, hydrolase- and/or glutathione S-transferase (GST) mediated mechanisms are involved in permethrin resistance in both the strains.

Sunaiyana et al.(2006) investigated the resistance to various insecticides from four major groups (OC, OP, Carbamate and Pyrethroid) in the field strain of *Cx. quinquefasciatus* from Nonthaburi province of Thailand and found that the tested strain was completely resistant to DDT and highly resistant to deltamethrin, permethrin, fenitrothion and propoxur, but still found to be highly susceptible to malathion. The study indicated that the vector is resistant to all insecticides tested except malathion and this should be an alternative for the control of *Cx. quinquefasciatus* in the area.

Sathantriphop et al. (2006) compared the behavioral responses of three colonized strains of *Cx. quinquefasciatus*, two from recent field collections in Thailand and one from a long established colony from the National Institute of Health (NIH), Thailand, during and after exposure to deltamethrin, propoxur and fenitrothion using an exito-repellancy escape chamber system. Results indicated striking differences in behavioral responses and exito-repellancy between mosquito strains and test

compounds. Most mosquitoes escaped the treated chambers without receiving a lethal dose, indicating limited physical contact with the chemically treated surfaces, followed by rapid behavioral avoidance. Deltamethrin was the most irritant insecticide, followed by fenitrothion. Comparatively, greater escape responses were observed in NIH strain. The authors concluded that irritant and repellent behavioral responses by *Cx. quinquefasciatus* are important components for assessing the impact of residual spraying in mosquito control programs.

Corbel et al. (2007) investigated the frequency and distribution of resistance in *Cx. quinquefasciatus* and *An.gambiae* in four localities selected on the basis of contrasting agricultural practices, use of insecticides and environment from Benin, West Africa and reported that both the species had developed high frequency of resistance to DDT and permethrin in three localities and to dieldrin in all four localities from Benin.

Selvi et al. (2007) studied the characterization of malathion and permithrin resistance and the variation of esterase activity with the life stages of *Cx. quinquefasciatus*. Larvae and adults were used for the tests undertaken for malathion resistant and permethrin resistant strains. The results showed a significant difference ($p < 0.05$) in esterase level in both the strains. Female malathion selected strain had the higher level of esterase activity than permithrin selected strain. The results indicated increased level of non-specific esterase playing an important role in resistant mechanism in malathion resistant strain. Permithrin selected strain exhibited non-specific esterase activity at a very low level throughout the different life stages compared to malathion selected strain. The study suggested that life stages play a

predominant role in conferring malathion and permethrin resistance in *Cx. quinquefasciatus*.

Pridgeon et al. (2008) studied the susceptibility level of *Ae. aegypti*, *Cx. quinquefasciatus* and *Anopheles quadrimaculatus* to 19 pesticides belonging to different groups, to assess their relative potency to control adult mosquitoes. On the basis of 24-h LD₅₀ values after topical application, the only pesticide that had higher activity than permethrin was fipronil. *Cx. quinquefasciatus* was found least susceptible to nine pesticides. The results indicated different susceptibility levels of three species to different pesticides tested, showing the need to select the most efficacious compounds for the least susceptible mosquito species to achieve successful mosquito control.

Swain et al. (2008) studied the influence of sunlight exposure on the survival of the laboratory reared malathion resistant (RR) and malathion susceptible (SS) fourth instar larvae of *Cx. quinquefasciatus*. The effect of varying temperature during the day time due to the incident sunlight was assessed by exposing the larvae which were placed in 500 ml beakers half-filled with distilled water, to direct sunlight. After six hours of sunlight exposure, the mortality of malathion susceptible strain (88%) almost doubled that of resistant strain (52%), indicating a significant difference ($p < 0.001$). The study revealed that resistant strain has shown relatively better thermal adaptation than the susceptible one as indicated by increased survival rate. Moreover, Yadav et al. (2005) have reported that temperature and insecticide selection pressure drastically alter the genetic structure of the population in the area.

Chapter 3: MATERIALS AND METHODS

The present study on *Culex quinquefasciatus* was aimed at research on bio-ecological aspects of the vector species in the area known to be endemic for lymphatic filariasis in Goa. The study was done both on immature and adult populations of *Cx. quinquefasciatus*. The nature of the study was qualitative as well as quantitative.

- ❖ **Qualitative studies** : To study 1. the prevalence of the vector species, locality-wise and seasonal distribution, 2. breeding behavior, 3. development from egg to adult – net emergence of adults, 4. feeding behavior, 5. resting behavior, 6. longevity (parity rate) of the species, 7. responses of both larval and adult populations of *Cx. quinquefasciatus* to different commonly used insecticides and 8. influence of meteorological variables on the populations of the vector species.

- ❖ **Quantitative studies** : To study 1. the density of immatures (per dip density and container index), 2. survival rate and mortality rate at different stages of development from egg to adult emergence and duration taken to complete each stage of development, 3. the density of adults, 4. proportion of females with different abdominal conditions (unfed, fed, semi-gravid and gravid), 5. proportion of adults (females and males) rested on different types of indoor structures, 6. biting rate, periodicity of biting and proportion of females landed on different body parts of human bait, 7. anthropophilic index, 8. parity rate and percent contribution by the number of parous females in different months, 9. susceptible/resistance level of both larvae and adults to different chemicals and 10. significance or non-significance of impact of meteorological variables on vector population.

Qualitative and quantitative studies were performed using standard materials and methods. The details of the study area, the materials used and the methodology followed for different aspects of the present study were as follows.

Study Area:

Panaji is the capital city of Goa state in India. It is situated between 15° 53' N latitude and 73° 52' E longitude on the western coast of India. The location of Goa and Panaji city are shown in Fig.6. Panaji has a moderate climate without any extremes of summer and winter. The meteorological data for three years from the 2005 to 2007 are given in Tables 2-4. Due to the close proximity to the sea, the area is generally humid. It receives rains from the southwest monsoons between the months of June and September. The whole year was divided into three seasons viz., Pre-monsoon (February to May), Monsoon (June to September) and Post-monsoon (October to January) to study the trends in the density of both larval and adult populations, breeding of *Cx. quinquefasciatus*, biting behavior and longevity of the species in different seasons.

The soil is alluvial, sandy, saline and marshy. Mangroves, maritime grasses and coconut palms are found along the shore. Paddy fields are found at the periphery. Paddy is cultivated during monsoon months. Vegetables are grown after the monsoon as second cash crop. Paddy and ragi are the main crops. The city has the piped water supply system with scheduled timing of release of water. Therefore, the people follow water storage practices, usually in underground cement tanks (sumps), overhead cisterns and other domestic containers.

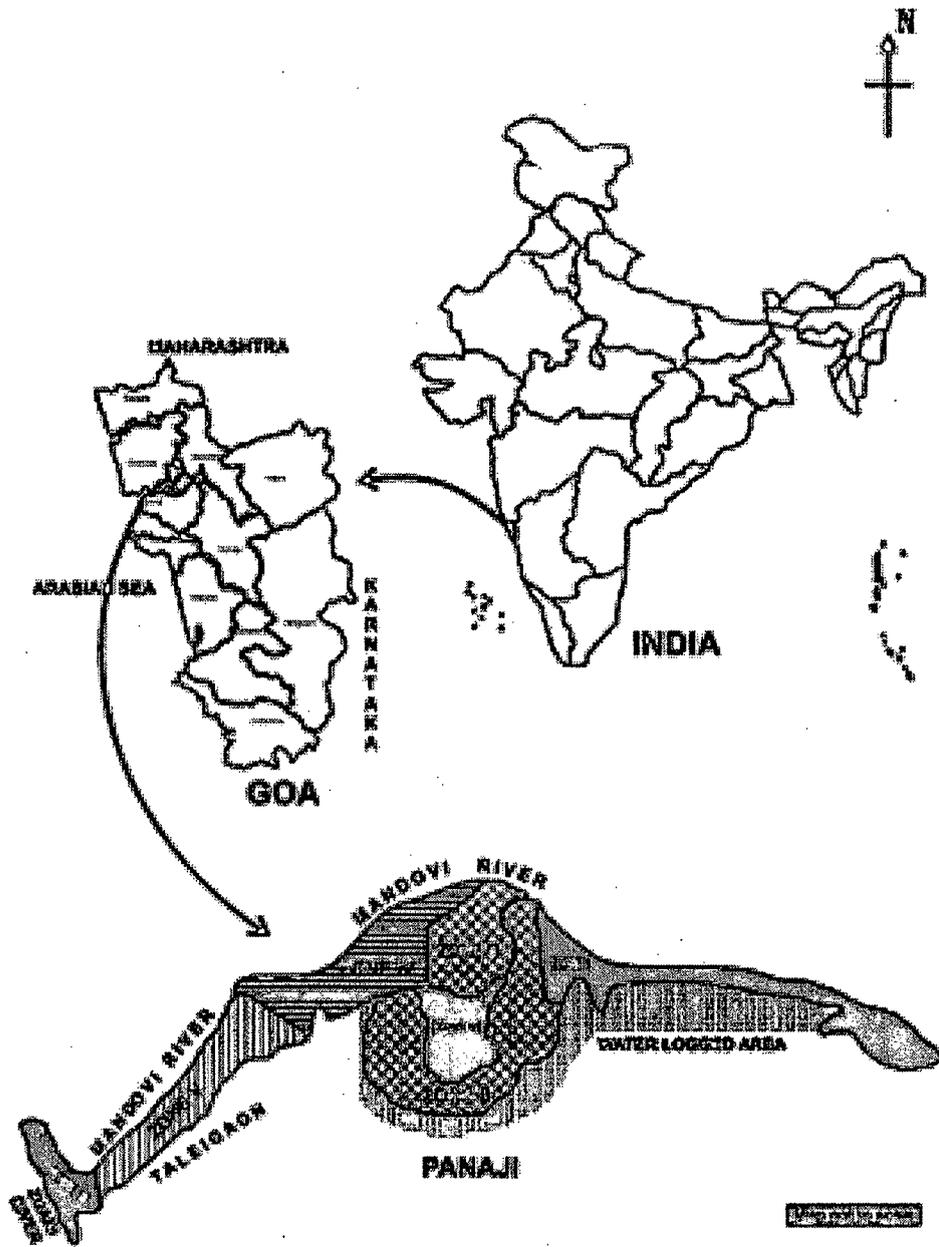


Fig. 1 - Map of India showing location of Goa and Panaji with different study zones (I to VI)

Table 2: Meteorological Data of Panaji for the Year 2005

Month	Temperature ° C		Actual Rainfall (mm)	Rainy days (No.)	Mean Relative Humidity (%)
	(Max)	(Min)			
Jan.	31.8	19.7	0.0	0	72.0
Feb.	32.0	20.1	2.0	1	72.5
Mar.	32.2	22.1	0.0	0	75.5
Apr.	33.5	24.9	45.0	2	73.5
May	34.2	26.0	42.7	1	71.5
Jun.	31.3	25.1	900.2	24	85.5
Jul.	29.3	24.1	1168.3	30	90.7
Aug	30.0	24.2	422.6	24	86.5
Sept.	29.6	23.7	638.5	25	90.0
Oct.	31.9	23.4	125.6	7	83.0
Nov.	34.0	21.0	0.0	0	67.0
Dec.	33.6	19.9	0.2	1	68.0
Total	-	-	3345.1	115	-

Table 3: Meteorological Data of Panaji for the Year 2006

Month	Temperature ° C		Actual Rainfall (mm)	Rainy days (No)	Mean Relative Humidity (%)
	(Max)	(Min)			
Jan.	34.0	19.6	0.0	0	65.5
Feb.	34.1	20.7	0.0	0	70.0
Mar.	32.2	22.9	44.3	2	72.5
Apr.	32.7	24.8	0.0	0	73.0
May.	32.9	26.0	416.7	8	78.0
Jun.	30.6	24.7	739.6	25	88.0
Jul.	30.0	25.0	371.1	30	86.0
Aug.	29.4	24.1	576.4	29	88.8
Sept.	30.0	24.0	422.5	17	88.5
Oct.	32.3	23.8	391.5	12	82.7
Nov.	34.0	23.6	6.2	5	77.0
Dec.	33.9	20.1	0.0	0	65.5
Total	-	-	2968.3	128	-

Table 4: Meteorological Data of Panaji for the Year 2007

Month	Temperature ° C		Actual Rainfall (mm)	Rainy days (No)	Mean Relative Humidity (%)
	(Max)	(Min)			
Jan.	33.5	20.2	0.0	0	72.0
Feb.	32.6	20.7	0.0	0	74.0
Mar.	32.6	23.6	0.0	0	75.0
Apr	34.0	26.3	0.0	0	74.0
May	34.0	26.6	113.8	8	71.0
Jun.	30.0	25.0	1077.4	29	89.0
Jul.	29.5	24.7	688.6	31	90.0
Aug.	26.1	24.1	887.0	29	91.0
Sept.	29.4	24.1	764.0	25	91.0
Oct.	31.5	24.2	81.9	11	85.5
Nov.	33.3	21.2	75.6	3	73.5
Dec.	33.3	20.9	0.7	1	73.0
Total	-	-	3689.0	137	-

There are open and closed drains which were blocked at places while at others they lacked proper gradient leading to the creation of ideal breeding grounds for mosquitoes. The city is also experiencing expansion and development, as a result a lot of multistoreyed building are being constructed which are creating additional breeding places both in and around these construction sites. Besides these, the drains, cesspits, cesspools, water storage containers, wells and other miscellaneous structures were the potential breeding habitats.

For the purpose of present study, Panaji was divided into six zones (Fig. 3.1). The zoning was done based on certain similarities within the localities. The areas covered under different zones were : Zone I – EDC and Patto ; Zone II – Mala and Bhatulem ; Zone III – Central Part and Market area ; Zone IV – Campal, Miramar, St. Inez and Tonca ; Zone V – Caranzalem ; Zone VI – Altinho and Donapaula. In zones IV and V, there is a wide nallah which is approximately 3 Kms. long and carries sullage water and domestic waste and meets Mandovi river which runs along the northern expanse of the city. Some areas of zones I and II are submerged in the back waters of this river.

3.1 Materials: The following materials, instruments and chemicals were utilized to carry out the present study.

1. Plastic white bowl (diameter-11 cm, holding capacity: 500 ml water) – to collect immature samples from field / for oviposition inside the cages
2. Steel Ladle (diameter – 9 cm, holding capacity: 150 ml. water) with 18 inches long handle – to collect immature samples from the field

4. Well net: An iron ring having diameter of 25 cm and a conical bag of nylon netting cloth were prepared. Nylon netting was fixed around the iron ring and excess nylon material was cut. The upper border of the bag was reinforced around the ring. Nylon string was tied to four points on the ring at equal distances and all four pieces of string were joined in such a way that the ring formed an angle of about 30°. A long rope of about 20 metres was tied to the well net for drawing samples from the well.
5. Hand net/Pond net: Hand net was prepared using ring (diameter-16 cm) of iron wire with long handle (3 feet), to which nylon bag was fixed around the ring. The depth of the net was 20 cm.
6. Pipette : This has a glass tube and at its one end the rubber bulb was fixed.
7. Plastic container with cap (500 ml capacity) - to carry field samples of immatures
8. Nylon netting cloth - to cover the plastic containers
9. Cotton wool – to plug the holes cut in the netting cloth which was used to cover the plastic containers containing the immature samples.
10. Aspirator (Sucking tube) : This consisted of plastic tube (approximate 15 inches long) attached to a flexible rubber tubing (about 20 inches long) and a small glass or plastic mouth-piece. A fine wire mesh/gauge separated the plastic and rubber tubes to prevent mosquitoes from entering the mouth while sucking the adult mosquitoes.
11. Test tubes (glass- borosil) – to introduce collected adult mosquitoes
12. Petridish (glass borosil)

13. Mosquito cage : This cuboidal cage was prepared using iron rods and cotton/nylon netting cloth. The size of the cage was 2ft. x 2 ft. x 2 ft. – to introduce the females for oviposition.
14. White enamel trays (15 x 10 x 3 inches) – to rear the larvae in the Insectory
15. White enamel basin – to transfer larvae collected from wells
16. Resin soaked with water – to feed mosquitoes inside the cage
17. Three celled torch with batteries – to search indoor resting adult mosquitoes
18. Permanent marker pen- to write on trays/containers, etc.
19. Micro slides (glass, size – 2.5 cm x 7.5 cm) – to prepare gel plates for blood meal assay
20. Whatman No. 1 circular filter papers (diameter – 10 cm) to collect blood meal samples of mosquitoes
21. Paper punch – to punch the blood meal from filter paper for assay
22. Gel puncher of 2 mm diameter – to punch the gel for making wells on gel plate
23. Glass rods – stirring purpose
24. Pasteur pipette – to take required quantity of serum, antiserum and known serum for assay
25. Glass pipettes (borosil) - 2 and 5 ml
26. Measuring cylinder (Glass borosil) - 100 and 500 ml capacity

27. Glass Bottle (borosil) - 100 ml capacity –2
28. Eppendorf tubes (Plastic) – 2 ml capacity
29. Conical flask (glass, borosil) - 250 ml capacity - 2
30. Conical flask (glass, borosil) - 100 ml capacity - 2
31. Plastic vials - 2 ml capacity - 5
32. Aluminium foil – 1 roll
33. Wet chamber/ Humid box : This was prepared by placing water soaked blotting paper/cotton cloth inside the rectangular plastic box with lid.
34. Water bath / hot plate
35. X - ray viewing machine to see precipitin arc to assess mosquito blood meal.
36. Hand Lens (x10) for identification of adult mosquitoes
37. Dissection microscope – to dissect mosquitoes
38. Compound Binocular microscope – to examine parasites and ovarioles for dilations.
39. Dissecting needles – These were prepared using sharp needles mounted in plastic holders.
40. Glass Beakers (borosil) – 500 ml capacity
41. Glass Beakers (borosil) – 100 ml capacity

42. WHO Adult Susceptibility test kit : The kit supplied by WHO included green dot and red dot plastic tubes of 125 mm (length) and 44 mm (breadth) with 16 mm mesh screen at one end; slide- units with screw cap on either side with a large orifice to transfer the mosquitoes and a small orifice for introduction of mosquitoes with aspirator; copper and steel clips; instruction sheet; log-probit papers; glass aspirator; roll of adhesive tape and white paper sheets (12 x 15 cm).

43. Larval food: A mixture of dog biscuit and Yeast extract in the ratio of 60:40 was used as larval food to rear them to pupa.

Chemicals: The following chemicals were used during the study.

1. Glucose 5% solution – to feed adult mosquitoes
2. Ethanol –to anaesthetize the adult mosquitoes to identify them up to species level.
3. Distilled water
4. Normal Saline (0.85%) – 0.85 g of Sodium chloride is dissolved in 100 ml distilled water.
5. Agarose – SRL Laboratories, Mumbai, India
6. 1 x TBE Buffer - Composition

Tris base - 10.8 g

Boric acid - 5.5 g

EDTA - 0.93 g

Made 1000 ml by adding distilled water.

7. Preparation of 1% Agarose gel

1 g of Agarose is dissolved in 100 ml of 1 x TBE Buffer by heating in a water bath for 15 minutes, till the solution becomes transparent.

8. PBS Buffer (pH = 7.4) - Composition

NaCl - 4.0 g

KCl - 0.1 g

Na₂HPO₄ - 0.72 g

KH₂PO₄ - 0.12 g

Dissolved in 100 ml of distilled water.

9. Test Sera (Mosquito blood meal samples collected from the field)

10. Anti – Sera (Human and Bovine)- Bought from Institute of Serology, Government of India, 3KYD Street, Kolkatta 700 016.

11. Known Sera (Human and Bovine)

12. Malathion - 31.25 mg/l, 781.25 mg/l

13. Fenitrothion - 6.25 mg/l, 31.25 mg/l

14. Fenthion - 6.25 mg/l, 31.25 mg/l

15. Temephos - 6.25 mg/l, 31.25 mg/l

16. Alcohol – to use for control sets

12-16 were the standard solutions of Insecticides for Larval Susceptibility Test, supplied by WHO in 50 ml plastic bottles

To conduct larval susceptibility tests, 4 different concentrations of each insecticide were prepared by mixing required quantity of the standard solution with the water taken in the beaker.

17. DDT 4% - Organochlorine insecticide (OC)
18. Malathion 5% - Organophosphate insecticide (OP)
19. Permethrin 0.25% - Synthetic pyrethroid insecticide (SP)
20. Deltamethrin 0.025% - Synthetic pyrethroid insecticide (SP)
21. OC – Control filter papers
22. OP – Control filter papers
23. SP – Control filter papers

17-23 Insecticide impregnated filter papers with diagnostic dosages for Adult Susceptibility Tests – Supplied by WHO.

24. Detergent powder – To clean all glass utensils, plastic tubes, enamel bowls, trays etc.

3.2 Methodology:

Mosquito Breeding Survey: Collection of immature stages of mosquitoes from the field

Breeding behavior differs in different mosquito species. The types and sizes of breeding habitats are highly variable. The mosquito breeding potential of any area mainly depends on the geo-physical characters of the area, water supply system, water storage practices adopted by the public, drainage system, ongoing developmental activities, ecological and seasonal changes.

Detection and collection of immature stages of mosquitoes (egg rafts, larvae and pupae) were done from different breeding habitats to determine the larval and pupal indices viz. habitat-wise per dip density and breeding index (container index) of *Cx. quinquefasciatus* and seasonal distribution of breeding.

Method: Mosquito breeding survey was carried out in different months covering pre-monsoon, monsoon and post-monsoon periods to detect and collect the egg rafts, larvae and pupae from different breeding habitats from all the six zones of Panaji during 2007 and 2008. The potential mosquito breeding sites such as drains, cesspits, cesspools, water storage tanks comprising cement tanks, plastic tanks and underground sumps, curing water collections, stagnant water collections and flower beds inside the buildings under construction, wells, plastic/iron barrels, vases, tires, discarded utensils, buckets, bottles, coconut shells, etc. were checked. The dipping, netting and pipetting methods were used for checking and collecting immatures (eggs, larvae and pupae) of mosquitoes from different breeding habitats (WHO, 1975; Service, 1976).

a. Dipping: This method was used most frequently. White plastic bowl and the ladle were used for dipping and collecting the larvae. Bowl/ladle were immersed in the breeding place at an angle of 45° to collect the immature stages. The care was taken not to fill the bowl/ladle completely to avoid any wash out of larvae/pupae. 5-10 dips were taken from each habitat checked. The number of larvae/pupae collected per each dip were counted and recorded.

b. Netting: Two types of nets – well net and pond net/hand net were used.

Use of well net: While using the well net, a small stone weighing about 50 g was fixed to keep the bottom of the net dipped and stretched under the water surface. To check the breeding in the well, the net was dipped slowly into the well in such a way that half of the ring is above the water surface. The net was kept in the same position for 2-3 minutes to allow the disturbed larvae to return to the surface. Then, the net was slowly moved around the edge of the well and withdrawn from the well. The withdrawn net was inverted in a white enamel basin containing water and the immature stages present were collected with a pipette. With a gap of every 2-3 minutes, the net was dipped five times to check a particular well.

Use of hand net / pond net: This net was used as a ladle to collect the immature stages from the inaccessible water surfaces of deep tanks and ponds. While collecting the larvae/pupae, the net was dipped at an angle. After dipping, the net was inverted and washed out in enamel basin containing water and from it the larvae / pupae were collected with a pipette.

c. Pipetting: Small pipettes (glass tubes with a rubber bulb) were used to collect the larvae/pupae from the surface of shallow waters, automobile tyres and other sites with little quantity of water in them.

The immature stages were collected along with the water of the breeding habitat in the plastic containers and brought to the laboratory. Soon after reaching the laboratory, the caps of the plastic containers were removed and covered with the nylon netting cloth and encircled with the rubber band. In the centre of the nylon netting, a hole was made and plugged with the cotton. The immature stages collected from each habitat were reared to adults in a separate container. The adults emerged from the pupae were captured through the hole in the net with the help of sucking tube and identified using the standard identification keys. Per-dip density and breeding index (container index) of *Cx. quinquefasciatus* for different habitats were calculated based on the results of adult emergence from each habitat.

Data Analysis: The data collected on the breeding was analysed habitat-wise and season-wise for *Anopheles*, *Culex*, *Aedes* and *Cx. quinquefasciatus* in particular. The breeding index (container index) and per dip density were calculated as shown below. The mean, standard deviation and the significance of habitat-wise breeding using Analysis of variance and Scheff's multiple comparison tests were done.

a. Per Dip Density: This is the number of larvae and /or pupae collected per dip in a breeding place. This was calculated as follows.

$$\text{Per dip Density} = \frac{\text{Total number of immature (larvae / pupae) collected}}{\text{Total number of dips taken from the breeding site}}$$

b. Breeding Index (BI) or Container Index (CI): This is the percentage of the breeding places/containers found with actual breeding of mosquitoes against the total number checked. Breeding Index or Container Index was calculated as follows.

$$\text{B.I. /CI} = \frac{\text{Number of breeding sites/container positive}}{\text{Total Number of Containers /Breeding sites checked.}} \times 100$$

Development of *Cx. quinquefasciatus* from egg to adult emergence in the Laboratory

Culex quinquefasciatus mosquito lays eggs in a cluster called egg raft. First stage of larvae hatch out from the eggs, grow to second stage followed by third stage and finally fourth stage larvae and then to pupae. Adults emerge out from the pupae. The development from egg up to adult emergence was studied for 38 egg rafts of *Cx. quinquefasciatus* during March to June, 2007 in the laboratory.

The development of *Cx. quinquefasciatus* from egg stage to adult emergence, survival and mortality at different stages of development, net emergence of adults, ratio of male to females and duration taken to complete each stage of development were studied at ambient temperature in the Laboratory.

Method: Freshly fed wild females of *Cx. quinquefasciatus* were collected between 2000-2100 h from the human dwellings. These mosquitoes were brought to the laboratory and introduced in the mosquito netting cage of 2 ft x 2 ft x 2 ft dimension in the Insectary. Cotton pad soaked with 5% glucose solution and resins soaked with water

were provided in the glass petridish as a source of sugar for the feeding of mosquitoes. After attaining the gravid stage, each female was individually kept inside the cotton cage. Plastic white bowl containing 300 ml of tap water was kept inside the cage for oviposition. After the oviposition, the number of eggs present in each egg raft were counted and noted. The oviposition never occurred in the day time. The observations for recording the egg laying, hatching of larvae, development to subsequent stages of larvae, pupae, survival, mortality and emergence to adults were made at 1000 h daily during the time of experiment. The observations were continued between 1000-1700 h every day. The duration from 1000 h to 1000 h of the next day was counted as one day and after 1000 h it was considered as the next day.

Rearing of Larvae: The first stage larvae hatched out from the eggs laid by each female were transferred to a white enamel tray (15 x 10 x 3 inches) and counted. Larvae were fed on the mixture of dog biscuit and Yeast Extract (60:40). Every day, any mortality in I to IV stage larvae and pupae were recorded. The larvae were kept in the tray until they grew to pupal stage.

Adult Emergence: All pupae were transferred to the plastic containers containing tap water. These containers were covered with a netting cloth with a hole in the centre to collect the adults emerged out from the pupae. The hole was covered by a cotton plug to prevent the escape of any adult through the hole and the netting cloth was held by a rubber band. Any mortality at pupal stage was recorded. The adults emerged from the pupae were collected by suction tube and transferred to test tubes. The number of males and females were counted.

Data Analysis: Number of eggs laid by each female, hatched to larvae, percent survival/mortality in eggs, I – IV stage larvae and pupal stage, time taken at different stages of development from egg to adult emergence and the net emergence of females and males against the number of eggs laid were noted. Mean and standard deviation were worked out.

Collection of Indoor Resting Adult Mosquitoes

To study the prevalence of *Cx. quinquefasciatus* in different zones of Panaji, relative monthly and seasonal abundance, and to obtain information on the movement and resting habits of *Cx. quinquefasciatus* mosquitoes of different physiological stages, the adult mosquitoes resting indoor were collected.

Mosquito Collection : The collections of indoor resting adult mosquitoes were carried out during April, 2005 to March, 2006 in all the six zones of Panaji. The standard method was followed to collect the indoor resting mosquitoes (WHO, 1975; Service, 1976). Ten mosquito catching sites were fixed in each zone. In all, sixty catching stations were fixed. While selecting the catching stations, care was taken to consider the similarities within the localities, mosquitogenic conditions, endemicity of filariasis and suitable representation of the different areas of Panaji city. The collection of indoor resting mosquitoes was done from 06.30 to 09.30 hours at fortnightly interval from sixty catching stations. Ten minutes were spent in each catching station to collect the mosquitoes resting on different surfaces viz., walls, hanging objects (clothes, gunny bags, wires, umbrellas, baskets, etc.), objects on the floor (cots, tables, chairs, benches, cycles etc.) and horizontal surfaces (shelves, wooden planks, ceiling etc). Adult

mosquitoes were searched with the aid of battery operated torch and sucked into the aspirator tube by holding the mouth of the aspirator tube close to a resting mosquito and gently but quickly sucking it from the mouth-piece.

The collected mosquitoes were prevented from escaping by immediately closing the mouth of the aspirator tube with the thumb and then gently blowed into the test tube marked with the type of resting site. Later, these mosquitoes were released into the respective cotton cage of 30 cm x 30 cm x 30 cm size. The number of anopheliline and culicine mosquitoes collected from each catching site were noted. Every month 20 man hours were spent to collect the mosquitoes from all the zones. 240 man hours were spent to collect the mosquitoes for 12 months. Mosquitoes were brought alive to the Laboratory for further analysis.

Identification of Mosquito species: Mosquitoes were transferred from cages to the test tubes and anaesthetized with solvent ether. Identification was done using the standard identification keys of Christopher (1933), Barraud (1934), Puri (1954), Rao (1984), Das et al. (1990) and Nagpal & Sharma (1994) based on morphological variations.

Classification of Abdominal status of female *Cx. quinquefasciatus*: Based on their abdominal conditions, *Cx. quinquefasciatus* females were classified as unfed, fed, semi-gravid and gravid by the external examination to ascertain the feeding status and ovary developmental status using the hand lens (10X). The objective of the abdominal classification of *Cx. quinquefasciatus* females was to obtain information on the resting behavior of mosquitoes of different physiological stages.

Data Analysis: The indoor resting density of mosquitoes was expressed as the number of males/females collected per man hour. Per man hour density (PMHD) was calculated as follows.

$$\text{PMHD} = \frac{\text{Total number of } Cx. \text{ quinquefasciatus Females / Males collected}}{\text{Total number of hours spent to collect the mosquitoes}}$$

The data on indoor resting mosquitoes was analyzed to know the prevalence of *Cx. quinquefasciatus* in all the zones, monthly abundance of the species, seasonal distribution, indoor resting behavior, influence of meteorological variables on the density pattern of *Cx. quinquefasciatus* and the prevalence of other mosquito species in different months of the year in Panaji. After the zone-wise analysis, the entire monthly data on *Cx. quinquefasciatus* from all six zones was pooled for further analysis. Two way factorial ANOVA was applied to analyze the significance of density variations in different months and zones, spatio-temporal variations in abdominal conditions and resting on different structures. Further, Tuckey a *post hoc* test was applied to justify the variations within different abdominal conditions and different resting sites. Regression analysis was done to analyze the impact of temperature, relative humidity, rainfall and number of rainy days on the abundance of *Cx. quinquefasciatus*. Correlation analysis was done to find out association between the abdominal status and the meteorological variables.

Collection of Adult Mosquitoes on Human Bait

The biting rate, periodicity of biting, seasonal variations in biting activity and the landing rate of *Cx. quinquefasciatus* on different parts of human bait were studied through whole night collection of mosquitoes landing on human bait.

Method: Two localities namely Mala and Kamrabhat are more prone to the problem of lymphatic filariasis in Panaji. These two areas were selected to collect the mosquitoes landing on human baits during July, 2005 to June, 2006.

Two teams were constituted for the collection of mosquitoes landing on human bait. Each team was comprised of two workers. Mosquitoes were collected from 1800 hours to 0600 hours with the help of battery operated torch and the aspirator tube (WHO, 1975; Service, 1976). Both the teams worked on shift of 3 hours at a time. When, one team was working, the second team was resting. A male volunteer from Mala locality and another male volunteer from Kamrabhat locality acted as human bait in the respective areas. Prior to starting of the study, the informed consent was taken from both the persons who acted as bait and they were provided with the prophylactic drugs against malaria and lymphatic filariasis as per the recommended doses of Chloroquine and Diethyl carbamazine tablets.

Every month two whole night landing collections, one collection each in both the localities were done for twelve months from July, 2005 to June, 2006. In all, landing collections were made for 24 whole nights in Panaji. Mosquitoes which landed on human bait and probing to bite were collected and transferred to the glass test tubes by gentle blowing. Immediately after blowing of mosquitoes into the test tube, the mouth of the test tube was closed with the help of thumb and plugged with the cotton wool to

prevent its escape. Glass test tubes were labeled with different body parts of human bait and different hours of the night from 1800 – 0600 hours. 3-4 sucking tubes were always used to catch the mosquitoes. Out of two workers in each team, one was collecting the landing mosquitoes and the other worker was assisting in transferring the mosquitoes and other related works. Mosquitoes landed on face, body, hands and legs for every hour were collected in different test tubes and brought alive to the laboratory. Identification of the mosquitoes was done using the standard identification keys as mentioned under indoor resting adult collection.

Data Analysis: The data on the biting activity of *Cx. quinquefasciatus* females from Mala and Kamrabhat localities were analyzed. After the areawise analysis of number and species of mosquitoes collected, the data from both the localities were pooled for further analysis. Average Landing rate (ALR) per bait per night in different months, seasons and for the whole year were calculated. The percent of *Cx. quinquefasciatus* females landed on human bait in different hours and quarters of the night, on different body parts, and the seasonal variations in percent landing were also worked out.

$$\text{Average Man-Landing Rate} = \frac{\text{Total No. of } Cx. \text{ quinquefasciatus} \text{ landed on Human bait}}{\text{Total No. of nights landing collection made}}$$

The seasonal average landing rate was also calculated as above, based on the total mosquitoes landed divided by total nights landing collections made during the respective season. The total number of mosquitoes landing per person per annum was calculated as follows-

$$\text{No. biting/Annum} = Cx. \text{ quinquefasciatus} \text{ Landed/Bait/Night} \times 365 \text{ days.}$$

The significance of spatio-temporal variations in biting activity between the areas, between the months, between the quarters of night, between the body parts of human bait and their different interactions were analysed using Two Way factorial ANOVA and Tuckey a *post hoc* test. The correlation of number of mosquitoes landing on human bait with the meteorological variables viz. temperature, relative humidity, actual rain fall and number of rainy days was done by Correlation analysis.

Analysis of Blood Meals of *Cx. quinquefasciatus*

The blood meals of *Cx. quinquefasciatus* were analysed to know the sources of blood meals for evaluating the feeding preference of the vector species and to find out the anthropophilic index.

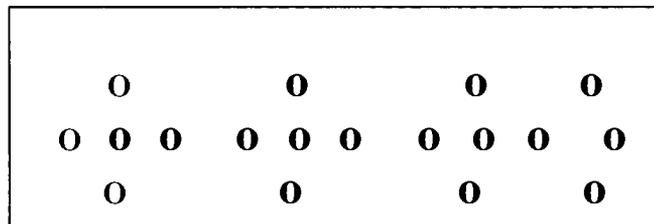
Gel diffusion (GD) technique as reported by Collins et al. (1986) was followed with few minor modifications to establish the source of mosquito blood meal. For the purpose of blood meal analysis, fully engorged mosquitoes were collected from human dwellings, cattlesheds and outdoor resting sites in Panaji.

Collection of samples: Blood from the abdomen of *Cx. quinquefasciatus* females were smeared on the circular Whatman No.1 filter paper (diameter – 10 cm) with the help of tip of alpin. 16 blood meal samples were smeared on one filter paper. The information regarding date, site and place of collection of mosquitoes was written on the back side of each blood smear. Filter papers with blood smears were stored in zipper polyethylene bags in a refrigerator until they were processed.

Preperation of Gel Plate: Glass slides were cleaned in soap water and rinsed with distilled water. Just before use, the glass slides were cleaned with spirit. Agarose gel

was warmed in water bath and 2.5 ml. of molten agarose was slowly poured on the glass slide. After 5-10 minutes, the gel got set and then these plates were kept inside the humid box and stored in the refrigerator at 4° C until used for the assay. Gel plates were prepared on the same day when the assay was done.

Making of wells in Gel Plate: Gel plates were taken out from the refrigerator just before use. Wells of 2 mm diameter were punched with the help of gel puncher. Pieces of gel were removed with the help of alpin. On each gel plate, 18 wells were made as per the well pattern shown in the Fig. 7, which allows testing of 12 blood meal samples against one anti-serum.



**Red well - Anti-Human/Anti-Bovine Serum ; Green well - Test Serum,
Blue well – Known Human Serum and Black well – Known Bovine Serum**

**Fig. 7: Analysis of Mosquito Blood meal = Pattern of wells
made on the gel plate.**

Reading of results: The results of gel diffusion tests were read by holding the plate against a black plate on an X-ray viewing machine. White precipitin arc was observed between the test serum and the anti-serum whenever the test serum reacted positively against the specific anti-serum.

Data analysis: The number of blood meals tested against antisera of human and bovine and found positive either to human or bovine blood were noted. To find out the percent of blood meals fed on human, the anthropophilic index was determined.

Anthropophilic Index (AI): This index is the percentage of mosquito blood meals of *Cx. quinquefasciatus* reacted against the anti human serum. Anthropophilic Index was calculated as follows.

$$\text{A. I.} = \frac{\text{Number of Blood meals positive for Human blood}}{\text{Total number of Blood meals tested for Human blood}} \times 100.$$

Dissection of *Cx. quinquefasciatus* Females

Female mosquitoes are dissected to find out the parity rates of the mosquitoes to determine the approximate age of mosquitoes and also to detect the filarial parasites inside the body of mosquitoes. Adult mosquitoes collected from the field, were dissected for this purpose.

Adult Mosquito Collection: Adult mosquitoes were collected between 06.00 h. and 09.00 h. in the morning from Mala, Bhatulem, Campal, St. Inez, Kamrabhat and Tonca areas in Panaji on weekly basis by spending 20 man hours every month during July, 2006 to June, 2007. In all, 240 man hours were spent to collect the adult

mosquitoes. Details of method of adult collection and identification are given under indoor resting collection. Per man hour density (PMHD) of *Cx. quinquefasciatus* females were calculated and some of *Cx. quinquefasciatus* females were dissected to determine the parity rate, infection rate and infectivity rate.

Dissection of Ovary - Parity Rate

Approximate age of the adult female mosquito is determined based on the number of dilations present on the ovariole (WHO, 1975). Females having one or more dilations are parous and females without any dilation are nulliparous mosquitoes. The number of dilations indicate the number of ovipositions that have already taken place. Females having one dilation are uniparous, two dilations - biparous, three dilations - triparous and so on. The ovaries of *Cx. quinquefasciatus* were dissected for establishing the parity rate and the physiological age of the vector mosquitoes in different months and seasons.

Dissection: *Cx. quinquefasciatus* females were anaesthetized with ether inside the test tubes. Each mosquito was taken on the glass slide, held by one wing and legs were removed one at a time. Both the wings were cut off. To avoid contamination of the slide, scales of wings or parts of the legs were removed before dissection.

Anaesthetized mosquito was placed on its back and a drop of 0.68% saline was put near the extremity of the abdomen. One dissecting needle was placed on the thorax muscles and using the second needle a small cut was made between the VI and VII sternites. Then, the second needle was gently moved to extract the ovaries. Once, the ovaries appeared, hindgut was removed and ovaries were separated. Ovarioles were

separated from the anterior, middle and posterior part of the ovary and examined with an ocular x5 and objective x40.

Data analysis: Per man hour density of *Cx. quinquefasciatus* collected in each month was calculated as mentioned under indoor resting adult collections. The number of females dissected, nulliparous and parous (with one or more dilations) found in different months were noted and the parity rate was determined as follows.

Parity Rate: This is the percentage of *Cx. quinquefasciatus* females with the parous condition in the adult female population of the species. Parity rate in different months and in different seasons of the year were calculated.

$$\text{Parity Rate} = \frac{\text{Number of Parous Females encountered}}{\text{Number of Total Females dissected}} \times 100.$$

To analyse the significance if any in the monthly variations in parity rate, Two Way ANOVA test was done. Further, Tuckey a *post hoc* test was done to justify the significance in variations in parity rate between the months. To analyse the correlation if any, between the parity rate and the per man hour density, the correlation analysis was done.

Dissection of Head, Thorax and Abdomen: Infection Rate and Infectivity Rate

Cx. quinquefasciatus females when bite and suck the blood from the microfilaria carrier, the filarial parasites enter the mosquito body and develop further to L-1, L-2 and L-3 stage larvae. The dissections of *Cx. quinquefasciatus* females are required to be

done for detecting the filarial parasites and to calculate the infection rate and infectivity rate in different months and seasons. Therefore, head, thorax and abdomen regions of the mosquito body were dissected and examined to detect the filarial parasites by the technique followed by earlier workers (Ramaiah et al. 1992; Kanhekar et al.1994).

Dissection: Anaesthetized *Cx. quinquefasciatus* females were trimmed by removing the wings and legs and placed on the glass slides. The body of mosquito was cut into head, thorax and abdomen. Three drops of normal saline (0.68%) at a distance from each other were put on the glass slide. Head, thorax and abdomen parts of mosquito were placed on each drop of saline. Each part was gently teased with the fine dissecting needles and examined under low power lens of compound microscope. The stage of filarial parasite, their number and the region of the mosquito body were recorded. While dissecting the abdomen, the malphigian tubules were taken out of the abdomen and examined separately under low power lens for any filarial larvae of animal origin.

Data analysis: The number of mosquitoes dissected and found with L1, L2 and L3 larvae, number of larvae per infected mosquito and the regions of the body where the parasites were detected in different months were noted. The infection rate, infectivity rate and estimated number of infected mosquitoes biting/person/month for different months were calculated as follows.

Infection Rate: This is the percentage of *Cx. quinquefasciatus* females found with mf, L-1, L-2 and L-3 stage of filarial parasites. The infection rate was calculated as follows.

$$\text{Infection Rate} = \frac{\text{Number of Mosquitoes with mf + L-1 + L-2 + L-3 larvae}}{\text{Number of total Mosquitoes dissected}} \times 100.$$

Infectivity Rate: This is the percentage of *Cx. quinquefasciatus* females found with only L-3 stage of filarial parasites. The infectivity rate was calculated as follows.

$$\text{Infectivity Rate} = \frac{\text{Number of Mosquitoes with L-3 Larvae}}{\text{Number of total Mosquitoes dissected}} \times 100.$$

Estimation of number of Infected/Infective biting: The estimation of number of infected/infective *Cx. quinquefasciatus* biting /person /month was done based on the mean landing rate/bait/night and the infection rate/infectivity rate of the corresponding month found in the present study. The estimated number was calculated as follows.

$$\text{Average man- landing rate/bait/night/month} \times \frac{\text{Infection rate or Infectivity rate}}{100}$$

Susceptibility status of *Cx. quinquefasciatus* Larvae and Adults to Insecticides

Adult females of *Cx. quinquefasciatus* were collected using sucking tube and battery operated torch. Adequate care was taken not to damage the mosquitoes while collecting, transferring to cages and conducting the tests. Fed females of *Cx. quinquefasciatus* were used for adult susceptibility tests. Semi-gravid and gravid

females were introduced inside the mosquito cages (2 ft x 2 ft x 2 ft.) for oviposition. The larvae hatched out from the eggs laid by the wild *Cx. quinquefasciatus* females were reared to late III / early IV stage larvae in the insectary and used for larval susceptibility tests. Both larvae and adult females of *Cx. quinquefasciatus* were exposed to the insecticides which are being used/approved for vector control to evaluate their current susceptibility/resistance status to the insecticides.

Larval Susceptibility Test: Larval tests were carried out using late III and early IV stage larvae of *Cx. quinquefasciatus* against four different concentrations of fenitrothion, malathion, fenthion and temephos as per WHO technique (WHO, 1981a). These tests were conducted between August, 2006 and February, 2007. The ambient mean temperature ranged from 26.6°C to 29.1°C and mean relative humidity ranged from 68.5% to 90.0% during the study period.

Procedure: Test concentrations were prepared by adding the required quantity of standard solution of each insecticide to the tap water free from chlorine, in 500 ml capacity glass beakers and making it equal to total 250 ml of test solution. The most diluted concentration was prepared first followed by the subsequent higher concentrations. After adding the insecticide, the water was stirred vigorously for 30 seconds. For control sets, required quantity of ethanol was added to make it to 250 ml of water.

Within 15-20 minutes of the preparation of the test concentrations, the larvae were introduced in the beakers. The experiment against each test concentration of the insecticide was conducted with four replicates of both experiment and control. 25 larvae

were used in each replicate and exposed to the insecticide for 24 hours. After every test, used articles were soaked in soap water, thoroughly cleaned and dried for further use. No larva turned to pupa during the exposure period. After the exposure period, the moribund and dead larvae were counted. The tests with a control mortality of 20% and above were discarded and fresh tests were done.

Adult Susceptibility Test: Adult tests were carried out with blood fed *Cx. quinquefasciatus* females against diagnostic concentrations – DDT 4%, malathion 5%, permethrin 0.25% and deltamethrin 0.025%, using WHO adult test kit and as per WHO technique (WHO, 1981b). These tests were conducted between January and March, 2007 inside the building free from insecticidal contamination and extremes of temperature, humidity, illumination and wind. The ambient mean temperature ranged from 26.6°C to 28.1°C and mean relative humidity from 72% to 75% during the study period.

Procedure: The plastic tubes with green dot were used for holding mosquitoes and for the control sets of exposure. Tubes with red dot were used for the exposure of mosquitoes to insecticides. The holding tubes were lined from inside with a plain white paper, fastened with a steel clip. Then, the holding tubes were fixed to the slide by threading into screw cap. To carry out the test, the green dot tubes were lined from inside with insecticide control papers and duly fastened with steel clips and the red dot tubes were lined with insecticide impregnated papers of the diagnostic doses and fastened with copper clip.

Prior to exposure to insecticides, the mosquitoes were introduced into the holding tubes through a small orifice in the slide and closed. Mosquitoes were kept in

the holding tubes for 30 minutes and provided with the glucose source in a cotton swab. After the holding period, injured or dead mosquitoes if any, were removed. The green dot tubes with insecticide control paper and the red dot tubes with insecticide impregnated paper were screwed to the respective holding tubes and the mosquitoes were gently transferred through big orifice into the control and test tubes. Mosquitoes were exposed for the specified period to each insecticide. During the exposure period, glucose source was removed. The tests against each insecticide were conducted with five replicates each for experiment and control. In each replicate, 20 fed females of *Cx. quinquefasciatus* were exposed. After the exposure period, mosquitoes were gently transferred back to the holding tubes, provided with glucose source and kept for 24 hours of post exposure holding period. After 24 hours of holding period, the mortality in the mosquitoes both in experiment and control tubes were observed and recorded. Any test with control mortality of 20% and above were rejected and fresh tests were done.

Data analysis: The number of dead and moribund larvae were noted after 24 hours of exposure period in the larval tests and the number of dead mosquitoes were noted after the 24 hours of holding period in the adult tests. In case of control mortality between 5% and 20% both in larval and adult tests, the percentage of test mortality was corrected by applying Abbott's formula.

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

LC₅₀ (the concentration which can kill 50% of the larvae exposed) and LC₉₀ (the concentration which can kill 90% of the larvae exposed) were determined from the

regression line. The regression equations for the tests with all four insecticides were constructed. For adult mosquitoes, as per the WHO criteria, the susceptibility status to the diagnostic dosages of insecticides was categorized as follows : 98 to 100% mortality – susceptible; 81 to 97% - verification required and <80% mortality - resistant to the insecticides.

Chapter IV: RESULTS

4.1 Breeding in different habitats and seasonal distribution

Mosquitoes need water sources for oviposition. Breeding behavior of different mosquito species differ. *Culex quinquefasciatus* is an ubiquitous mosquito. Although, it prefers to breed in habitats having organically rich matter, its breeding habitat range also covers a clean fresh water habitats as well. The type and size of these breeding habitats are highly variable. The potential mosquito breeding sites checked to detect the egg rafts, larvae and pupae included drains, cesspits, cesspools, water storage tanks comprising cement tanks, plastic tanks and underground sumps, curing water collections, stagnant water collections and flower beds inside the buildings under construction, wells, plastic/iron barrels, vases, tyres, discarded utensils, buckets, bottles, coconut shells etc. A total of 10363 potential mosquito breeding sites from six zones of Panaji were surveyed between June, 2007 and May, 2008 covering pre-monsoon, monsoon and post-monsoon periods.

The samples of immatures of mosquito were collected, brought to the laboratory and reared to the adults for species identification and to calculate the breeding index (container index) and per-dip density of *Cx. quinquefasciatus* for different habitats.

Breeding of *Culex*, *Anopheles* and *Aedes* Mosquitoes.

Mosquito species belonging to three genera- *Culex*, *Anopheles*, and *Aedes* were found breeding in Panaji. Out of total 10363 potential breeding sites checked, *Culex* were found breeding in 574, *Anopheles* in 315, and *Aedes* in 258 habitats (Table 5). Total breeding index of *Culex*, *Anopheles* and *Aedes* mosquitoes was 5.53 percent, 3.04 percent and 2.49 percent respectively (Fig. 8).

Table 5: Breeding of *Culex*, *Anopheles* and *Aedes* Mosquitoes encountered in different Habitats in Panaji , Goa

Sr. No.	Type of Breeding Habitat	Number checked	Number breeding			Breeding Index		
			<i>Culex</i>	<i>Anopheles</i>	<i>Aedes</i>	<i>Culex</i>	<i>Anopheles</i>	<i>Aedes</i>
1	Drain	2215	302	17	11	13.63	0.76	0.49
2	Cess pit	784	86	23	00	10.97	2.93	0.0
3	Cess pool	430	29	11	00	6.74	2.56	0.0
4	Water Storage Tanks	566	23	32	38	4.06	5.65	6.71
5	Curing water collections	4089	73	147	109	1.81	3.60	2.67
6	Well	473	19	26	12	4.02	5.50	2.54
7	Barrels	621	15	21	32	2.42	3.38	5.15
8	Miscellaneous	1185	27	38	56	2.19	3.21	4.73
	Total	10363	574	315	258	5.53	3.04	2.49

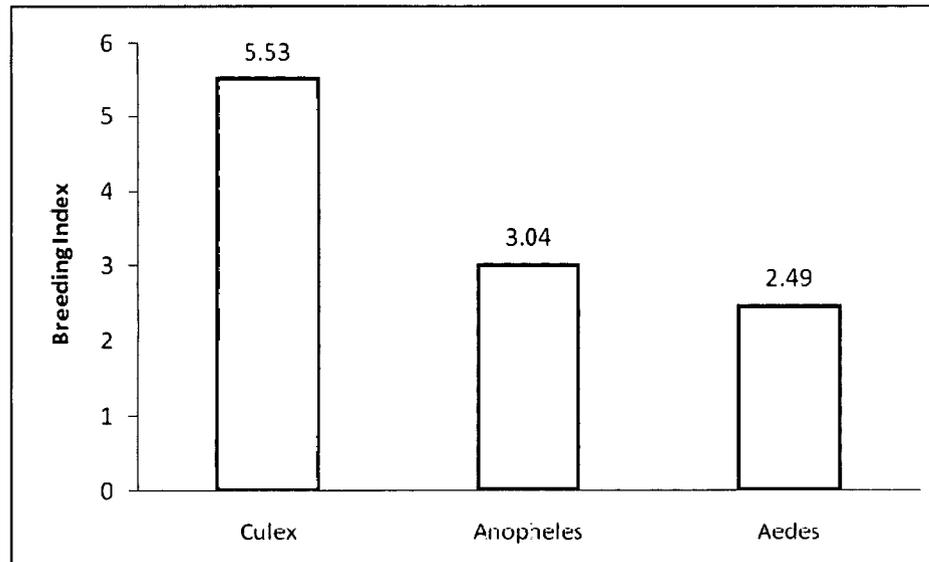
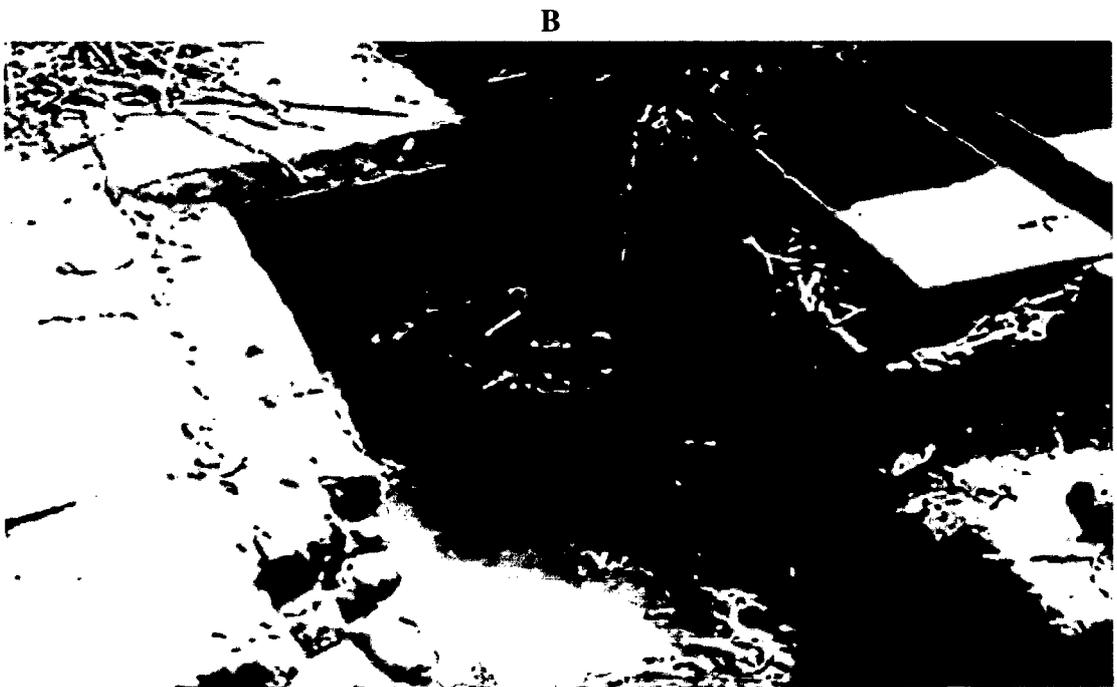
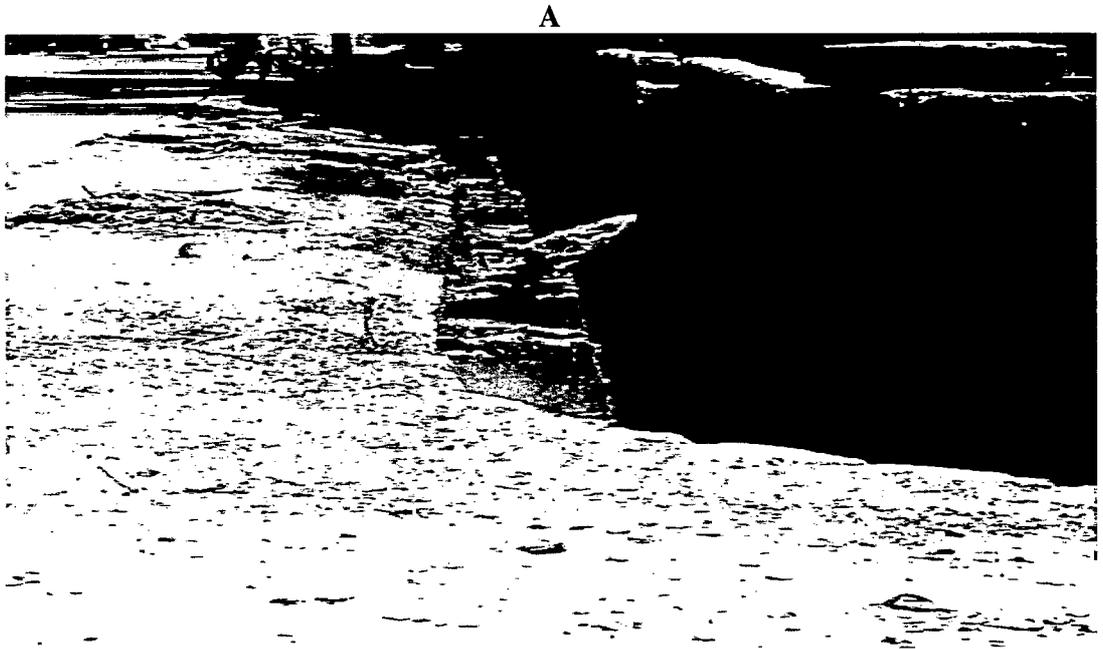


Fig. 8: Breeding Index of *Culex*, *Anopheles* and *Aedes* mosquitoes in Panaji, Goa.



**Fig. 9 (A&B): Polluted Drains- Breeding sites
of *Cx. quinquefasciatus*.**

A



B



Fig. 10 (A&B) : Polluted Nullah & Cesspit - Breeding sites

A

A



B

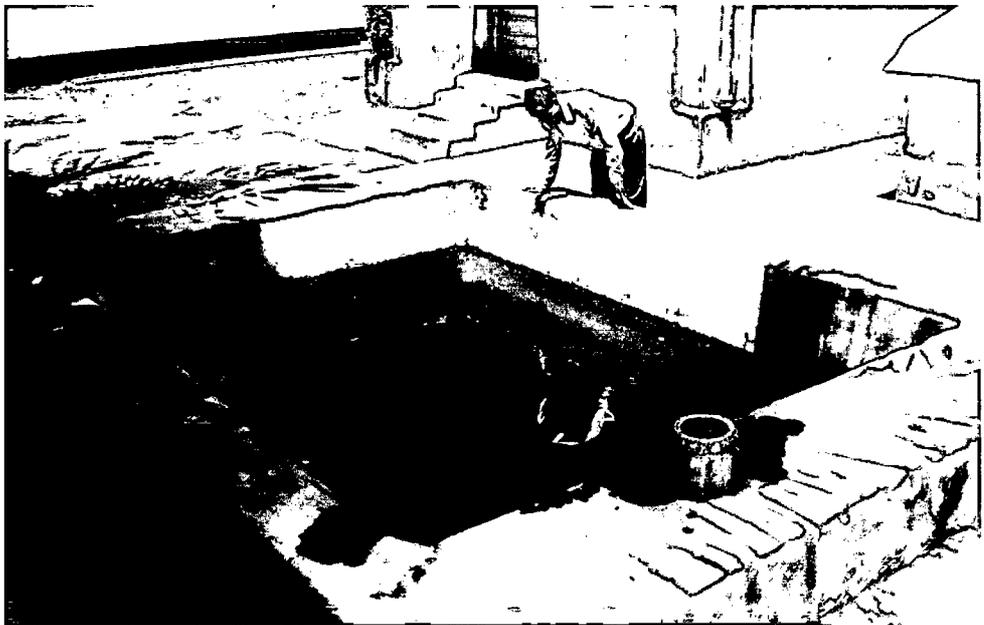


Fig. 11 (A&B): Curing water inside the Building under construction & Cement Tank- Breeding sites

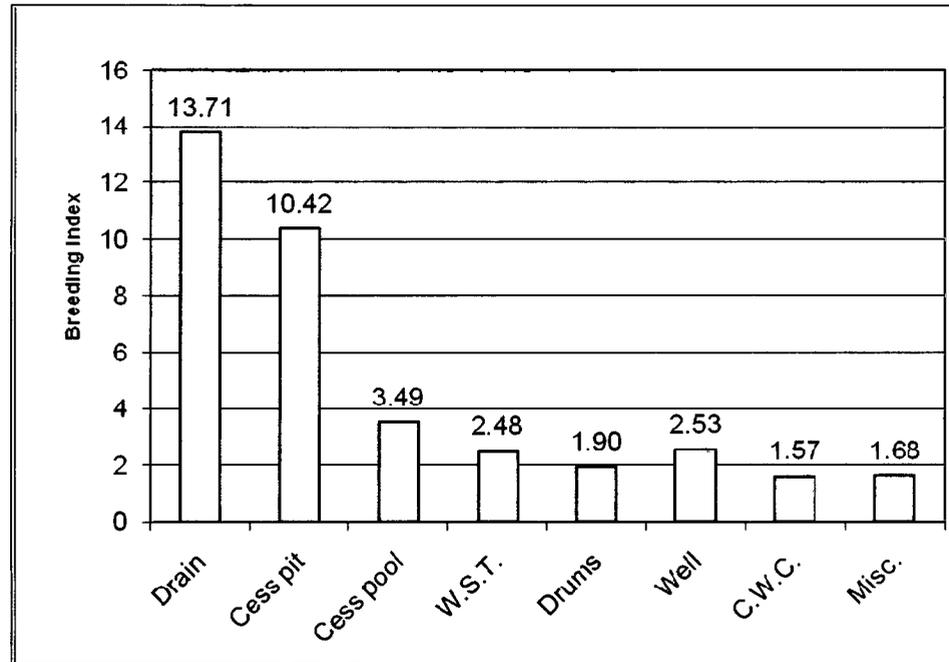
The habitat-wise highest breeding index of *Culex* mosquitoes was found in drains (13.71%) and the lowest in curing water collections (1.81%). The highest breeding index of *Anopheles* mosquitoes was found in water storage tanks (5.65%) and the lowest in drains (0.76%). The highest breeding index of *Aedes* mosquitoes was also found in water storage tanks (6.71%) and the lowest in the drains (0.49%). The cesspits and cesspools were totally negative for *Aedes* breeding. *Anopheles* and *Aedes* breeding was found more in clean and fresh water sources while *Culex* breeding was found more in the breeding places which were rich in organic matter.

Breeding of *Culex quinquefasciatus* in Different Habitats.

Among different mosquito species encountered in the present study, *Culex quinquefasciatus* was the most predominant species and found breeding in different habitats with both polluted and clean water sources. The results of detection of *Cx. quinquefasciatus* breeding in different habitats is presented in Table 6. Out of total 10363 sites checked, *Cx. quinquefasciatus* was found breeding in 522 (5.05%) habitats. The most preferred breeding sites for *Cx. quinquefasciatus* were found to be the drains followed by the cesspits and they were found to be statistically significant (Table 7 & 8). Out of 2215 drains and 784 cesspits checked, 302 drains (13.71 ± 7.10) and 81 cesspits (10.42 ± 4.50) respectively were found with *Cx. quinquefasciatus* breeding (Fig. 12). Also, 3.49% of cesspools, 2.53% of wells, 2.47% of water storage tanks, 1.93% of curing water collections, 1.57% of barrels and 1.68% of miscellaneous habitats were found with *Cx. quinquefasciatus* breeding. Of the total 522 different habitats found with *Cx. quinquefasciatus* breeding, 57.85% were contributed by drains and 15.46% by cesspit (Fig. 13).

Table 6: Number of various Breeding sites checked, found with breeding, Breeding index and Per dip density of *Cx. quinquefasciatus* in different Habitats in Panaji, Goa

Sr. No.	Type of Breeding Habitat	Number Checked	Number breeding	Breeding Index with SD	Per dip Density (Range)	
					Larva	Pupa
1	Drain	2215	302	13.71 ± 7.10	21 - 144.5	5 - 22.5
2	Cesspit	784	81	10.42 ± 4.50	12 - 30.5	3 - 11
3	Cesspool	430	15	3.49 ± 3.11	5 - 14	2 - 5
4	Water Storage Tank	566	14	2.48 ± 0.28	2 - 9.5	1 - 3
5	Curing water collection	4089	67	1.57 ± 0.49	2 - 8	1 - 4
6	Well	473	12	2.53 ± 0.61	3 - 7.5	1 - 2
7	Barrel (Iron/Plastic)	621	12	1.59 ± 0.67	5.5 - 12	2 - 5
8	Miscellaneous	1185	19	1.68 ± 0.86	3 - 5	2 - 3
-	Total	10363	522	4.84±5.17	6.7 - 28.9	2.1 - 6.9



Note: W.S.T.-water storage tank, C.W.C.-curing water collection,
Misc.-miscellaneous.

**Fig. 12: Breeding Index of *Cx. quinquefasciatus*
in different Habitats in Panaji, Goa.**

The highest per dip density both for larvae and pupae was encountered in drains followed by cesspits. The highest per dip density of larvae in different habitats ranged between 5.0 to 144.5 and the lowest per dip density ranged between 2.0 to 21.0. The highest per dip density of pupae ranged between 2.0 to 22.5 and the lowest between 1.0 to 5.0 (Table 6). The variations in breeding index between the different habitats were found to be significant ($F=6.2694$; $p<0.05$) (Table 7). The pairwise comparison of breeding habitat with respect to breeding index by scheffes multiple comparison tests procedure further justified the statistical significance of habitatwise variations in breeding of *Cx. quinquefasciatus* (Table 8).

Seasonal Distribution of *Cx. quinquefasciatus* Breeding.

Season-wise detection of breeding of *Cx. quinquefasciatus* in different habitats is presented in Table 9. Seasonally, there were variations in the breeding indices of *Cx. quinquefasciatus* in different habitats (Fig.14). The breeding index in drains which was 19.05% during pre-monsoon and 16.48% in post-monsoon, had dropped down to 5.65% in monsoon. Likewise, the breeding index in cesspits which was 14.35% in pre-monsoon and 11.40% in post-monsoon had dropped down to 5.52% in monsoon. Similar reduction was observed in cesspools also. The breeding index in other habitats seasonally varied between 1.74% and 7.81% in cesspools, 2.19% and 2.75% in water storage tanks, 1.08% and 2.33% in curing water collections, 1.95% and 3.16% in wells, 1.42% and 2.39% in barrels and 0.88% and 2.59% in miscellaneous habitats.

Table 7: Comparison for different breeding habitats with respect to Breeding Index by Analysis of variances.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Between breeding habitat	7	450.79	64.3987	6.2694	0.0012*
Within breeding habitat	16	164.35	10.2719		
Total	23	615.14			

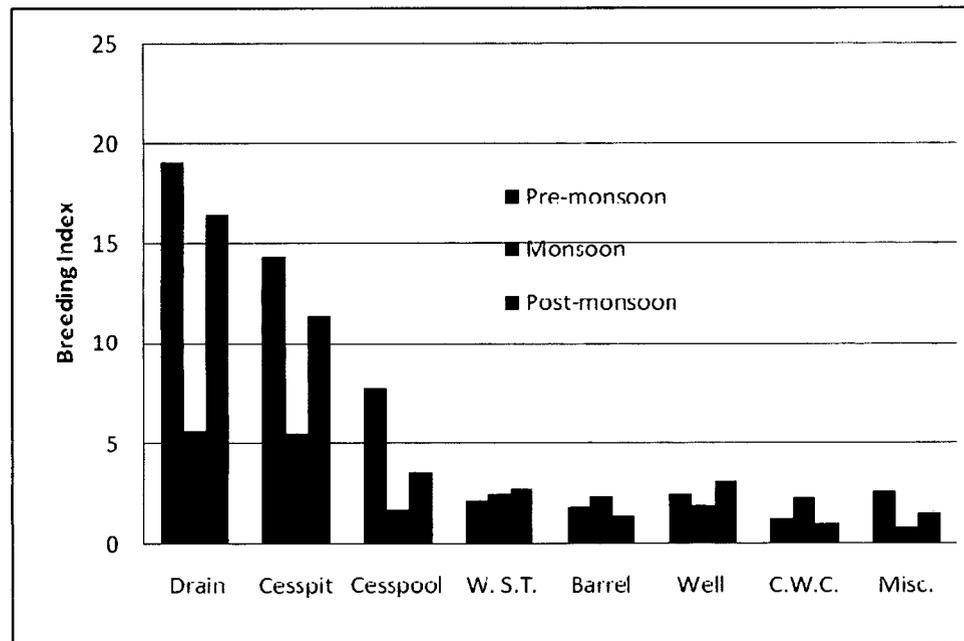
*Note: Significant at 5% level of significance ($p < 0.05$)

Table 8: Pairwise comparison of breeding habitat with respect to breeding index by scheffes multiple comparison tests procedure.

Habitat	Drain	Cesspit	Cesspool	W. S.T.	Barrel	Well	C. W.C.	Misc.
Means	13.7100	10.4230	4.3867	2.4767	1.9033	2.5333	1.5733	1.6767
Drain	-							
Cesspit	P=0.9733	-						
Cesspool	P=0.1534	P=0.6276	-					
W. S.T.	P=0.0516*	P=0.3044	P=0.9990	-				
Barrel	P=0.0365*	P=0.0499*	P=0.9947	P=1.0000	-			
Well	P=0.0534*	P=0.3123	P=0.9992	P=1.0000	P=1.0000	-		
C. W.C.	P=0.0298*	P=0.1965*	P=0.9889	P=1.0000	P=1.0000	P=1.0000	-	
Misc.	P=0.0318*	P=0.0471*	P=0.9911	P=1.0000	P=1.0000	P=1.0000	P=1.0000	-

Note: W.S.T.-water storage tank, C.W.C.-curing water collection,

Misc.- Miscellaneous.*Significant at 5% level of significance ($p < 0.05$)



Note: W.S.T.-water storage tank, C.W.C.-curing water collection, Misc.-Miscellaneous.

Fig.14: Seasonal variations in Habitatwise breeding index of *Cx. quinquefasciatus* in Panaji, Goa.

Table 9 : Seasonal Variations in Breeding Index of *Cx. quinquefasciatus* in different Habitats in Panaji, Goa.

Type of Breeding Habitat	Premonsoon			Monsoon			Postmonsoon		
	Number Checked	Number Positive	Breeding Index	Number Checked	Number Positive	Breeding Index	Number Checked	Number Positive	Breeding Index
Drain	714	136	19.05	726	41	5.65	785	129	16.48
Cesspit	223	32	14.35	254	14	5.52	271	37	11.40
Cesspool	64	5	7.81	172	3	1.74	194	7	3.61
Water Storage Tank	183	4	2.19	201	5	2.49	182	5	2.75
Barrel	158	3	1.90	251	6	2.39	212	3	1.42
Well	161	4	2.49	154	3	1.95	158	5	3.16
Curing water coll.	1374	18	1.31	1417	33	2.33	1298	14	1.08
Miscellaneous	347	9	2.59	453	4	0.88	421	4	1.56
Total	3224	211	6.54	3626	107	2.95	3513	204	5.79

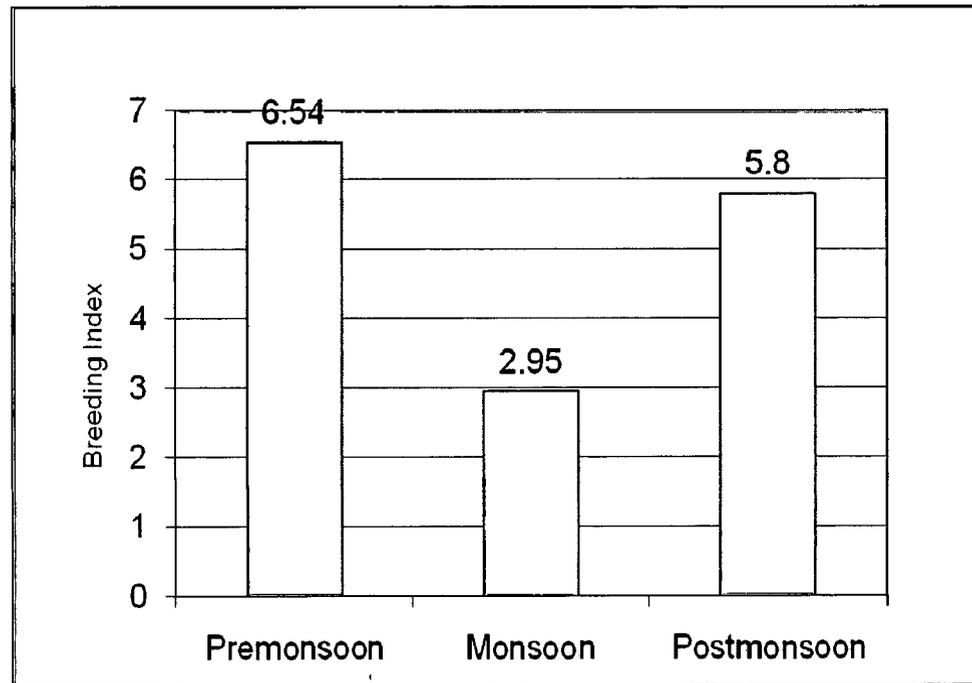


Fig. 15: Seasonal Distribution of *Cx. quinquefasciatus* breeding in Panaji, Goa.

Some tilt in the breeding behavior of *Cx. quinquefasciatus* towards the indoor breeding foci like curing water collections and other clean and fresh water breeding sources was observed during the monsoon season as the vector faced difficulty in outdoor breeding foci due to rainfall.

During pre-monsoon and post-monsoon periods, the drains followed by cesspits were the most important breeding sites for *Cx. quinquefasciatus*. Seasonally, highest breeding index of 6.54% was found in pre-monsoon followed by 5.8% in post-monsoon period. The lowest breeding index of 2.95% was found in monsoon (Fig. 15). The flushing and over flowing of outdoor breeding foci due to heavy rains, particularly the drains and cesspits during rainy season had resulted in the reduction of breeding index during monsoon period.

4.2 Life Table of *Cx. quinquefasciatus* - Development from Egg to Adult

The life cycle of mosquito comprises of 4 distinct stages viz., egg, larva, pupa and adult. A female mosquito needs blood meal for the development and maturation of eggs inside the ovary. Blood fed females after the maturation of the eggs, search for suitable water source to lay the eggs. The first stage of larva hatches out from the egg and grows from first to fourth stage larva after 3 moults and further to pupa. Finally, adult female and male mosquitoes emerge out from the pupae. The number of eggs laid by each female, survival/mortality rate at different stages of development and net adult emergence are variable. The duration required to complete the development from egg laying to adult emergence may also be variable depending upon the temperature. The results of the study on development from egg laying to adult emergence of *Cx. quinquefasciatus* strain from Panaji are presented here.

Number of Egg rafts from *Cx. quinquefasciatus*.

Thirty eight females of *Cx. quinquefasciatus* laid eggs in 38 egg rafts and the study on development was continued with these egg rafts. The number of eggs laid by each individual female ranged between 50 and 218 eggs (120.11 ± 43.90) (Table 10). Out of 38 egg rafts, the larvae were hatched out from 27 egg rafts (71.05%). The eggs from remaining 11 egg rafts (28.94%) did not hatch to larvae until three weeks from the date of egg laying and later, these egg rafts degenerated.

Table 10: Number of eggs laid per raft, larvae hatched and percent of eggs hatched to larvae from 38 *Cx. quinquefasciatus* females.

Egg Raft	No. of eggs laid	No. of larvae hatched out	% of eggs hatched
1.	196	196	100.0
2.	198	198	100.0
3.	210	210	100.0
4.	85	0	0.0
5.	188	188	100.0
6.	218	192	88.07
7.	188	188	100.0
8.	136	136	100.0
9.	127	5	3.93
10.	132	123	93.18
11.	114	8	7.01
12.	112	0	0.0
13.	104	75	72.11
14.	146	94	64.38
15.	147	0	0.0
16.	88	72	81.81
17.	73	0	0.0
18.	144	144	100.0
19.	184	184	100.0
20.	102	89	87.25
21.	102	0	0.0
22.	94	78	82.97
23.	76	76	100.0
24.	94	94	100.0
25.	128	119	92.96
26.	84	72	85.71
27.	66	66	100.0
28.	102	102	100.0
29.	80	45	56.25
30.	87	7	8.04
31.	98	0	0.0
32.	139	121	87.05
33.	98	0	0.0
34.	99	0	0.0
35.	112	97	86.60
36.	50	0	0.0
37.	71	0	0.0
38.	92	0	0.0
X±SD	120.11±43.90	78.39±72.14	57.82±44.80

All larvae hatched out from 4 egg rafts (10.52%) died in I to III stage larval development. The mean number of eggs laid by 38 females and percent of eggs hatched to first stage larvae were: 78.39 ± 72.14 and 57.82 ± 44.80 respectively. As the development progressed, a proportion of larvae hatched out from 23 egg rafts survived up to adult emergence. The developmental study on *Cx. quinquefasciatus* from egg laying up to adult emergence was continued with 23 egg rafts.

Survival of Eggs, Larvae and Pupae of *Cx. quinquefasciatus*.

The number of i) eggs laid, ii) I - stage larvae hatched out, iii) I – stage larvae developed to II stage, III stage and IV stage larvae, iv) pupae formed and v) adults emerged from each egg raft of 23 females are presented in Table 11. The mean number of eggs laid, hatched to first stage larvae, grown to II to IV stage larvae, pupae formed and the adults emerged were: 131.26 ± 47.21 , 109.04 ± 64.13 , 104.57 ± 62.59 , 95.87 ± 59.36 , 90.00 ± 58.58 , 80.83 ± 58.10 and 77.35 ± 53.89 respectively.

The total number (%) of eggs laid by 23 females, the total number survived at I, II, III, IV stage larvae, pupae and adults emerged is presented in Fig. 16. Out of total 3018 eggs laid, only 2507 eggs (83.1%) hatched to first stage larvae, 2404 larvae (79.7%) were grown to II stage, 2205 larvae (73.1%) to III stage, 2070 larvae (68.6%) to IV stage, 1859 (61.6%) to pupae and 1779 (58.9%) emerged to adults.

The number of female and male *Cx. quinquefasciatus* emerged and percent of eggs emerged to female and males from 23 rafts are given in Table 12.

Table 11 : Number of eggs laid per raft, larvae (I-IV), Pupae and Adults emerged from 23 *Cx. quinquefasciatus* females.

Egg raft No.	Eggs laid	I- Larva	II- Larva	III- Larva	IV- Larva	Pupa	Adult
1.	196	196	196	158	155	155	155
2.	199	199	199	156	156	156	146
3.	210	210	198	198	190	190	171
4.	188	188	184	182	182	176	159
5.	218	192	166	155	155	48	48
6.	188	188	185	182	156	156	146
7.	136	136	132	131	128	121	117
8.	127	5	3	3	3	3	3
9.	132	123	112	108	96	84	84
10.	114	8	8	8	7	7	7
11.	104	75	75	73	73	52	52
12.	146	94	81	74	72	72	72
13.	88	72	70	62	55	51	45
14.	144	144	144	144	142	142	136
15.	76	76	76	76	65	50	50
16.	94	94	94	94	76	65	65
17.	128	119	118	118	118	118	114
18.	84	72	65	52	39	38	38
19.	66	66	66	34	30	28	28
20.	102	102	102	79	68	51	49
21.	80	45	33	33	29	28	26
22.	87	7	7	7	7	7	7
23.	112	97	91	78	68	61	61
X±SD	131.26±47.21	109.04±64.13	104.57±62.59	95.87±59.36	90.00±58.58	80.83±58.10	77.35±53.89

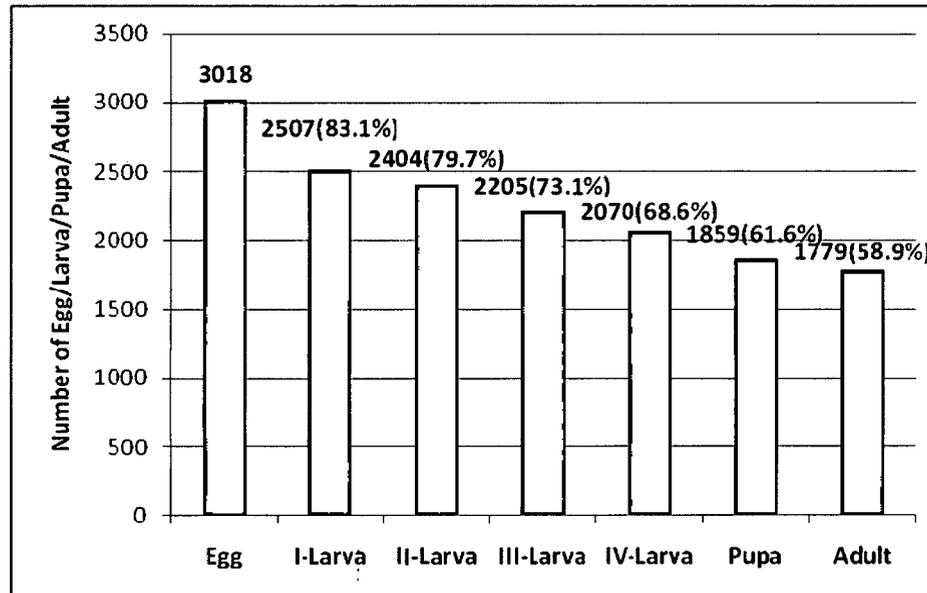


Fig.16 : Number of eggs laid, I-stage larvae hatched, II-IV stage larvae survived, pupae formed and adults emerged from 23 females.

**Table 12 : Proportion (%) of Eggs developed to Adult
Male and Female *Cx. quinquefasciatus***

Egg raft No.	No. of Adults emerged			Percent of Eggs emerged to		
	Male	Female	Total	Male	Female	Total
1.	80	75	155	40.81	38.26	79.08
2.	72	74	146	36.18	37.18	73.36
3.	89	73	162	44.28	37.14	81.42
4.	72	80	152	40.42	44.14	84.57
5.	21	27	48	09.63	12.38	22.01
6.	74	72	146	39.57	38.50	78.07
7.	59	62	121	41.60	43.79	85.40
8.	2	1	3	01.57	0.78	02.36
9.	39	45	84	29.54	34.09	63.63
10.	4	3	7	03.50	02.63	06.14
11.	27	25	52	25.96	24.04	50.00
12.	36	36	72	24.48	24.48	48.97
13.	24	21	45	27.58	24.13	51.72
14.	73	69	142	48.61	45.83	94.44
15.	26	24	50	33.76	31.16	64.93
16.	35	30	65	37.23	31.91	69.14
17.	58	60	118	43.75	45.31	89.06
18.	20	18	38	24.09	21.68	45.78
19.	14	14	28	21.21	21.21	42.42
20.	26	25	51	24.50	23.52	48.03
21.	12	14	26	15.18	17.72	32.91
22.	6	1	7	06.89	01.14	08.04
23.	32	29	61	28.57	25.89	54.46
X±SD	39.2±26.9	38.2±26.5	77.4±53.3	29.9±13.7	29.1±13.8	58.9±27.3

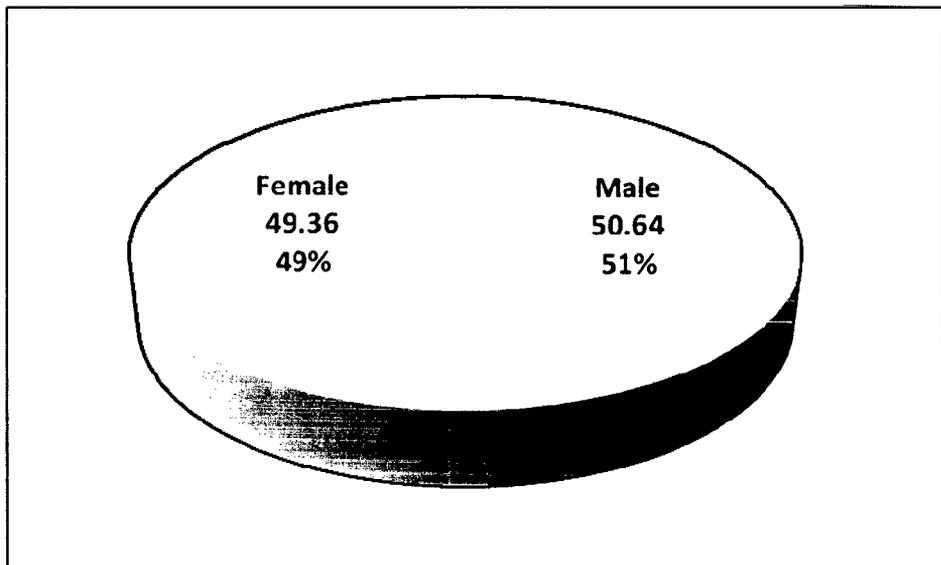


Fig. 17: Percent contributed by Female and Male to total emergence of adults from 23 Females.

Total number of females and males emerged was 878 (49.36%) and 901 (50.64%) (Fig. 17) respectively. The proportion of female emergence ranged between 0.78% and 45.83% and male emergence between 1.57% and 48.61%. The mean percent of eggs emerged to females and males was 29.1 ± 13.8 and 29.9 ± 13.7 respectively. The mean number of female to male ratio was found to be 1:1.03.

Percent mortality at different Developmental stages

The percent mortality observed at each stage of development from egg to adult emergence for 23 egg rafts is given in Table 13. The percent mortality at egg level ranged between 6.81% to 96.06% and in 11 out of 23 egg rafts, there was no mortality at egg level and all the eggs hatched to larvae. At I- stage larval development (I instar), the mortality varied between 0.78% to 15% and in 10 egg rafts there was no mortality. At II- stage larval development (II instar), the mortality ranged between 0.73% and 48.48% in 14 egg rafts and there was no mortality in the remaining 9 egg rafts. At III- stage larval development (III instar), the mortality ranged between 0.87% and 19.14% and there was no mortality in 7 egg rafts. At IV stage larval development (IV instar), the mortality varied between 1.19% and 49.08% and in 10 egg rafts there was no mortality. At pupal stage, the mortality varied between 1.96% and 9.04% and in 13 egg rafts there was no mortality.

In the development of number of eggs laid by 23 females, the average mortality rate observed at different stages of development was : 16.99 ± 31.66 at egg stage, 3.25 ± 4.40 at I stage larvae, 7.21 ± 11.69 at II stage larvae, 5.30 ± 5.99 at III stage larvae, 6.57 ± 11.32 at IV stage larvae and 2.17 ± 3.02 at pupal stage (Table 13).

Table 13: Percent mortality per raft at different stages of development of *Cx. quinquefasciatus*

Egg raft No.	No. of eggs laid	Egg stage	I-stage Larva	II-stage Larva	III-stage Larva	IV-stage Larva	Pupa
1.	196	0.0	0.0	19.38	1.53	0.0	0.0
2.	199	0.0	0.0	21.21	0.0	0.0	5.05
3.	210	0.0	5.71	0.0	3.80	0.0	9.04
4.	188	0.0	2.12	1.06	0.0	3.19	9.04
5.	218	11.92	11.92	5.04	0.0	49.08	0.0
6.	188	0.0	1.59	1.59	13.82	0.0	5.31
7.	136	0.0	2.94	0.73	2.20	5.14	2.94
8.	127	96.06	1.57	0.0	0.0	0.0	0.0
9.	132	6.81	8.33	3.03	9.09	9.09	0.0
10.	114	92.98	0.0	0.0	0.87	0.0	0.0
11.	104	27.88	0.0	1.92	0.0	20.19	0.0
12.	146	35.61	8.90	4.79	1.36	0.0	0.0
13.	88	18.18	2.27	9.09	7.95	4.54	6.81
14.	144	0.0	0.0	0.0	1.38	0.0	4.16
15.	76	0.0	0.0	0.0	14.47	19.73	0.0
16.	94	0.0	0.0	0.0	19.14	11.70	0.0
17.	128	7.03	0.78	0.0	0.0	0.0	3.12
18.	84	14.28	8.33	15.47	15.47	1.19	0.0
19.	66	0.0	0.0	48.48	6.06	3.03	0.0
20.	102	0.0	0.0	22.54	10.78	16.66	1.96
21.	80	43.75	15.0	0.0	5.0	1.25	2.5
22.	87	91.95	0.0	0.0	0.0	0.0	0.0
23.	112	13.39	5.35	11.60	8.92	6.25	0.0
X±SD	131.3±47.2	17.0±31.7	3.3±4.4	7.2±11.7	5.3±6.0	6.6±11.3	2.2±3.0

The total mortality from egg to adult emergence observed in the course of development of eggs laid by 23 females was 41.1%, resulting in 58.9% of eggs developing to the adults.

Net Emergence of Adult *Cx. quinquefasciatus*.

In the present study, the total number of eggs laid by 38 females of *Cx. quinquefasciatus* was 4564, out of these eggs, the total number of larvae hatched out was 2985 (65.4%), pupae formed was 1859 (40.7%) and finally the adults emerged was 1779 (38.9%) (Fig.18). The proportions of net emergence of females (19.23%) and males (19.74%) was almost equal. As observed in this study, all the eggs laid by 11 females did not hatch to larvae, may be due to non-viable eggs or the mortality at the egg stage. The total mortalities observed at egg, larval and pupal stages were 34.59%, 24.67% and 1.76% respectively.

Duration of Development from Egg to Adult.

The duration (number of days) taken at each stage of development from egg to adult emergence and the total period for complete development from egg to adult emergence of *Cx. quinquefasciatus* from each batch of eggs laid by 23 females is presented in Table 14.

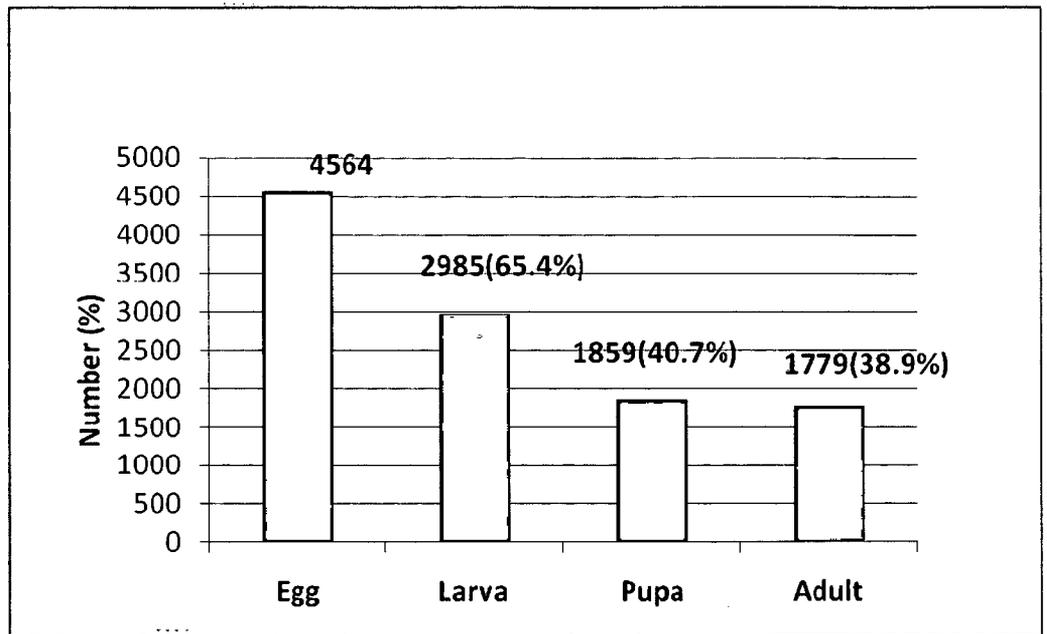


Fig. 18: Percent of eggs hatched to larvae, pupae formed and adults emerged from the eggs laid by 38 females (total number of eggs laid by 38 females).

Table 14 : Duration (days) taken for different stages of development of *Cx. quinquefasciatus*

Egg raft -No.	Egg to I - stage Larva	I to IV stage Larva	IV-stage Larvae to Pupa	Pupal stage to Adult	Total Period Eggs to Adult
1.	2	7	1	1	11
2.	1	8	1-2	1-3	11-14
3.	1	8	1-2	1-2	11-13
4.	2	8	1	1-2	12-13
5.	2	8	1-2	1	12-13
6.	1	6	1-2	1	9-10
7.	2	7	1	1	11
8.	2	7	1	1	11
9.	2	7	1-2	1	11-12
10.	2	8	1	1	12
11.	4	5	1-2	1-2	11-13
12.	3	6	1-2	1-2	11-13
13.	2	6	2	1	11
14.	1	6	1-2	1	9-10
15.	2	6-7	2	1-2	11-13
16.	2	7	1-2	1	11-12
17.	1	8	1-2	1	11-12
18.	1	8	1-2	1	11-12
19.	2	7	1-2	1-2	11-13
20.	2	7	1-2	1-2	11-13
21.	2	6	2	1	11
22.	3	7	1	1	12
23.	3	6	1	1-2	11-12
X±SD	1.96±0.77	6.93±0.98	1.41±0.33	1.22±0.29	-

The observed length of gonotrophic cycle of *Cx. quinquefasciatus* was 3-4 days. The duration taken for hatching of eggs to I stage larvae ranged between 1 and 4 days; I - stage to IV stage larval development between 5 and 8 days; IV - stage larvae to pupal stage between 1 and 2 days and pupal stage to adult emergence between 1 and 3 days. The average duration taken was 1.96 ± 0.77 days for hatching of eggs, 6.93 ± 0.88 days for I to IV- stage larval development, 1.41 ± 0.33 days for IV- stage to pupal stage and 1.22 ± 0.29 days for pupal stage to adult emergence. The total number of days taken for development from egg laying to adult emergence ranged between 9 and 14 days. The larval stage (I-IV) development took the maximum period (57.72%) of the total duration from egg to adult emergence of *Cx. quinquefasciatus*.

4.3 Adult Density, Seasonal Distribution Abdominal conditions of females and Indoor Resting Behavior.

Adult mosquitoes rested on different types of resting sites inside sixty fixed mosquito catching stations from six zones of Panaji, were collected on fortnightly basis during April, 2005 to March 2006. In all, 144 mosquito collections were made by spending 240 man hours. The mean temperature during the study period ranged from 26.7° C to 30.1° C and relative humidity ranged from 65.5% to 90.7%. The total rainfall was 3387.4 mm during the study period. The number of rainy days during different months was: April'05-2, May-1, June-24, July-30, August-24, September-25, October-7 and March,06-2.

Mosquito species collected from Panaji.

A total of 11,673 mosquitoes belonging to sixteen different species were collected. Six species each belonged to genus *Culex* and genus *Anopheles*, two species to genus *Aedes* and one species each to genus *Armigeres* and genus *Mansonia*. *Cx. quinquefasciatus* was the most predominant species among the indoor resting mosquitoes in Panaji. A total of 9571 *Cx. quinquefasciatus* were collected and this species contributed 81.99% to the total number of mosquitoes collected indoor (Fig. 19). Out of 9571, 7018 (73.32%) were females and 2553 (26.67%) were males (Fig. 20).

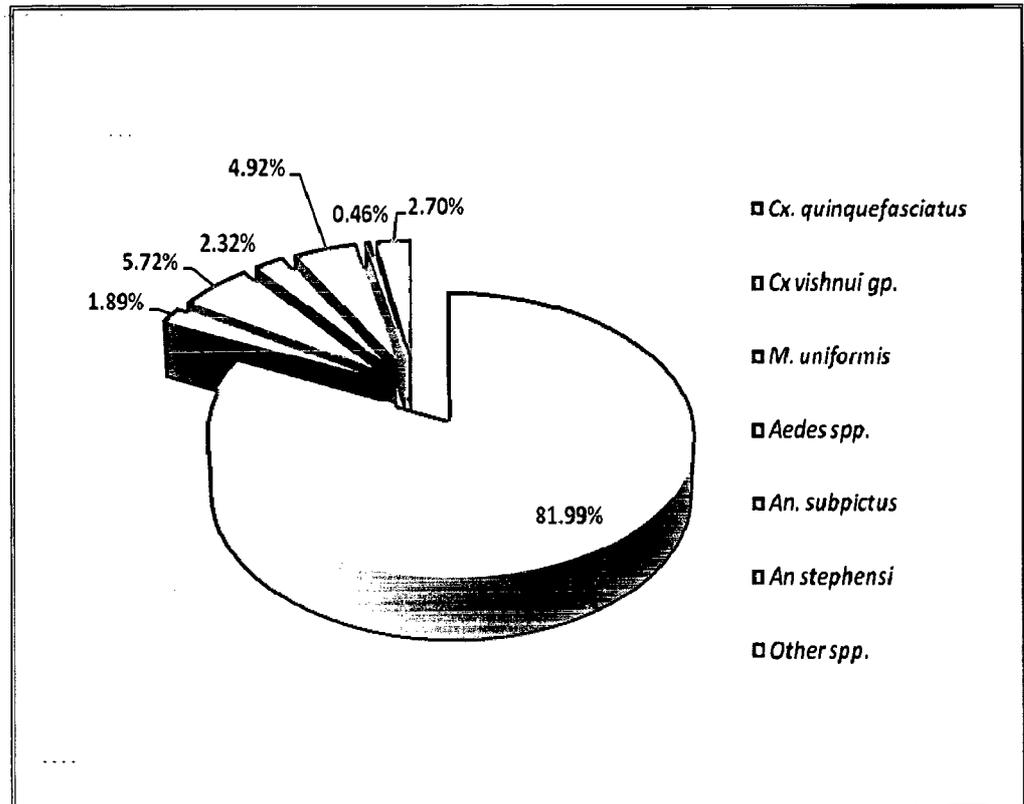


Fig. 19: Percent contributed by *Cx. quinquefasciatus*, vectors of Japanese encephalitis, Malayan filariasis, Dengue/Chikungunya, Malaria and other mosquito species to the total number of mosquitoes collected indoor in Panaji, Goa, during April, 2005 to March, 2006.

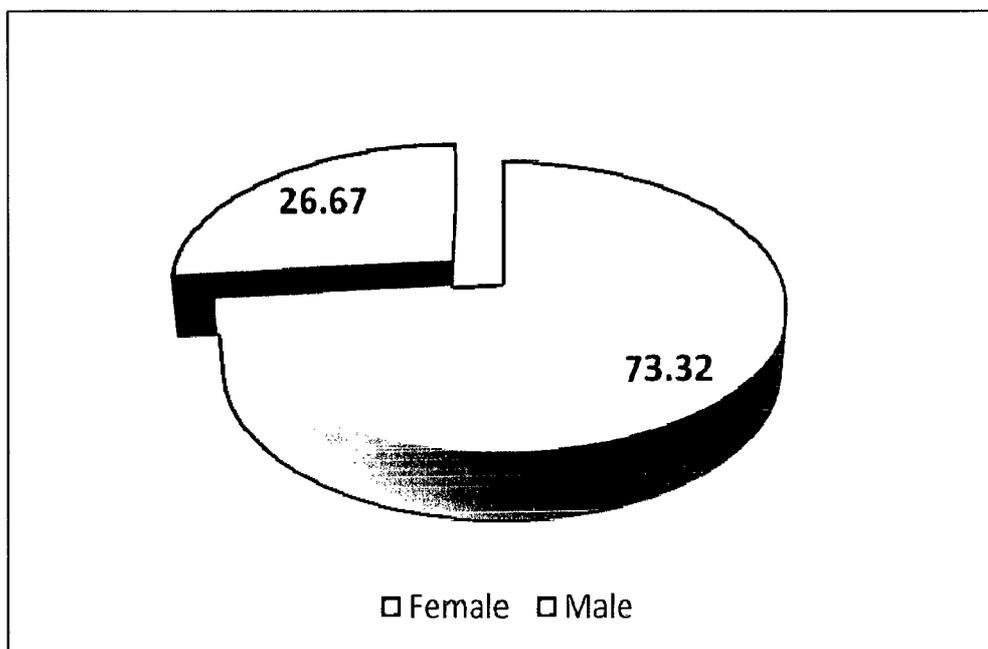


Fig. 20: Percent contribution of Male (n=2553) and Female (n=7018) *Cx. quinquefasciatus* in indoor collection during April, 2005 to March, 2006 in Panaji.

Prevalence of *Cx. quinquefasciatus* in Six Zones of Panaji.

Total number of *Cx. quinquefasciatus* females and males collected from zones I to VI was: 1149 and 572, 1191 and 402, 1016 and 342, 1249 and 509, 1279 and 387, 1134 and 341 respectively. Per man hour density of female and male *Cx. quinquefasciatus* collected from six zones in different months during April, 2005 to March, 2006 is presented in Table 15. All six zones in Panaji had high prevalence of *Cx. quinquefasciatus*. In zones IV and V, comparatively more number of female *Cx. quinquefasciatus* were collected.

Period of peak prevalence of *Cx. quinquefasciatus* females showed some difference in six zones. Zone I had the highest per man hour density (PMHD) of 52.7 during March and the lowest density of 10.5 during September and October months. Zone II had the highest density of 54.9 in December and the lowest density of 8.1 in August. Zone III had the highest density of 51.9 in February and the lowest density of 3.6 in September. Zone IV had the highest density of 56.4 in March and the lowest density of 8.7 in September.

Zone V recorded two almost equal peaks in two different seasons- one in February (54.3) and the other in July (55.3) which dropped down to the lowest density (1.8) in September. Among the six zones, Zone VI recorded highest density (69.1) in July and lowest density (1.2) in September. *Cx. quinquefasciatus* male per man hour density in different months ranged between 4.5 and 28.5, 2.7 and 22.8, 2.1 and 29.1, 1.5 and 34.5, 2.7 and 15.6, 1.8 and 21.8 from zones I to VI respectively.

Table 15 : Per man hour density (PMHD) of Females and Male *Cx.quinquefasciatus* encountered in the fortnightly collection from Zones I to VI in different months in Panaji, Goa during April, 05 to March, 06

Month	Zone I		Zone II		Zone III		Zone IV		Zone V		Zone VI	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
April' 05	16.8	8.1	20.1	06.0	16.8	6.9	20.4	9.3	17.1	6.6	16.5	5.7
May	30.6	12.0	21.3	07.8	20.7	8.7	26.7	12.0	30.6	12.9	24.0	9.3
June	33.9	14.4	23.7	09.3	31.5	9.0	36.6	19.8	33.0	15.6	37.2	13.2
July	26.1	8.4	33.9	10.8	16.8	4.8	38.1	12.6	55.8	12.6	69.0	11.4
August	13.2	4.5	08.1	03.6	05.7	2.7	13.5	5.4	24.6	11.4	26.1	6.0
September	10.5	10.8	14.1	02.7	03.6	2.1	8.7	1.5	1.8	0.0	1.2	0.0
October	10.5	10.8	15.3	12.0	14.1	3.6	9.9	3.3	3.3	2.7	3.3	1.8
November	29.7	28.5	46.8	11.4	32.4	9.3	20.7	11.1	25.8	3.6	24.9	8.4
December	32.7	14.7	54.9	17.4	42.9	8.7	44.7	11.7	44.4	10.2	33.6	9.6
January,06	42.3	21.0	38.1	03.9	23.9	11.1	45.6	15.6	42.9	12.3	27.3	4.8
February	46.2	15.6	43.5	12.9	51.9	6.6	53.4	15.9	54.3	15.3	42.3	21.6
March' 06	52.7	22.8	37.5	22.8	44.7	29.1	56.4	34.5	50.1	12.9	34.8	10.5
Total	28.72	14.3	29.77	10.05	25.4	8.55	31.22	12.72	31.97	9.67	28.35	8.52

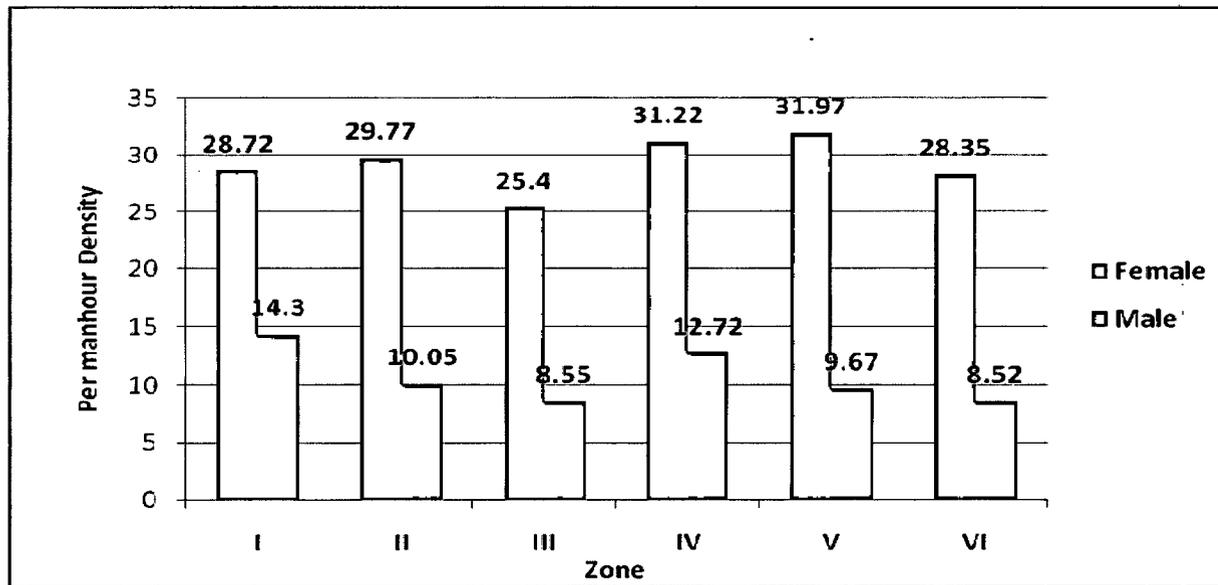


Fig. 21: Average Per man hour density of Female and Male *Culex quinquefasciatus* in six zones of Panaji during April, 2005 to March, 2006.

The average man hour density of both females and males in six zones of Panaji is presented in Fig. 21.

Prevalence of *Cx. quinquefasciatus* in different Months

The data on the collection of *Cx. quinquefasciatus* from all zones was pooled for further analysis. The total number of females and males collected and per man hour densities in different months during April 2005 to March 2006 are presented in Table 16.

Cx. quinquefasciatus was found prevalent in all the months of the study in Panaji. The highest per man hour density of adult females (PMHD = 48.65) was in February and the lowest density (PMHD = 6.65) in September. Two peaks of *Cx. quinquefasciatus* population were observed; first in July and second in February. After the first peak, the population showed declining trend from August and reached the lowest prevalence in September. The next ascending phase of *Cx. quinquefasciatus* population started in October-November hitting the second peak in February, followed by a declining phase in March. (Fig. 22).

Two way ANOVA test revealed that the density variations of females between the months were significant ($F = 15.3$; $p < 0.05$) and the density variations between different zones were not significant (Table 17). Similarly, the density variations of male population between the months were significant ($F = 15.36$; $p < 0.05$) and the variations between different zones were not significant (Table 19).

Table 16: Number and Per manhour Density (PMHD±SD) and Sex Ratio of Male and Female *Cx. quinquefasciatus* encountered in the fortnightly indoor resting collections of mosquitoes in different months in Panaji during April, 05 to March, 06.

Month	Female		Male		Sex Ratio M : F
	Number	PMHD	Number	PMHD	
April' 05	359	17.96+1.79	142	07.10+1.36	1 : 2.5
May	513	25.67+4.39	209	10.46+2.11	1 : 2.5
June	653	32.67± 4.89	288	14.41 ±3.41	1 : 2.3
July	799	39.98 ±19.32	194	10.11± 3.03	1 : 4.1
August	304	15.21± 8.43	112	05.60 ±3.03	1 : 2.7
September	138	06.65± 5.24	31	02.85± 4.05	1 : 4.5
October	188	08.91± 5.95	114	05.70± 4.48	1 : 1.6
November	596	24.82± 14.81	258	12.91± 8.47	1 : 2.3
December	844	42.28 ±2.22	241	12.06 ±3.36	1 : 3.5
January,06	733	36.72 ±8.99	229	11.46 ±6.49	1 : 3.2
February	972	48.65± 5.26	293	14.66± 4.89	1 : 3.3
March' 06	919	45.99± 8.52	442	22.12 ±9.20	1 : 2.1

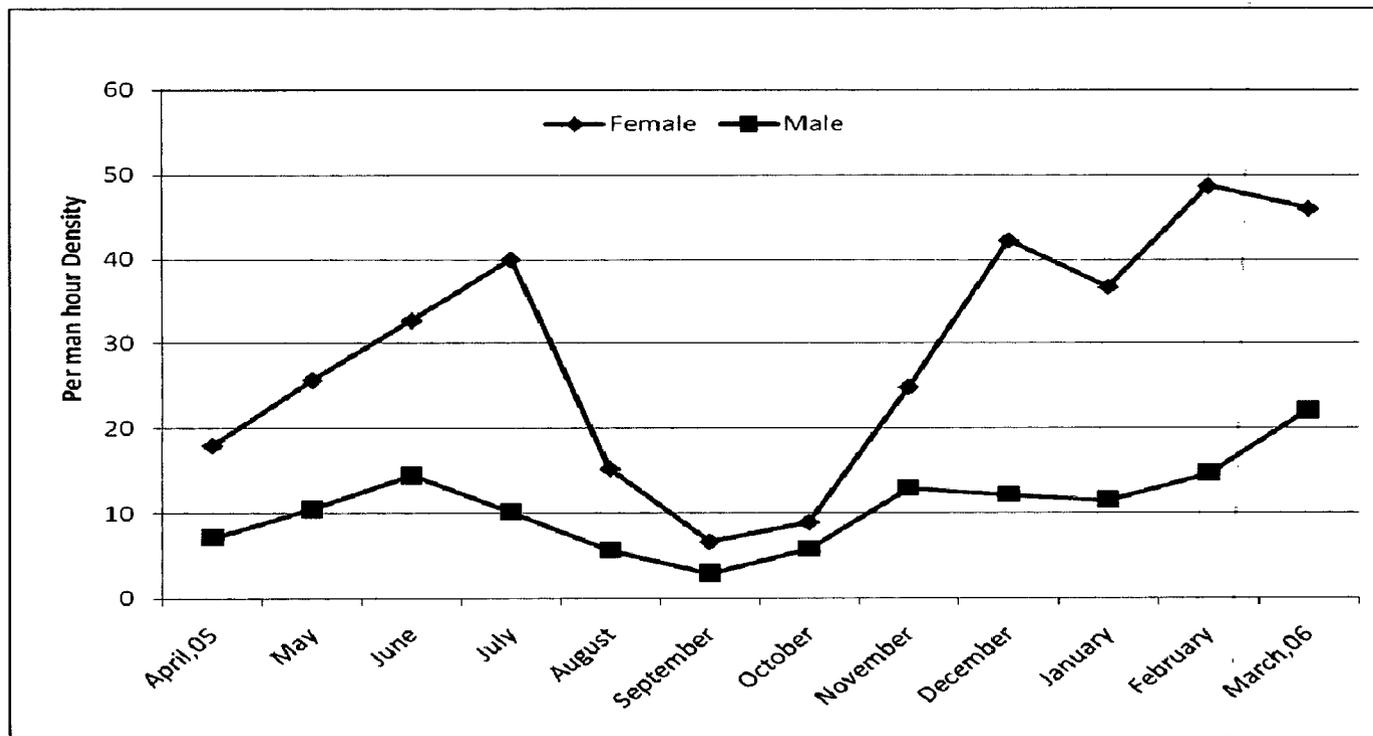


Fig. 22: Monthly variations in Per man hour density of Male and Female *Cx. quinquefasciatus* in the fortnightly indoor resting collections of mosquitoes in Panaji, Goa during April, 2005 to March, 2006

**Table 17 : Two way Analysis (ANOVA) between months
and zones – *Cx.quinquefasciatus* females.**

Source of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value	Significance
Between months	13720.5452	11	1247.322	15.3591	0.0000	S
Between zones	642.6093	5	128.5219	1.5826	0.1803	NS
Error	4466.5968	55	81.2109	-	-	-
Total	18829.7513	71		-	-	-

Note- S= Significant, NS= Not significant

Table 18 : Scheffes multiple comparison test procedure showing the level of difference in the densities of *Cx.quinquefasciatus* females between pairs of months.

Months	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March
Mean	17.963	25.673	32.678	39.983	15.210	6.6533	8.905	24.825	42.238	36.7180	48.6450	45.9920
April,05	-											
May	0.3237	-										
June	0.0373*	0.1937	-									
July	0.0016*	0.0451*	0.3624	-								
August	0.6073	0.2132	0.0145*	0.0005*	-							
September	0.1577	0.0090*	0.0003*	0.0001*	0.2511	-						
October	0.2136	0.0209*	0.0006*	0.0001*	0.2414	0.6742	-					
November	0.2028	0.8741	0.3106	0.0462*	0.1768	0.0100*	0.0207*	-				
December	0.0006*	0.0232*	0.2862	0.6737	0.0002*	0.0001*	0.0001*	0.0212*	-			
January	0.0073*	0.1042	0.4514	0.5424	0.0022*	0.0001*	0.0002*	0.1263	0.5573	-		
February	0.0001*	0.0013*	0.0438*	0.3722	0.0002*	0.0001*	0.0002*	0.0010*	0.4564	0.1799	-	
March,06	0.0002*	0.0043*	0.1046	0.5010	0.0001*	0.0002*	0.0001*	0.0036*	0.4840	0.3122	0.6204	-

Note - *indicates significant at 5% level of significance ($p < 0.05$).

Table 19 : Two way Analysis (ANOVA) between months and zones – *Cx. quinquefasciatus* – males.

Source of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value	Significance
Between months	13602.6077	11	1236.601	15.3672	0.0000.	S
Between zones	639.2268	05	127.8454	1.5887	0.1786	NS
Error	4425.8493	55	80.4700	-	-	-
Total	18667.6838	71	-	-	-	-

Note- S= Significant, NS= Not significant

Table 20 : Scheffes multiple comparison test procedure showing the level of difference in the densities of *Cx.quinquefasciatus* females between pairs of months

Months	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March
Mean	17.9630	25.6730	32.6780	39.9830	15.2100	6.6533	9.4050	24.8250	42.2380	36.7180	48.645	45.99
April,05	-											
May	0.3206	-										
June	0.0362*	0.1918	-									
July	0.0015*	0.0438*	0.3593	-								
August	0.60557	0.2099	0.0140*	0.0005*	-							
September	0.1549	0.0086*	0.0003*	0.0001*	0.2481	-						
October	0.2480	0.0260*	0.0008*	0.0001*	0.2783	0.6060	-					
November	0.2009	0.8736	0.3075	0.0448*	0.1743	0.0096*	0.0257*	-				
December	0.0006*	0.0224*	0.2825	0.6724	0.0002*	0.0001*	0.0001*	0.0204*	-			
January	0.0069*	0.1022	0.4494	0.5407	0.0021*	0.0001*	0.0002*	0.1238	0.5545	-		
February	0.0001*	0.0012*	0.0423*	0.3684	0.0002*	0.0001*	0.0002*	0.0009*	0.4534	0.1765	-	
March,06	0.0002*	0.0041*	0.1021	0.4980	0.0001*	0.0002*	0.0001*	0.0034*	0.4821	0.3085	0.6189	-

Note - *indicates significant at 5% level of significance ($p < 0.05$).

Table 21 : Regression analysis of dependent variables and density of female *Cx. quinquefasciatus*

Variable	Regression coefficient	SE of Regression coefficient	t- value	p- value	Significance
Intercept	242.7936	90.8814	2.6715	0.0319	S
Relative Humidity %	1.3509	0.9728	1.3887	0.2075	NS
Actual Rainfall	0.0558	0.0212	2.6346	0.0337	S
Temperature °C	4.0730	3.1074	1.3108	0.2313	NS
No. of Rainy days	1.2468	1.1053	1.1280	0.2965	NS
R=0.8353, R ² = 0.6977, Adjusted R ² = 0.5251, F= 4.0405 p 0.05, S, Std. Error of estimate:9.7726					

*Note - S=Significant, NS= Not significant

The results of Scheffes multiple comparison test procedure between pairs of months on the density of female and male are given in Tables 18 & 20 which showed significant difference in density when compared between two different months of the year. Regression analysis revealed that the actual rain fall significantly influenced the population density pattern of female *Cx. quinquefasciatus* ($t = 2.63$; $p < 0.05$) (Table 21). There did not appear any significant impact of temperature, relative humidity and number of rainy days on *Cx. quinquefasciatus* densities in Panaji. The influence of meteorological variables on male population was not significant.

***Cx. quinquefasciatus* Male to Female Ratio.**

Male to Female *Cx. quinquefasciatus* ratio in different zones and also in different months varied. In zones I – VI, the ratio was 1: 2.00, 1: 2.96, 1: 2.97, 1: 2.45, 1: 3.30 and 1: 3.32 respectively. The ratio in different months in Panaji ranged between 1:1.6 (October) and 1: 4.5 (September). It was high during July and September months. Average M : F ratio was 1 : 2.1 (Table 16).

Abdominal Conditions of *Cx. quinquefasciatus* Females.

The number and percent contributed by each group of abdominal condition in different months during April, 2005 to March, 2006 are given in Table 22. Total number of unfed, fed, semigravid and gravid female *Cx. quinquefasciatus* were 1033, 4547, 861 and 577 respectively. The percentage of *Cx. quinquefasciatus* found with different abdominal conditions are presented in Fig. 23.

Table 22 : Abdominal conditions of *Cx. quinquefasciatus* females collected in different months in Panaji from April,2005 to March,2006

Month	Unfed		Fed		Semigravid		Gravid	
	Number collected	% to total Females						
April,05	56	15.6	215	59.9	60	16.7	28	7.8
May	56	10.9	317	61.8	92	17.9	48	9.4
June	66	10.1	394	60.3	124	19.0	69	10.6
July	143	17.9	422	52.8	147	18.4	87	10.9
August	49	16.1	177	58.2	43	14.1	35	11.5
September	21	15.2	70	50.7	28	20.3	19	13.8
October	23	12.2	104	55.3	27	14.4	34	18.1
November	127	21.3	372	62.4	44	7.4	53	8.9
December	145	17.2	587	69.5	73	8.6	39	4.6
January,06	133	18.1	498	67.9	59	8.0	43	5.9
February	97	10.0	722	74.3	90	9.3	63	6.5
March	117	12.7	669	72.8	74	8.1	59	6.4
Total	1033	14.71	4547	64.79	861	12.26	577	8.22

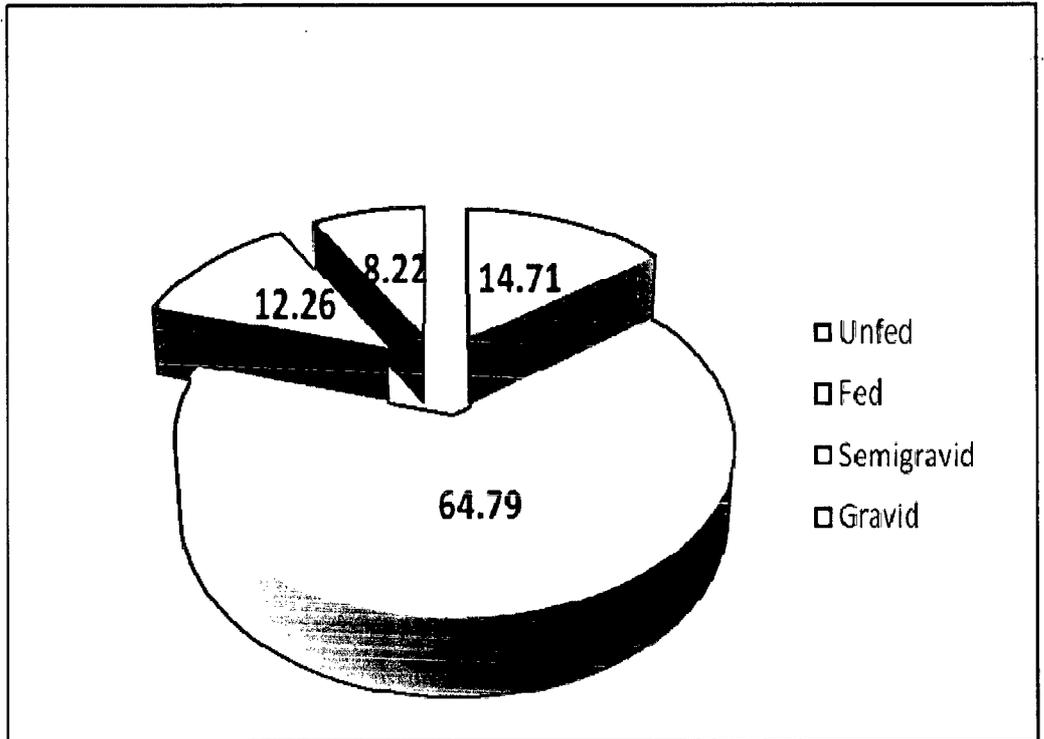


Fig. 23: Percent of unfed, fed and semigravid and gravid females of *Cx. quinquefasciatus* encountered in indoor collection during April, 2005 to March, 2006 in Panaji Goa)

The females with fully fed abdominal status were the maximum and gravid females were the least in number. The fed, semigravid and gravid females captured indoors together contributed 85.28% indicating the strong endophilic nature of the species.

Two way factorial ANOVA using month and abdominal status on number of *Cx. quinquefasciatus* females (Table 23) revealed that the variations in abdominal status in different months ($F=25.02$; $p=0.0000$), the variations within different abdominal status ($F= 345.33$; $p=0.0000$) and also the two way interactions of month and abdominal status ($F=11.84$; $p=0.0000$) were significant. Similar analysis using zones and abdominal status revealed that the variations within the abdominal status was significant ($F=102.66$; $p=0.0000$) and it was not significant both in the zones and two way interactions of zones and abdominal status (Table 24). Further, a *post hoc* Tukey test (Table 25) justified the differences between the different abdominal status ($p<0.05$). The difference between semigravid and gravid groups was not significant. The results of statistical analysis to assess the impact of relative humidity, actual rainfall, number of rainy days and the temperature on the number of different abdominal status of *Cx. quinquefasciatus* are presented in Table 26.

Table 23 : Two way factorial ANOVA using month and abdominal status on number of female *Cx. quinquefasciatus*

Source of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value	Significance
Main effects						
Month	38069.0369	11	3460.8215	25.0254	0.0000	S
Abdominal status	143271.5391	3	47757.1797	345.3349	0.0000	S
2- way Interactions						
Month x Abdominal status	54073.1708	33	1638.5809	11.8487	0.0000	S
Error	33190.1660	240	138.2924			
Total	268603.9127	287				
S= Significant						

Table 24 : Two way factorial ANOVA using zone and abdominal status on number of female *Cx. quinquefasciatus*

Source of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value	Significance
Main effects						
Zones	667.2674	5	133.4535	0.2869	0.9200	NS
Abdominal status	143271.5391	3	47757.1797	102.6617	0.0000	S
2- way Interactions						
Month x Abdominal status	1855.0243	15	123.6683	0.2658	0.9976	NS
Error	122810.0801	264	465.1897			
Total	268603.9109	287				

Table 25: Tukey a *post hoc* test between different abdominal conditions of female *Cx. quinquefasciatus*

Abdominal status	Unfed	Fed	Semigravid	Gravid
Mean	14.29167	62.97222	11.77778	8.888889
Unfed	1.0000			
Fed	0.0000*	1.0000		
Semigravid	0.5740	0.0000*	1.0000	
Gravid	0.0298*	0.0000*	0.4533	1.0000

* Note - p- value less than 0.05 are significant at 5% level ($p < 0.05$)

Table 26 : Correlation between Abdominal status of female *Cx. quinquefasciatus* with Relative Humidity (%), Actual Rainfall, No of Rainy days and Temperature °C.

Variable	Abdominal condition	Correlation coefficient	t - value	p- value	S/NS
Relative Humidity (%)	Unfed	0.1568	0.5019	0.6266	NS
	Fed	0.7958	4.1553	0.0020	S
	Semigravid	0.7783	3.9204	0.0029	S
	Gravid	0.7311	3.3887	0.0069	S
Actual Rainfall	Unfed	0.0167	0.0528	0.9589	NS
	Fed	0.6474	2.6863	0.0228	S
	Semigravid	0.6946	3.0555	0.0121	S
	Gravid	0.4094	1.4188	0.1864	NS
No. of Rainy Days	Unfed	0.0201	0.0637	0.9505	NS
	Fed	0.0035	0.0111	0.9914	NS
	Semigravid	0.7065	3.1570	0.0102	S
	Gravid	0.5311	1.9820	0.0756	NS
Temperature °C	Unfed	0.4640	1.6564	0.1286	NS
	Fed	0.0035	0.0111	0.9914	NS
	Semigravid	0.3349	1.1238	0.2873	NS
	Gravid	0.0150	0.0473	0.9632	NS

Note - S = Significant, NS= Not significant

There was a significant correlation between the fed group with relative humidity ($r = 0.79$, $p < 0.05$), actual rain fall ($r = 0.65$, $p < 0.05$) and number of rainy days ($r = 0.73$, $p < 0.05$). Similar correlation was found in semigravid group with relative humidity ($r = 0.77$, $p < 0.05$), actual rain fall ($r = 0.69$, $p < 0.05$) and number of rainy days ($r = 0.70$, $p < 0.05$). In the case of gravid group, there was a significant correlation only with relative humidity ($r = 0.73$, $p < 0.05$).

Seasonal Distribution of *Cx. quinquefasciatus*.

The total number of female and male collected in different seasons were : 2763 and 1086 in pre-monsoon, 1894 and 625 in monsoon and 2361 and 842 in post-monsoon season. The seasonal distribution of *Cx. quinquefasciatus* females and males has been presented in Fig. 24. The abundance of *Cx. quinquefasciatus* females both during pre-monsoon (PMHD = 34.5) and post-monsoon (PMHD = 29.5) was high. The abundance was low (PMHD = 23.8) during monsoon season. Seasonally, per man hour densities of males were 13.58, 7.81 and 10.53 during pre-monsoon, monsoon and post-monsoon seasons respectively.

Indoor Resting Behavior of *Cx. quinquefasciatus*.

The collection of *Cx. quinquefasciatus* from different resting sites revealed that 1502 females and 567 males rested on walls, 4379 females and 1661 males on hanging objects, 723 females and 229 males on the objects on floor and 414 females and 96 males on horizontal surfaces (Table 27).

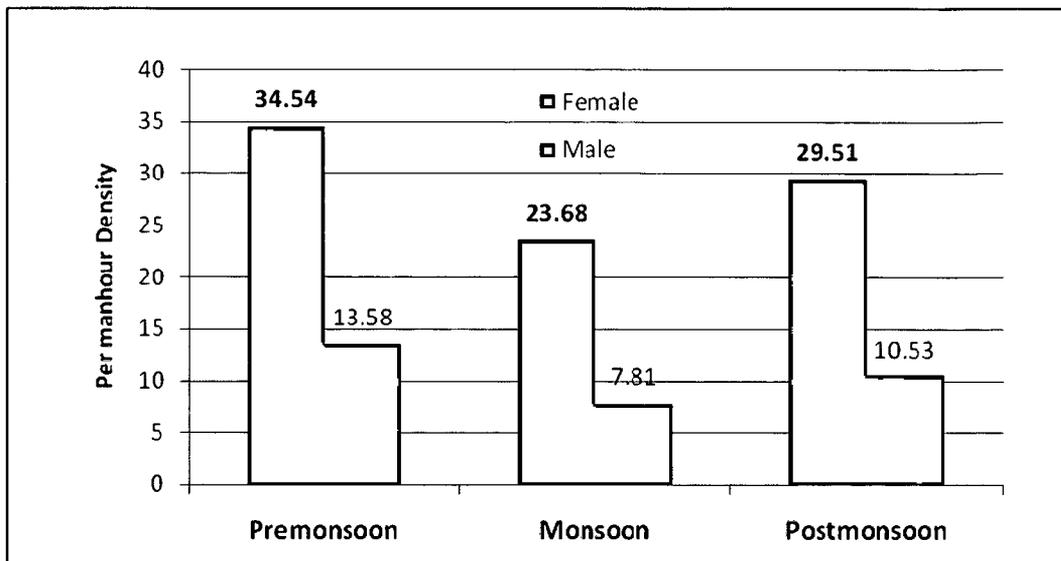


Fig. 24: Seasonal distribution of Females and Males of *Cx. quinquefasciatus* during April, 2005 to March, 2006 in Panaji, Goa

The percent of *Cx. quinquefasciatus* females and males rested on different sites is presented in Fig. 25. The resting behavior of *Cx. quinquefasciatus* revealed that the hanging objects were the most preferred resting sites and horizontal surfaces were the least preferred for both females and males. 62.4% of females and 65.1% of males rested on hanging objects. 5.9% of females and 3.8% of males rested on horizontal surfaces.

Two way factorial ANOVA using month and resting sites on number of *Cx. quinquefasciatus* females (Table 28) revealed significant variations in the number resting in different months ($F=36.71$; $p=0.0000$) and the significant variations within different resting sites ($F= 490.24$; $p=0.0000$). Two way interactions of month and resting sites ($F=9.77$; $p=0.0000$) was also significant. Similar analysis using zones and resting sites on females revealed that the variations within the resting sites was significant ($F=136.50$; $p=0.0000$) but, it was not significant both in the zones and two way interactions of zones and resting sites (Table 29). Further, a *post hoc* Tukey test (Table 30) justified the differences between the different resting sites ($p<0.05$).

Two way factorial ANOVA using month and resting sites on number of *Cx. quinquefasciatus* males (Table 31) revealed significant variations in the number resting in different months ($F=11.89$; $p=0.0000$) and the significant variations within different resting sites ($F= 193.1845$; $p=0.0000$). Two way interactions of month and resting sites ($F=3.84$; $p=0.0000$) was also significant.

Table 27 : Number and Percent of *C. quinquefasciatus* Females and Males rested on different types of Structures during April, 2005 to March, 2006

Resting Habitat	Female		Male		Female + Male	
	Number Collected	% of total females	Number Collected	% of total Males	Number Collected	% of total Collected
Wall	1502	21.4	0567	22.2	2069	21.6
Hanging Objects	4379	62.4	1661	65.1	6040	63.1
Objects on Floor	0723	10.3	0229	09.0	0952	09.9
Horizontal Surfaces	0414	05.9	0096	03.8	0510	05.3

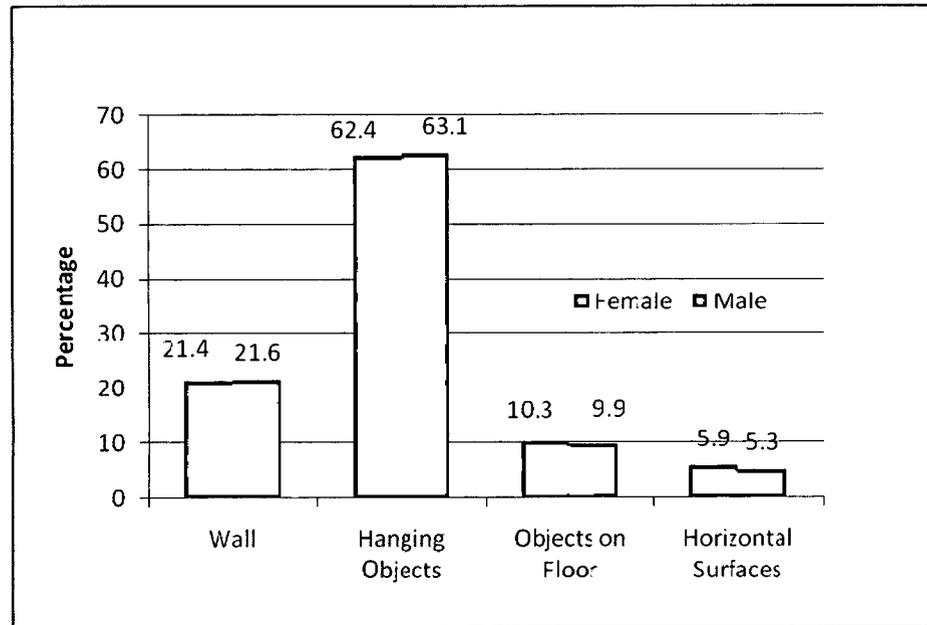


Fig. 25: *Cx. quinquefasciatus* Females and Males resting on different indoor resting sites during April, 2005 to March, 2006 in Panaji, Goa.

Table 28: Two way factorial ANOVA using month and resting sites on number of *Cx. quinquefasciatus* females

Source of Variation	Degrees of freedom	Sum of square	Mean sum of square	F-value	p-value	Significance
Main effects						
Month	11	37445.3723	3404.1248	36.7101	0.0000	S
Resting site	3	136381.9219	45460.6406	490.248 1	0.0000	S
2- way Interactions						
Month x Resting site	33	29915.6984	906.5363	9.7761	0.0000	S
Error	240	22255.1660	92.7299			
Total	287	225998.1586				

Note -S = Significant

Table 29: Two way factorial ANOVA using zone and resting sites on number of *Cx. quinquefasciatus* females

Source of Variation	Degrees of freedom	Sum of square	Mean sum of square	F-value	p-value	Significance
Main effects						
Zone	5	919.7257	183.9451	0.5523	0.7365	NS
Resting site	3	136381.9219	45460.6406	136.506 4	0.0000	S
2- way Interactions						
Month x Resting site	15	776.7604	51.7840	0.1555	0.9999	NS
Error	264	87919.7505	333.0294			
Total	287	225998.1586				

Note -S = Significant. NS= Not significant.

Table 30 : Tukey a *post hoc* test between different resting sites of *Cx. quinquefasciatus* females

Resting site	Wall	Hanging object	Object on floor	Horizontal surface
Mean	20.86111	60.81944	10.01389	5.736111
Wall	1.0000			
Hanging object	0.0000*	1.0000		
Object on floor	0.0000*	0.0000*	1.0000	
Horizontal surface	0.0000*	0.0000*	0.0385*	1.0000

* p-value significant at 5% level ($p < 0.05$).

Table 31: Two way factorial ANOVA using month and resting sites on number of *Cx. quinquefasciatus* males

Source of Variation	Degrees of freedom	Sum of square	Mean sum of square	F-value	p-value	Significance
Main effects						
Month	11	4731.0104	430.0919	11.8967	0.0000	S
Resting site	3	20952.0659	6984.0220	193.1845	0.0000	S
2- way Interactions						
Month x Resting site	33	4584.1424	138.9134	3.8425	0.0000	S
Error	240	8676.5002	36.1521			
Total	287	38943.7190				

Note -S = Significant

Similar analysis using zones and resting sites on number of males revealed that the variations within the zones ($F=3.03$; $p<0.05$) and variations within the resting sites ($F=114.08$; $p=0.0000$) were significant (**Table 32**), but two way interactions of zones and resting sites was not significant. Further, a *post hoc* Tukey test (**Table 33**) justified the differences between the different resting sites ($p<0.05$). The difference between resting on objects on floor and horizontal surfaces was not significant.

Prevalence of other Mosquito species.

Mosquito species other than *Cx. quinquefasciatus* prevalent indoor in different months during April, 2005 to March, 2006 in Panaji are presented in **Table 34**. While collecting the indoor resting mosquitoes, the vector mosquitoes spreading malaria (*Anopheles stephensi* Liston), dengue fever and chikungunya fever (*Aedes aegypti* Lin. and *Ae. albopictus* Skuse), Japanese encephalitis (*Culex vishnui* group of mosquitoes comprising *Cx. vishnui* Theobald, *Cx. pseudovishnui* Colles and *Cx. tritaeniorhynchus* Giles) and Malayan filariasis (*Mansonia uniformis* Theobald) were also encountered.

The total number and percent contributed by each species to all the indoor resting mosquitoes collected were as follows- *Culex tritaeniorhynchus* Giles- 176 (1.5%), *Cx. gelidus* Theobald - 37 (0.3%), *Cx. vishnui* Theobald- 26 (0.2%), *Cx. pseudovishnui* Theobald - 19 , (0.2%), *Cx. bitaeniorhynchus* Giles - 9 (0.09%),

Table 32 : Two way factorial ANOVA using zone and resting sites on number of *Cx. quinquefasciatus* males

Source of Variation	Degrees of freedom	Sum of square	Mean sum of square	F-value	p-value	Significance
Main effects						
Zone	5	928.7813	185.7563	3.0343	0.0111	S
Resting site	3	20952.0659	6984.0220	114.0842	0.0000	S
2- way Interactions						
Zone x Resting site	15	901.2882	60.0859	0.9815	0.4750	NS
Error	264	16161.5838	61.2181			
Total	287	38943.7192				

Note -S = Significant, NS= Not significant

Table 33 : Tukey a *post hoc* test between resting Sites of *Cx. quinquefasciatus* males

Resting site	Wall	Hanging object	Object on floor	Horizontal surface
Mean	7.861111	23.05556	3.180556	1.361111
Wall	1.0000			
Hanging object	0.0000*	1.0000		
Object on floor	0.0000*	0.0000*	1.0000	
Horizontal surface	0.0000*	0.0000*	0.2659	1.0000

Note -* **p-value** significant at 5% level ($p < 0.05$).

Table 34 : Number and Percent contribution by other Mosquito species encountered in the fortnightly collection of indoor resting mosquitoes during April, 2005 to March, 2006..

Sr. No.	Mosquito species	No. collected	% of total collection
1.	<i>Culex tritaeniorhynchus</i>	176	1.50
2.	<i>Culex vishnui</i>	026	0.22
3.	<i>Culex pseudovishnui</i>	019	0.16
4.	<i>Culex bitaeniorhynchus</i>	009	0.07
5.	<i>Culex gelidus</i>	037	0.31
6.	<i>Mansonia uniformis</i>	668	5.72
7.	<i>Aedes aegypti</i>	257	2.20
8.	<i>Aedes albopictus</i>	014	0.11
9.	<i>Armegeles sublbatus</i>	114	0.97
10.	<i>Anophelis subpictus</i>	575	4.92
11.	<i>Anopheles vagus</i>	129	1.10
12.	<i>Anopheles stephensi</i>	054	0.46
13.	<i>Anopheles jamsi</i>	012	0.10
14.	<i>Anopheles barbirostris</i>	009	0.07
15.	<i>Anopheles hyrcanus</i>	003	0.03

Mansonia uniformis Theobald- 668 (5.7%), *Aedes aegypti* Linnaeus -257 (2.2%), *Ae. albopictus* Skuse- 14 (0.1%), *Armigeres sublbatus*- 114 (1.0%), *Anopheles subpictus* Grassi- 575 (4.9%), *An. vagus* Donitz- 129 (1.1%), *An. stephensi* Liston -54 (0.5%), *An. jamesi* Theobald- 12 (0.1%), *An. barbirostris* Van der Wulp- 9 (0.07%) and *An. hyrcanus* variety *nigerimus* Giles- 3 (0.03%).

4.3 Collection of Adult Mosquitoes on Human Bait

Man-vector contact rate and periodicity of biting of vector mosquitoes are the important aspects of the study. The results of the whole night collections of adult mosquitoes landed on human bait to bite and suck the blood between 1800 h- 0600 h in Mala and Kamrabhat localities of Panaji were analysed. The mean temperature during the study period ranged from 26.7° C to 29.4° C and relative humidity ranged from 65.5% to 90.7%. The total rainfall was 3555.8 mm. The number of rainy days during different months were: July, 05- 30, August- 24, September- 25, October- 7, March, 06- 2, May- 7 and June- 25.

After the locality-wise analysis of data on biting activity from Mala and Kamrabhat, it was found that there was no significant difference between them. Therefore, the data from both the localities were pooled for further analysis.

***Cx. quinquefasciatus* and other Mosquito-species landing for biting on Human Bait.**

1848 mosquitoes belonging to 13 mosquito species landed on human bait in Mala area. 6 species of mosquitoes belonged to genus *Culex*, 2 species to genus *Aedes*, 1 species each to genus *Mansonia* and genus *Armegeres* and 3 species to genus *Anopheles*. Among all the species, *Cx. quinquefasciatus* was the most predominant species. 1435 *Cx. quinquefasciatus* female mosquitoes were collected on human bait and this species alone contributed 77.65% to the total landing collections. Remaining 12 species altogether contributed 32.35%.

A total of 2066 mosquitoes belonging 11 different mosquito species landed on human bait in Kamrabhat area. 5 species belonged to genus *Culex*, 2 species each to genus *Aedes* and genus *Anopheles*, 1 species each to genus *Mansonia* and genus *Armegères*. In Kamrabhat area also, *Cx. quinquefasciatus* was the most predominant species and total number of *Cx. quinquefasciatus* females landing on human bait was more than Mala area. 1642 *Cx. quinquefasciatus* females were collected and this species alone contributed 79.48% to the total landing collections. Remaining 10 species altogether contributed 20.52%.

A total of 3914 mosquitoes were collected on human bait from both the localities in Panaji. 3077 *Cx. quinquefasciatus* females were encountered in 24 whole night landing collections and this species alone contributed 78.61% to the total mosquitoes collected on bait (Fig.26).

Hourly/Quarterly Landing of *Cx. quinquefasciatus* on Human Bait.

Results of hourly collections revealed that biting activity of *Cx. quinquefasciatus* was continued throughout the night both in Mala and Kamrabhat areas. Total number of *Cx. quinquefasciatus* collected in different hours from 1800 h to 0600 h were: 244, 221, 229, 248, 310, 350, 186, 226, 274, 240, 262 and 287 respectively (Table 35).

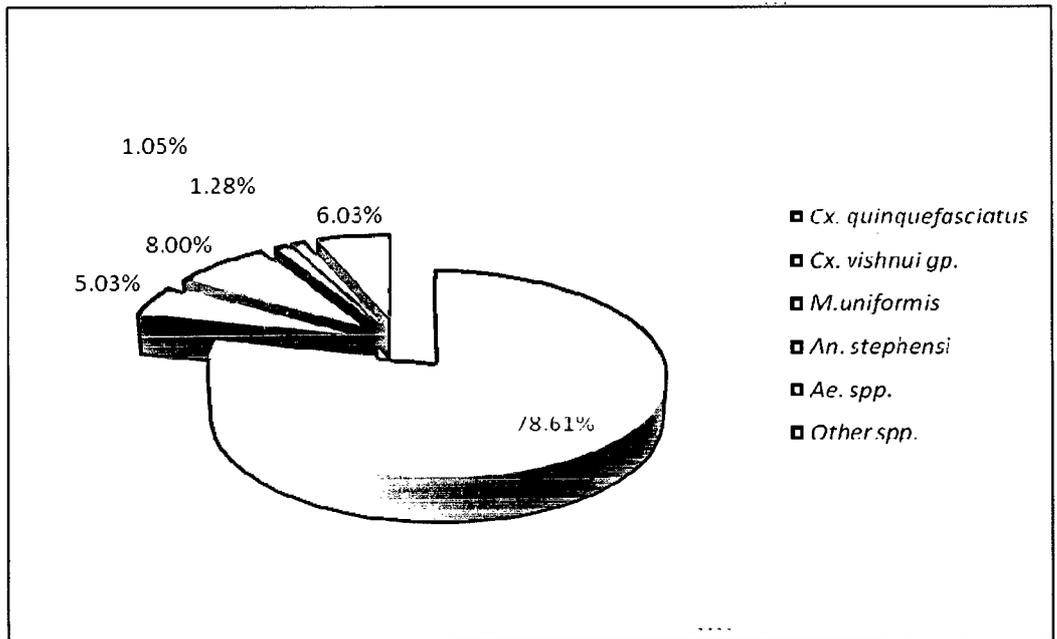


Fig. 26: Percent contributed by *Cx. quinquefasciatus*, *Cx. vishnui* group, *M. uniformis*, *An. stephensi*, *Ae. spp.* (*Ae. aegypti*+ *Ae. albopictus*) and other species to total number of mosquitoes Landed on Human bait in Panaji, Goa during July, 2005 to June, 2006.

Table 35: The total number of *Cx. quinquefasciatus* Females landing on Human bait in different Hours encountered during 24 whole night collections in Panaji during July, 2005 to June,2006.

Different Hours	July'05	Aug.	Sept.	Oct.	Nov.	Dec.	Jan,06	Feb.	Mar.	Apr.	May	June' 07	Total
1800 to 1900	30	15	10	26	31	37	19	15	16	12	16	17	244
1900 to 2000	16	22	06	17	23	34	25	11	18	15	14	20	221
2000to 2100	40	09	08	18	23	47	09	14	10	11	20	20	229
2100 to 2200	21	15	14	23	24	49	22	18	11	11	16	24	248
2200 to 2300	18	11	04	36	46	40	21	17	30	26	34	27	310
2300 to 2400	16	19	20	40	51	42	21	26	22	26	33	34	350
2400 to 0100	11	09	06	14	15	11	26	18	12	20	23	21	186
0100 to 0200	17	18	16	22	24	18	14	19	13	13	25	27	226
0200 to 0300	19	10	07	38	40	22	21	18	21	22	28	28	274
0300 to 0400	10	11	08	11	17	24	29	35	21	19	26	29	240
0400 to 0500	11	15	16	18	23	17	24	17	23	26	27	45	262
0500 to 0600	16	14	33	18	20	25	21	36	14	28	31	31	287
Total	225	168	150	281	337	366	252	244	211	227	293	323	3077

The highest hourly biting (11.37%) occurred at 2300-2400 h. Surprisingly in the next hour (2400-0100), the percent of biting dropped to the lowest (6.04%). However, it showed increasing trend in the latter hours. Average hour-wise man-landing rate per bait ranged between 93.0 (2400-0100 h) to 175.0 (2300-2400 h) during July, 2005 to June, 2006 (Fig. 27). 694, 901, 789 and 693 females landed on human baits during first to fourth quarters respectively. The percent of females landed in different quarters ranged from 22.52% to 29.28% (Fig. 28). The highest biting in Panaji occurred in the second quarter (2100 h- 2400 h) of the night. Biting dropped down in the third quarter and it again increased in the fourth quarter.

Average Per Bait Per Night Biting Rate of *Cx. quinquefasciatus* in different months.

Cx. quinquefasciatus was found biting in all months of the year. Total number of *Cx. quinquefasciatus* landed on human bait from Mala and Kamrabhat areas together in different months during July, 2005 to June, 2006 were 225, 168, 150, 281, 337, 366, 252, 244, 211, 227, 293 and 323 respectively. Average per bait per night landing rate of *Cx. quinquefasciatus* during July, 2005 to June, 2006 was : 112.5, 84.0, 75.0, 140.5, 168.5, 183.0, 126.0, 122.0, 105.5, 113.5, 146.5 and 161.5 respectively. The highest per bait per night biting rate was recorded in December month and the lowest in September (Fig. 29).

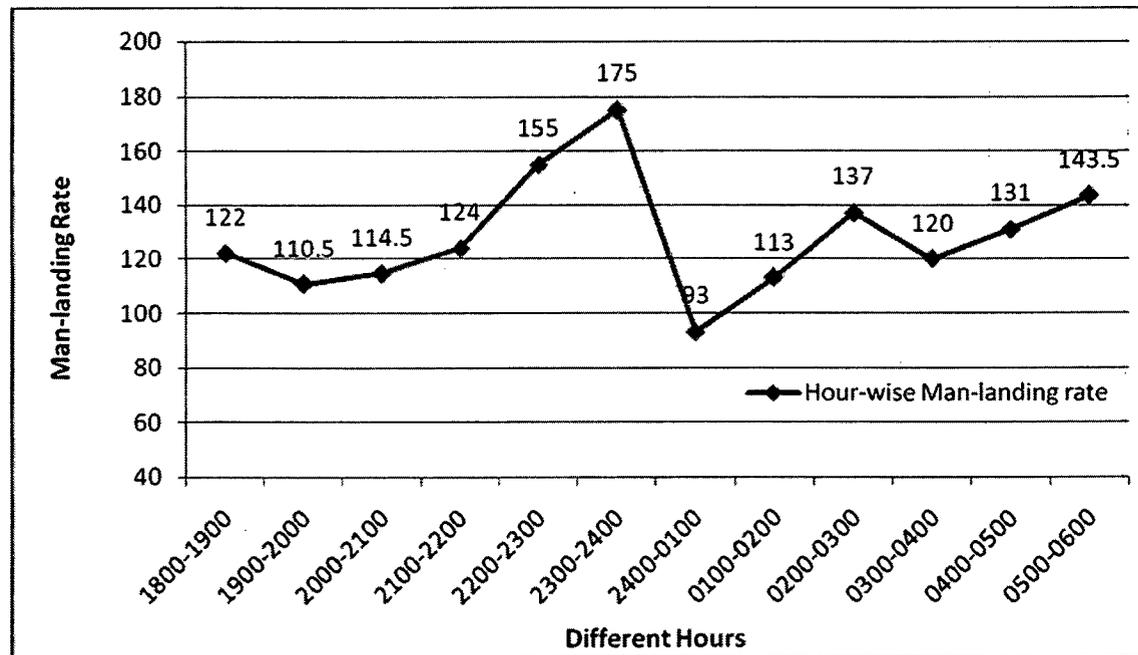


Fig. 27: Average Hour-wise Man-landing Rate per bait of *Cx. quinquefasciatus* females during different hours of the night in Panaji during July, 2005 to June, 2006

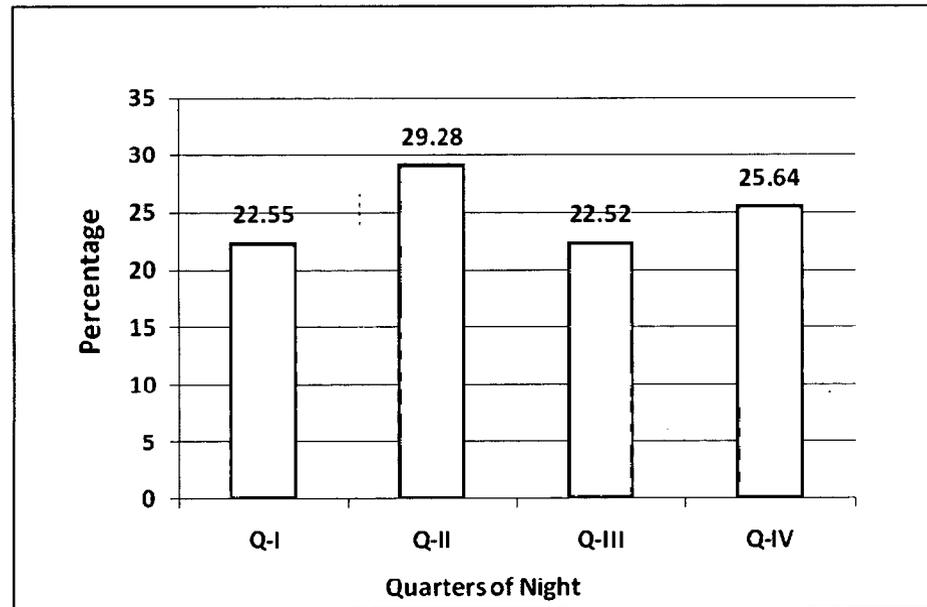


Fig. 28: Landing (%) of *Cx. quinquefasciatus* on Human Bait in different Quarters of the night in Panaji during July, 2005 to June, 2006.

The percent contributions by each month to total number landed from July, 2005 to June, 2006 were : 7.31%, 5.45%, 4.87%, 9.13%, 10.95%, 11.89%, 8.18%, 7.92%, 6.85%, 7.37%, 9.52% and 10.49% respectively. Average per bait per night biting rate of *Cx. quinquefasciatus* in Panaji was high in May, June and October to December months. It was moderate in January to April and July months and low in August and September months.

Two way factorial ANOVA using month and areas on the number of *Cx. quinquefasciatus* females landing on human bait revealed that the variations between different months were significant ($F=3.8480$; $p=0.0002$), but, the variations between the two localities and also the two way interactions of month and area were not significant (Table 36). Two way factorial ANOVA using month and quarter of the night on number of *Cx. quinquefasciatus* females landing on human bait for biting revealed that the variations in quarterly landing in different months ($F=6.3998$; $p=0.0000$), the variations within different quarters ($F=6.4992$; $p=0.0009$) and also the two way interactions of month and quarters ($F=2.1379$; $p=0.008$) were significant (Table 37). Similar analysis using areas and quarters of the night revealed that the variations within the quarters was significant ($F=3.0911$; $p=0.0311$) and it was not significant both in the areas and two way interactions of areas and quarters (Table 38). Further, Tukey a *post hoc* tests (Table 39 & 40) justified the significant variations between the different months ($p<0.05$) and also the different quarters ($p<0.05$).

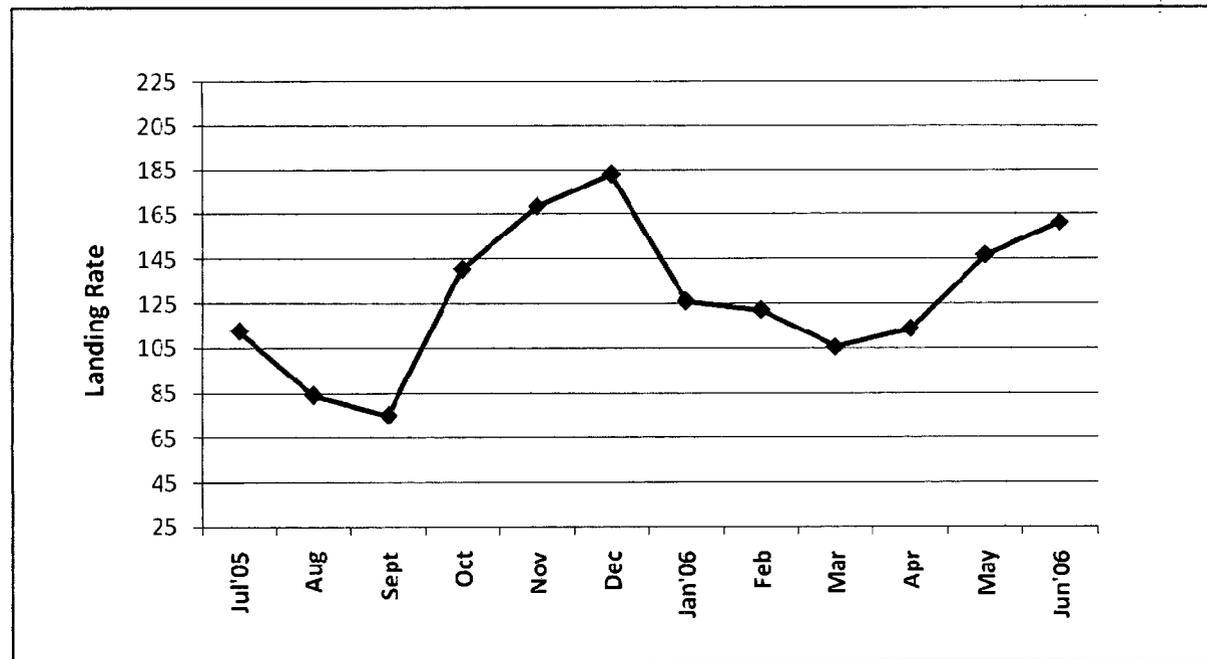


Fig. 29: Per bait per night Landing rate of *Cx. quinquefasciatus* on Human bait in different months in Panaji, Goa during July, 2005 to June, 2006.

Table 36: Two way factorial ANOVA using month and area on number of *Cx. quinquefasciatus* landing on human bait

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of square	F-value	p-value
Main effects					
Months	11	5290.8647	480.9877	3.8480	0.0002*
Areas	1	263.3438	263.3438	2.1068	0.1510
2-way interactions					
Months x Areas	11	1112.0313	101.0938	0.8088	0.6311
Error	72	8999.7501	124.9965		
Total	95	15665.9898			

* **p-value** significant at 5% level ($p < 0.05$).

**Table 37: Two way factorial ANOVA using Month and Quarter
on number of *Cx. quinquefasciatus* Biting**

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of square	F-value	p-value
Main effects					
Months	11	5290.8647	480.9877	6.3998	0.0000*
Quarter	3	1465.3646	488.4549	6.4992	0.0009*
2-way interactions					
Months x Quarter	33	5302.2605	160.6746	2.1379	0.0080*
Error	48	3607.5000	75.1563		
Total	95	15665.9898			

Note -*p-value significant at 5% level ($p < 0.05$).

**Table 38: Two way factorial ANOVA using Area and Quarter
on number of mosquitoes biting.**

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of square	F-value	p-value
Main effects					
Area	1	263.3438	263.3438	1.6665	0.2001
Quarter	3	1465.3646	488.4549	3.0911	0.0311*
2-way interactions					
Area x Quarter	3	31.5313	10.5104	0.0665	0.9775
Error	88	13905.7496	158.0199		
Total	95	15665.9892			

Note - *p-value significant at 5% level ($p < 0.05$).

Table 39 : Tuckey a *post hoc* test to justify the variations in number biting between the months.

Months	5	6	7	8	9	10	11	12
Means	36.625	40.375	28.125	21.0000	18.75000	28.25000	42.12500	42.12500
5								
6	0.9992							
7	0.7160	0.2019						
8	0.0320*	0.0027*	0.8837					
9	0.0074*	0.0006*	0.5828	1.0000				
10	0.7339	0.2138	1.0000	0.8716	0.5633			
11	0.9793	1.0000	0.0430*	0.0008*	0.0002*	0.0488*		
12	0.9793	1.0000	0.0430*	0.0008*	0.0002*	0.0488*	1.0000	

Note : 5 – 12 : May to December months; * p-value significant at 5% level (p<0.05).

**Table 40: Tuckey a *post hoc* test to justify the variations
between the Quarters.**

Quarters	I	II	III	IV
Means	26.83333	37.00000	28.54167	32.33333
I	-			
II	0.0313*	-		
III	0.9654	0.0489*	-	
IV	0.4327	0.5743	0.7237	-

Note :* p-value significant at 5% level ($p < 0.05$).

Landing of *Cx. quinquefasciatus* on Different Body Parts of Human Bait.

The number of *Cx. quinquefasciatus* females landed on different body parts in different months are given in Table 41. Total females landed on face, body, hands and legs were 202, 570, 1083 and 1222 respectively. Percent of *Cx. quinquefasciatus* landed on different body parts of human bait are presented in Fig. 30. The percent of *Cx. quinquefasciatus* landed varied between 6.59% and 39.68%. The legs and hands were found to be the preferred body parts for *Cx. quinquefasciatus* in Panaji.

Two way factorial ANOVA using month and Body part of human bait on the number of *Cx. quinquefasciatus* females landing on human bait for biting (Table 42) revealed that the variations in landing in different months ($F=10.6911$; $p=0.0000$), the variations in the landing on different body parts ($F=180.5920$; $p=0.0000$) were significant. But, the two way interactions of month and body parts were not significant. Tukey a *post hoc* tests (Table 43 & 44) further justified the significances in variations between different months ($p<0.05$) and different body parts ($p<0.05$). Similar analysis using areas and body parts of human bait revealed that the variations within the areas ($F=3.9883$; $p=0.0489$) and also within the body parts ($F=82.5157$; $p=0.0000$) were significant (Table 45). Further, Tukey a *post hoc* tests (Table 46) justified the significances in variations between different body parts within the two localities ($p<0.05$).

Table 41 : *Cx. quinquefasciatus* females landing on different Body Parts of Human bait in different months in Panaji, Goa during July, 2005 to June, 2006.

Month	Face	Body	Hand	Leg
July,05	19	48	74	84
August	13	34	57	64
September	09	32	47	62
October	22	52	104	103
November	15	56	124	142
December	24	68	128	146
January,06	15	49	82	106
February	14	40	85	105
March	13	44	73	81
April	14	42	81	90
May	22	45	108	118
June, 06	22	60	120	121
Total	202	570	1083	1222

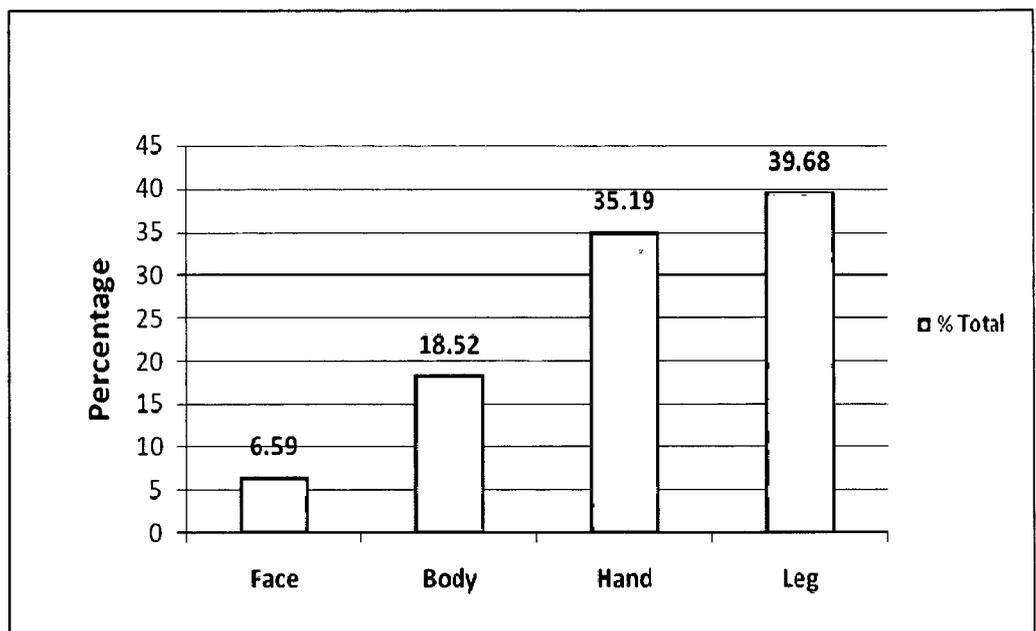


Fig. 30: Percent of total number of *Cx. quinquefasciatus* landed on different Body parts of Human bait in Panaji, Goa during July, 2005 to June, 2006.

Table 42: Two way factorial ANOVA using Month and Body parts on number of *Cx. quinquefasciatus* biting.

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of square	F-value	p-value
Main effects					
Months	11	6013.6149	546.6923	10.6911	0.0000*
Body Part	3	27703.9482	9234.6494	180.5920	0.0000*
2-way interactions					
Months x Body Part	33	2612.6772	79.1720	1.5483	0.0821
Error	48	2454.5001	51.1354		
Total	95	38784.7403			

Note : ***p-value** significant at 5% level ($p < 0.05$).

Table 43: Tuckey a *post hoc* test to justify the variations between the months.

Months	1	2	3	4	5	6	7	8	9
Mean	31.50000	30.50000	26.37500	28.37500	36.62500	40.37500	28.12500	21.00000	18.75000
6	0.3751	0.2290	0.0135*	0.0409*	0.9955				
7	0.9982	0.9999	1.0000	1.0000	0.4402	0.0511			
8	0.1608	0.2787	0.9325	0.6501	0.0036*	0.0002*	0.6958		
9	0.0355*	0.0424*	0.6033	0.2615	0.0006*	0.0001*	0.2966	1.0000	
10	0.9967	0.9761	0.3963	0.7605	1.0000	0.9420	0.7179	0.0122	0.0019
11	0.1493	0.0488*	0.0032*	0.0165*	0.9219	1.0000	0.0135*	0.0001*	0.0001*
12	0.0111*	0.0049*	0.0002*	0.0008*	0.3346	0.9325	0.0007*	0.0001*	0.0001*

Note- 1- 9: January to September; 6-12: June to December; * **p-value significant** at 5% level ($p < 0.05$).

Table 44: Tuckey a *post hoc* test to justify the variations in biting between the Body Parts

Body Part	Face	Body	Hand	Leg
Means	8.416667	23.75000	45.12500	50.91667
Face	-			
Body	0.0002*	-		
Hand	0.0002*	0.0002*	-	
Leg	0.0002*	0.0002*	0.0353*	-

Note - *p-value significant at 5% level ($p < 0.05$).

Table 45: Two way factorial ANOVA using Area and Body part on the number of mosquitoes biting

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of square	F-value	p-value
Main effects					
Area	1	446.3438	446.3438	3.9883	0.0489*
Body Part	3	27703.9482	9234.6494	82.5157	0.0000*
2-way interactions					
Area x Body Part	3	786.0312	262.0104	2.3412	0.0787
Error	88	9848.4167	111.9138		
Total	95	38784.7399			

Note: *p-value significant at 5% level ($p < 0.05$).

Table 46: Tcckey a *post hoc* test to justify the variations in biting between Body Part.

Body Part	Face	Body	Hand	Leg
Means	8.416667	23.75000	45.12500	50.91667
Face	-	-	--	-
Body	0.0002*	-	-	-
Hand	0.0001*	0.0001*	-	-
Leg	0.0001*	0.0001*	0.2371	-

Note: *p-value significant at 5% level of significance (p<0.05)

Seasonal Variations in Biting Activity

Seasonal distribution of *Cx. quinquefasciatus* landing on human bait from both the areas in Panaji revealed that a total of 975 females in pre-monsoon, 866 in monsoon and 1236 in post-monsoon season landed on human bait. Seasonally, the highest percentage of *Cx. quinquefasciatus* females landed during the post-monsoon and the lowest number in monsoon (Fig. 31). Average per bait per night biting rate of *Cx. quinquefasciatus* for the season was calculated as 121.88 in pre-monsoon, 108.25 in monsoon and 154.50 in post-monsoon period in Panaji. The estimated number of *Cx. quinquefasciatus* landing per bait per annum is 46796.7.

Analysis of seasonal data on *Cx. quinquefasciatus* landing during four quarter of night from both the areas of Panaji over the period from July, 2005 to June, 2006, revealed variations in biting activity in all three seasons (Fig. 32). Biting activity was low (17.64%) in the first quarter of night, it increased during second quarter (29.23%), and thereafter, with a drop in the third quarter (22.05%), attained the maximum (31.07%) in the fourth quarter of night during premonsoon period.

During Monsoon period the biting activity was almost uniform (23.55% to 24.59%) in first three quarters of night and it showed some increase (27.59%) in the fourth quarter of the night. Biting activity was high (25.0%) in the first quarter and attained the peak (33.33%) in the second quarter, thereafter, it declined in the third (21.68%) and fourth quarters (19.98%) of the night during post-monsoon season.

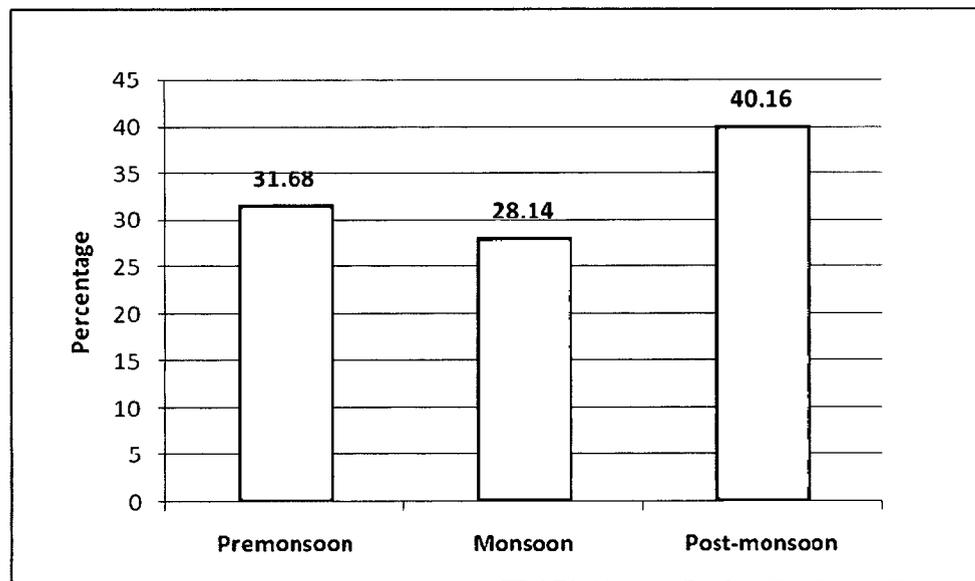


Fig. 31: Seasonal distribution (%) of *Cx. quinquefasciatus* landing on Human bait in Panaji, Goa during July, 2005 to June, 2006.

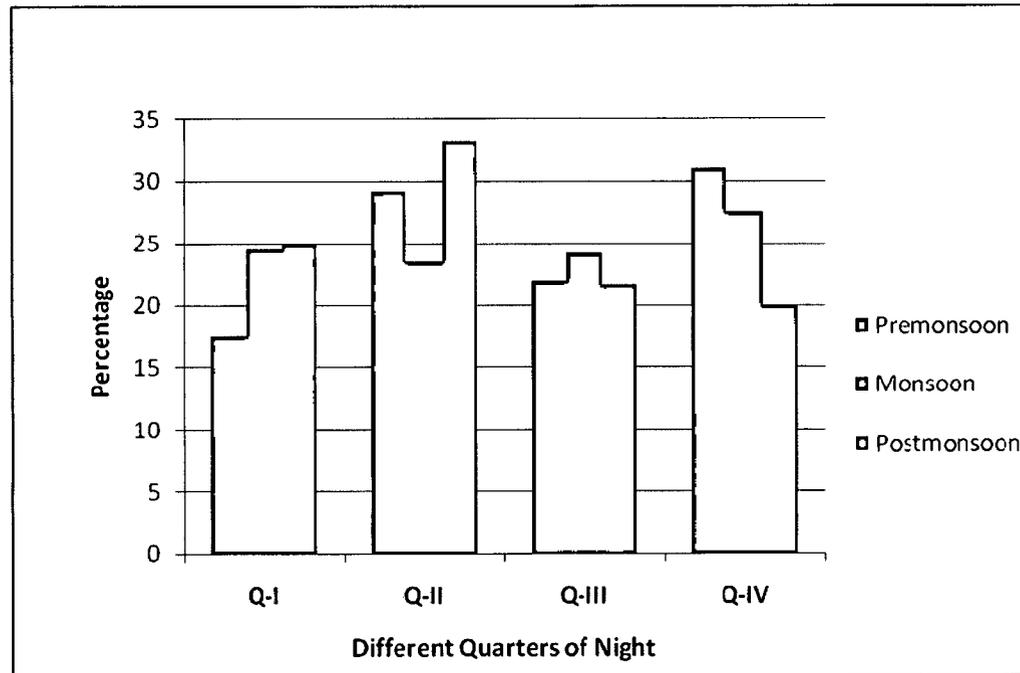


Fig. 32: Seasonal variations in biting of *Cx. quinquefasciatus* in different Quarters of night in Panaji, Goa during July, 2005 and June, 2006.

Other Mosquito species Landing on Human Bait.

Total number of mosquitoes collected in different months from both the areas is given in **Table. 48**. Seven species of mosquitoes landed on human bait in pre-monsoon, 13 species in monsoon and 12 species in post-monsoon season. *Mansonia uniformis* is the vector of Malayan filariasis and contributed 8% to the total number of mosquitoes landed on human bait. *Cx. vishnui*, *Cx. pseudovishnui* and *Cx. tritaeniorhynchus* are the vectors of Japanese encephalitis and these three species together contributed 5.03%. *Ae. aegypti* and *Ae. albopictus* are the vectors of Dengue fever and Chikungunya fever and two species together contributed 1.28%. *Anopheles stephensi* is the vector of urban malaria and accounted for 1.05% to the total collection.

Table 47: Mosquito species other than *Cx. quinquefasciatus* landed on Human bait in different months during July, 2005 to June, 2006 in Panaji

Mosquito species	Jul.'05	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.'06	Feb.	Mar.	Apr.	May	Jun.'06	Total
<i>Cx. tritaeniorhynchus</i>	12	22	31	25	16	10	17	14	06	03	04	07	167
<i>Cx. vishnui</i>	08	08	0	0	0	0	0	0	0	0	0	0	016
<i>Cx. pseudovishnui</i>	0	0	0	0	0	0	05	04	0	0	0	05	014
<i>C.x bitaeniorhynchus</i>	01	0	0	01	01	0	0	0	0	0	0	0	03
<i>Cx. gelidus</i>	08	23	25	08	0	0	0	0	0	0	0	0	064
<i>M. uniformis</i>	0	73	162	70	08	0	0	0	0	0	0	0	313
<i>Ae. aegypti</i>	0	0	03	07	03	03	04	04	05	03	03	0	35
<i>Ae. albopictus</i>	0	0	0	0	02	02	04	03	0	0	0	04	15
<i>Ar. sublbatus</i>	03	04	09	011	04	05	06	07	03	04	02	0	58
<i>An. stephensi</i>	04	07	02	02	02	01	04	06	03	03	02	05	41
<i>Anopheles subpictus</i>	07	03	09	08	11	02	20	16	09	07	05	10	107
<i>An. vagus</i>	0	0	02	02	0	0	0	0	0	0	0	0	04
<i>Total</i>	43	140	243	134	47	23	60	54	26	20	16	31	837

4.4 Analysis of Blood meals of *Cx. quinquefasciatus*

Mosquitoes suck blood both from human host and variety of other animal hosts. However, some species show preference for human host (anthropophilic) and some other species show preference for animal host (zoophilic). Feeding behavior of vector mosquitoes showing preference for human host, directly influences the intensity of transmission of mosquito borne diseases. High anthropophilic index of the vector species increases the risk of transmission.

110 samples of blood meals of *Cx. quinquefasciatus* collected from Panaji were analyzed to find out the source of blood meal of the vector species. Out of 110 samples tested, 95 blood meals positively reacted against the human antiserum, six blood meals reacted against bovine antiserum and remaining 8 samples did not react both against antihuman and antibovine sera.

The positive controls (known serum of human and bovine) reacted against the respective antiserum (human or bovine). The results revealed very high anthropophilic index of 86.36% for *Cx. quinquefasciatus*. The test samples found negative to both antihuman and antibovine sera indicated that the source of blood meals may be from some other animals.

4.6. Parity rate, Infection rate and Infectivity rate

A total of 6032 *Cx. quinquefasciatus* females were collected from thirty fixed catching stations in Patto, Mala, Bhatulem, St. Inez, Kamrabhat and Tonca areas in Panaji. Mosquito collections were made on weekly basis by spending 10 minutes in each catching station. 20 man hours were spent in each month. Total number of *Cx. quinquefasciatus* females collected in different months during July, 2006 to June, 2007 were : 501, 346, 191, 304, 520, 735, 891, 690, 581, 422, 368 and 483 respectively. For the same months, per man hour densities of *Cx. quinquefasciatus* were 25.05, 17.30, 9.55, 15.20, 26.00, 36.75, 44.55, 34.50, 29.05, 21.10, 18.40 and 24.15. The peak density was observed in January and the lowest density in September (Fig. 33). The results of the dissections of ovary to find out parity rate and dissections of head, thorax and abdomen to detect the filarial parasites are presented here.

Dissection of ovary - Parity Rate.

Approximate age of the adult female mosquito is determined by dissecting the ovary to find out the number of dilations present on the ovariole. Females having one or more dilations are parous mosquitoes and females without any dilation are nulliparous mosquitoes. The presence of number of dilations indicate the number of ovipositions that have already taken place. Females having one dilation are uniparous, two dilations are biparous and so on. The number of parous mosquitoes determine the parity rate (%) of the species in the population and indicate the age structure of the population which can be related to the transmission risk of the disease causative organism.

Parous and Nulliparous *Cx. quinquefasciatus* Females.

Cx. quinquefasciatus females comprised of both parous and nulliparous mosquitoes. The number dissected, number of nulliparous and parous females and parity rate in each month to the total parous females found from July, 2006 to June, 2007 are given in Table. 48. Out of 1980 females dissected 659 females (33.28%) were parous and the remaining 1321 females (66.72%) were nulliparous. The number of females with different parous conditions and their percent contribution to total parous conditions are given in Table. 49. Among the parous mosquitoes, 459 females (23.18%) were uniparous, 147 females (7.42%)- biparous, 42 females (2.12%)- triparous and 11 females (0.55%) – quadriparous, indicating the different age structure of the species.

Monthly Parity Rate of *Cx. quinquefasciatus* Females

Monthly parity rate and per man hour density during different months are presented in Fig. 33. Monthly parity rate ranged between 26.11 (February) and 46.60 (September). The percent contribution by the number of parous females in each month to the total parous females for 12 months ranged between 6.37% (March) to 10.31% (January). Though, parity rate was highest in September, the percent contribution to total parous females by the number of parous females during the month was low (7.28%).

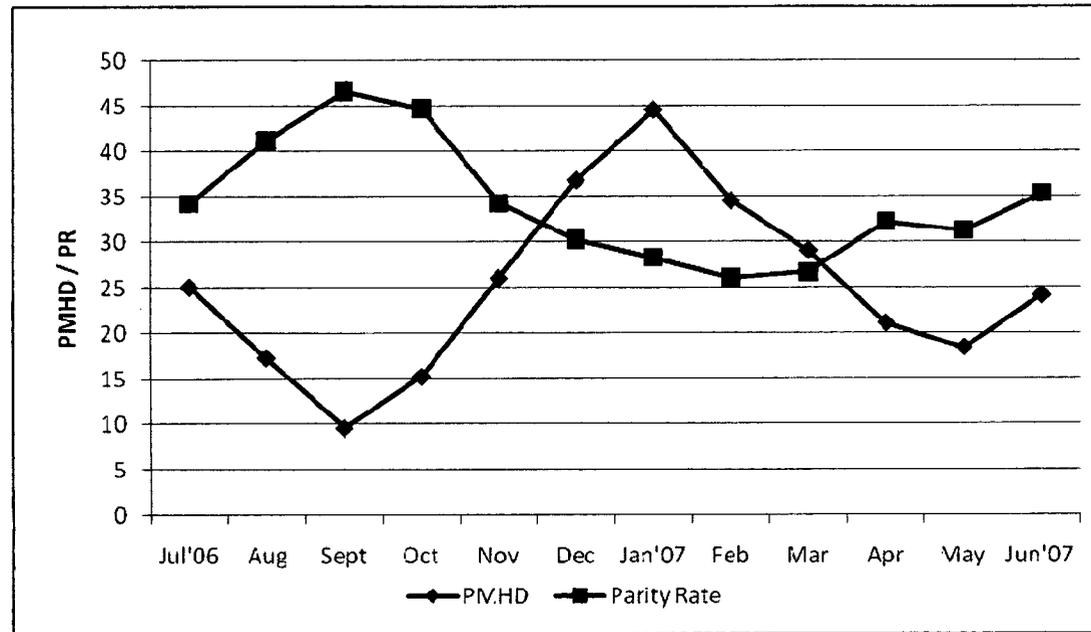


Fig. 33: Correlation of Per man hour density (PMHD) and Parity rate (PR) of *Cx. quinquefasciatus* in different months in Panaji, Goa during July, 2006 to June, 2007

Table 48: Number and Percent Contribution of Nulliparous and Parous *Cx. quinquefasciatus* in different Months in Panaji, Goa during July, 2006 to June, 2007

Month	Number dissected	Nulliparous Females		No. of Parous Females	Parity Rate
		Number	% to total dissected		
July, 06	181	119	65.75	62	34.25
August	148	087	58.78	61	41.22
September	103	055	53.40	48	46.60
October	123	068	55.28	55	44.72
November	178	117	65.73	64	34.27
December	221	154	69.68	67	30.32
January,07	240	172	71.67	68	28.33
February	180	133	73.89	47	26.11
March	157	115	73.25	42	26.75
April	152	103	67.76	49	32.24
May	147	101	68.71	43	31.29
June, 07	150	97	64.67	53	35.33
Total	1980	1321	66.72	659	33.28

Table 49: Percent contributed by different Parous conditions to total Parity rate of *Cx. quinquefasciatus* in different months during July, 2006 to June, 2007

Month	Uniparous		Biparous		Triparous		Quadriparous	
	No.	% to total	No.	% to total	No.	% to total	No.	% to total
July, 06	39	62.90	18	29.03	04	6.45	01	1.61
August	45	73.77	13	21.31	03	4.92	00	0.00
September	33	68.75	10	20.83	03	6.25	02	4.17
October	41	74.55	11	20.00	02	3.64	01	1.82
November	44	68.75	12	18.75	05	7.81	03	4.69
December	53	79.10	09	13.43	04	5.97	01	1.49
January,07	43	63.24	17	25.00	06	8.82	02	2.94
February	30	63.83	12	25.53	04	8.51	01	2.13
March	31	73.81	08	19.05	03	7.14	00	0.00
April	33	67.35	14	28.57	02	4.08	00	0.00
May	29	67.44	12	27.91	2	4.65	00	0.00
June, 07	38	71.70	11	20.75	04	7.55	00	0.00
Total	459	69.65	147	22.31	42	6.37	11	1.67

In December and January months, parity rates were low. But, both the months contributed high percentages to total parous females. The dry months (February to May) had both low parity rates (26.11 to 32.24) and low percent contributions (6.37% to 7.43%).-Month of March had both low parity rate and lowest percent contribution.

The significance in the variations of parity rate in different months was analysed by Two Way Analysis of variance. The results showed the significance in the monthly parity rates ($F=4.2758$; $p=0.0004$) (Table 50). Tuckey a *post hoc* test (Table 51) further justified the significance in variations in parity rate between different months ($p<0.05$). The results showed the significant difference between the parity rates in January- March months and September-October months. The correlation analysis carried out to find out any correlation between parity rate and per man hour density showed the correlation as with the increase in per man hour density increased, the parity showed the decrease (Table 52).

Seasonal fluctuations in Per man hour Density and Parity Rate.

The number and mean per man hour density of *Cx. quinquefasciatus* females during different seasons were: 2061 (PMHD=25.76) in pre-monsoon, 1521 (PMHD=19.01) in monsoon and 2450 (PMHD=30.62) in post-monsoon season. Per man hour density was the lowest in monsoon and the highest in post-monsoon season. Total number dissected, number found nulliparous and parous and parity rates in three seasons are given in Table. 53.

Table 50: Significance of variations in Parity Rate between the months.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Between months	11	2364.63	214.9663	4.2758	0.0004*
Within months	36	1809.92	50.2756	-	-
Total	47	4174.55	-	-	-

Note: p-value *significant at 5% level of significance ($p < 0.05$)

Table 51: Tuckey a *post hoc* test to justify variations in Parity Rate between months.

Months	January	February	March
Means	28.5300	26.3920	26.5850
September	0.0275*	0.0090*	0.0100*
October	0.0364*	0.0121*	0.0134*

Note: *p-value significant at 5% level of significance ($p < 0.05$)

Table 52: Correlation between Monthly Parity Rate and PMHD.

Variables	Parity Rate	PMHD
Parity Rate	1.0000	-0.5424
PMHD	-0.5424*	1.0000

Note: *p-value significant at 5% level of significance ($p < 0.05$)

Table 53: Seasonal Variations in Parity Rate of *Cx. quinquefasciatus* during July, 2006 to June, 2007 in Panaji, Goa.

Season	No. Dissected	Nulliparous		No. Parous	Parity Rate
		No.	% total		
Premonsoon	636	452	71.1	181	28.45
Monsoon	582	358	61.5	224	38.48
Postmonsoon	762	511	67.1	254	33.33
Total	1980	1321	66.7	659	33.28

Seasonally, parity rate was highest in monsoon (38.48%) and lowest in pre-monsoon (28.45%). During post-monsoon season, it was 33.33%. The percent contribution by each season to total parous *Cx. quinquefasciatus* females was different. The percent contribution by the number of parous mosquitoes was found to be the highest (38.54%) in the post-monsoon season followed by the monsoon season (33.99%) and it was the least (27.46%) in pre-monsoon season indicating the highest number of parous females in post-monsoon.

Dissection of Head, Thorax and Abdomen - Infection Rate and Infectivity Rate of *Cx. quinquefasciatus*.

Cx. quinquefasciatus females when bite and suck the blood from the microfilaria carrier, the filarial parasites enter the mosquito body and develop further to L-1, L-2 and L-3 stage larvae. Head, thorax and abdomen regions of the mosquito body were dissected and examined to detect the filarial parasites.

A total of 3562 *Cx. quinquefasciatus* females collected from the field, were dissected during July, 2006 to June, 2007 and the results are given in Table 54. The results of dissections revealed the presence of L-1 and L-2 stage parasites in the thorax region and L-3 stage in head region of the mosquito body. Twelve *Cx. quinquefasciatus* females were found positive for filarial infection. Out of 12, 9 were positive for L-1 and L-2 stage and 3 were positive for L-3 stage parasites. The number of L-1 and L-2 larvae per infected mosquito ranged from 3 to 7 and L-3 larvae per infective mosquito from 2 to 5. The number of infected mosquitoes found in different months were – 1 each in July and September, 4 in October, 4 in January and 2 in February month. The infection rates in different months were: 0.30 in July, 0.59 in September, 1.60 in October, 0.87 in January and 0.52 in February. The infected mosquitoes were found during monsoon (July and September), post-monsoon (October and January) and pre-monsoon month (February). The estimated numbers of infected *Cx. quinquefasciatus* biting / person / month were found to be 10.64, 13.48, 69.95, 34.33 and 17.97 in July, September, October, January and February months (Table 55).

**Table 54: *Cx. quinquefasciatus* - Infection Rate and Infectivity Rate
in different Months during July,2006 to June,2007 in Panaji, Goa.**

Month	No. Females collected	No. Dissected	No. Positive for L1, L2, L3	No. Positive Only for L3	Infection Rate	Infectivity Rate
July, 06	501	328	01	0	0.30	0
August	346	247	0	0	0	0
September	191	167	01	0	0.59	0
October	304	249	04	02	1.60	0.80
November	520	340	0	0	0	0
December	735	430	0	0	0	0
January.07	891	455	04	01	0.87	0.21
February	690	380	02	0	0.52	0
March	581	296	0	0	0	0
April	422	235	0	0	0	0
May	368	202	0	0	0	0
June, 07	483	233	0	0	0	0
Total	6032	3562	12	03	0.33	0.08

Table 55: Estimation of number of infected and infective *Cx. quinquefasciatus* mosquitoes biting in different months based on mean landing rate/bait/night and infection rate in Panaji, Goa.

Month (a)	Infection Rate (d)	Infectivity Rate	X No. biting/ bait/night (e)	Estimaed No. biting/ bait/night/month (f)	Estimated No. of infected bites /person / month (g)	Estimated No. of infective bites /person / month (g)
July, 06	0.305	0.0	112.5	3487.5	10.64	0.0
September	0.599	0.0	75.0	2250.0	13.48	0.0
October	1.606	0.80	140.5	4355.5	69.95	34.84
January.07	0.879	0.21	126.0	3906.0	34.33	08.20
February	0.526	0.0	122.0	3416.0	17.97	0.0

Cx. quinquefasciatus females with L-3 stage of parasites were found during two months- October and January. Two infective mosquitoes were found in October and one mosquito in January. Infective rates for these months were 0.80 and 0.21 respectively. The estimated numbers of infective *Cx. quinquefasciatus* biting / person / month were found to be 34.84 and 8.20 during October and January months.(Table 55). Both infected and infective mosquitoes were encountered in October and January months of post-monsoon season.

4.7 Larval and Adult Susceptibility Status to Insecticides.

The larval and adult populations of *Cx. quinquefasciatus* were tested to assess their current susceptibility/resistance status to different commonly used chemical insecticides and the results of the tests are presented here.

***Cx. quinquefasciatus* - Larval Susceptibility Status.**

The results of susceptibility tests conducted with the III-IV stage larvae of *Cx. quinquefasciatus* against different concentrations of Fenitrothion, Malathion, Fenthion and Temephos chemicals are given in Table 56. The details of results of tests conducted with 4 replicates against each concentration of Fenitrothion, Malathion, Fenthion and Temephos are given under Tables 1-4 in Appendix I.

Tests against Fenitrothion : The exposure of 100 larvae each to 0.025 mg/l. concentration of Fenitrothion resulted in 13.0% mortality, 0.05 mg/l. concentration in 46.0% mortality and 0.075 mg/l. in 69.8% mortality. The highest mortality of 89.0% was produced by 0.125 mg/l. conc. of Fenitrothion. The observed mortality in control sets was only 4%. LC₅₀ and LC₉₀ values were found to be 0.0632 mg/l and 0.1131 mg/l respectively. The results indicated a low level of resistance developed by the larvae of *Cx. quinquefasciatus* to Fenitrothion.

Table 56: Susceptibility status of *Cx.quinquefasciatus* III and IV stage larvae to different concentrations of Fenitrothion, Malathion, Fenthion and Temephos larvicides in Panaji, Goa.

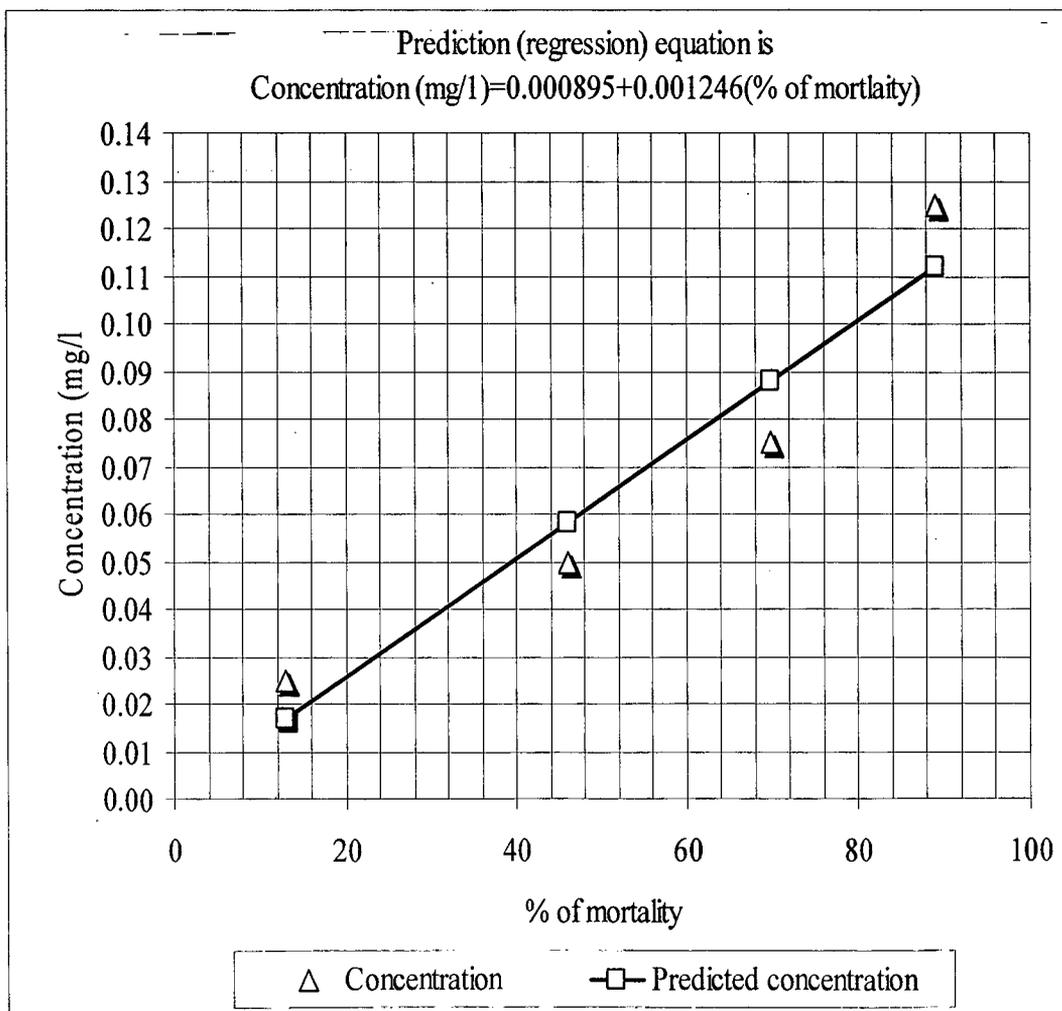
Different Concentration (mg/l.)	Number of Replicates	Control		Experiment		% Corrected mortality
		No. Exp.	No. Dead	No. Exp.	No. Dead	
Fenitrothion						
0.025	4	100	0	100	13	13.00
0.05	4	100	0	100	46	46.00
0.075	4	100	4	100	71	69.80
0.125	4	100	0	100	89	89.00
Malathion						
0.0625	4	100	0	100	0	0.0
0.125	4	100	0	100	6	6.00
0.500	4	100	2	100	17	15.30
3.125	4	100	0	100	57	57.00
Fenthion						
0.0125	4	100	0	100	2	02.0
0.025	4	100	0	100	43	41.8
0.05	4	100	0	100	91	91.0
0.125	4	100	0	100	100	100.0
Temephos						
0.0125	4	100	0	100	2	2.00
0.03125	4	100	0	100	54	54.00
0.0625	4	100	0	100	85	85.00
0.125	4	100	0	100	100	100.0

Tests against Malathion : The exposure of 100 larvae to 0.0625 mg/l. concentration of Malathion did not show any larval mortality. The exposure to 0.125 mg/l. concentration resulted 6.0% mortality and 0.500 mg/l resulted 15.3% mortality. The highest mortality of 57.0% mortality was produced by 3.125 mg/l. concentration of malathion, Only 2% of mortality was noticed in control sets. LC₅₀ and LC₉₀ values were found to be 2.6654 mg/l and 4.9174 mg/l respectively. The results indicated that *Cx. quinquefasciatus* larvae have developed very high degree of resistance to malathion.

Tests against Fenthion : The exposure of 100 larvae each to 0.0125 mg/l., 0.025 mg/l., 0.05 mg/l. and 0.125 mg/l. concentrations of Fenthion resulted in 2.0%, 41.8%, 91.0% and 100.0% of *Cx. quinquefasciatus* larvae respectively. There was nil mortality in control sets. LC₅₀ and LC₉₀ values were found to be 0.0453 mg/l and 0.0814 mg/l respectively. The results revealed the susceptible status of the *Cx. quinquefasciatus* larvae to 0.125 mg/l. concentration of Fenthion.

Tests against Temephos : The exposure of 100 larvae each to 0.0125 mg/l., 0.03125 mg/l., 0.0625 mg/l. and 0.125 mg/l. concentrations of Temephos resulted in 2.0%, 54.0%, 85.0% and 100.0% of *Cx. quinquefasciatus* larvae respectively. There was nil mortality in control sets. LC₅₀ and LC₉₀ values were found to be 0.0476 mg/l and 0.0874 mg/l respectively. The results revealed the susceptible status of the *Cx. quinquefasciatus* larvae to 0.125 mg/l. concentration of Temephos.

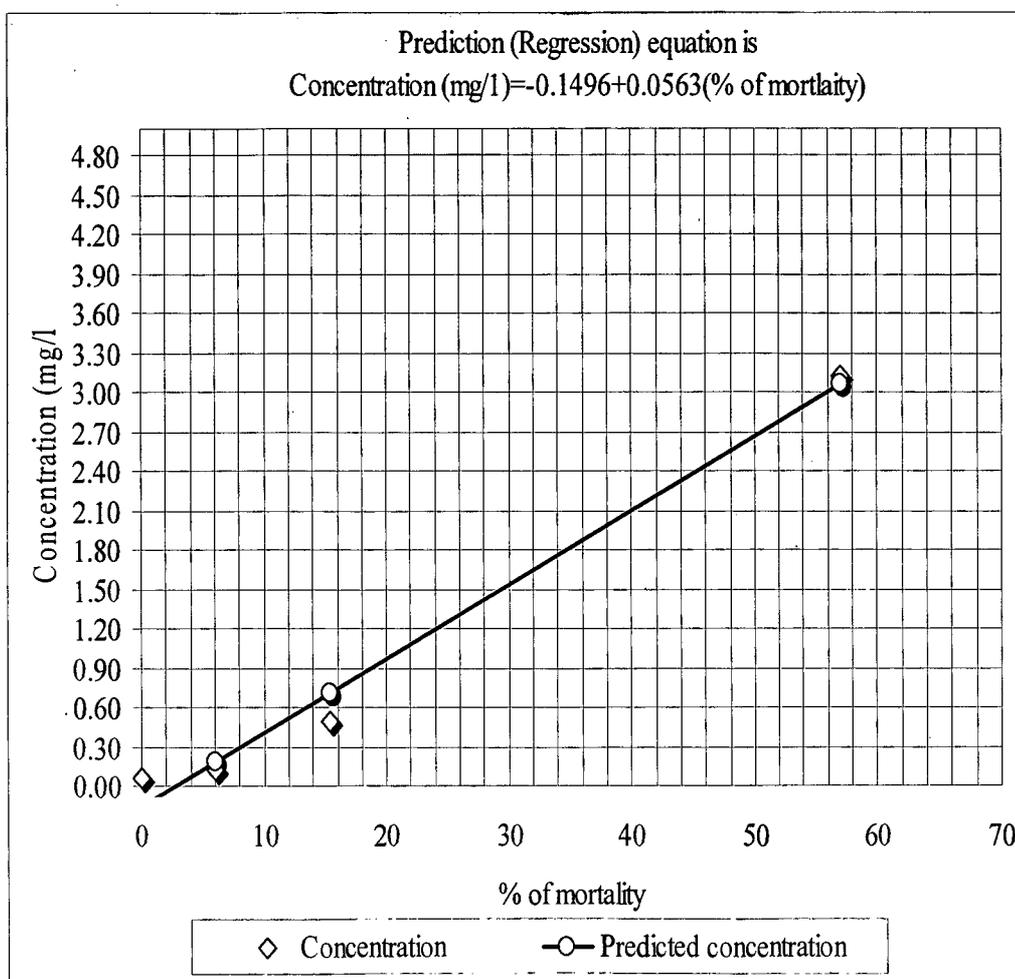
Fig.34: Relationship between concentration and % of mortality in Fenitrothion Insecticide



LC₅₀ values = 0.0632 mg/l.

LC₉₀ values = 0.1131 mg/l.

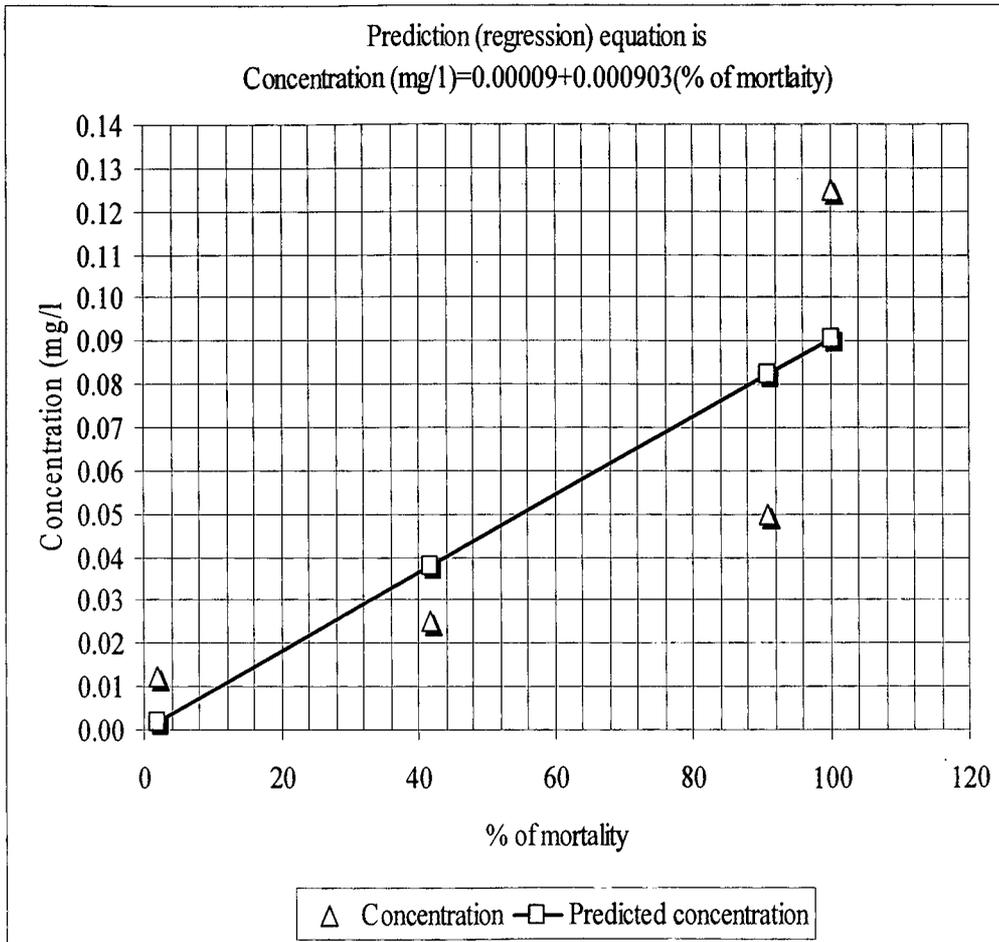
Fig.35: Relationship between concentration and % of mortality in Malathion Insecticide



LC₅₀ values = 2.6654 mg/l.

LC₉₀ values = 4.9174 mg/l.

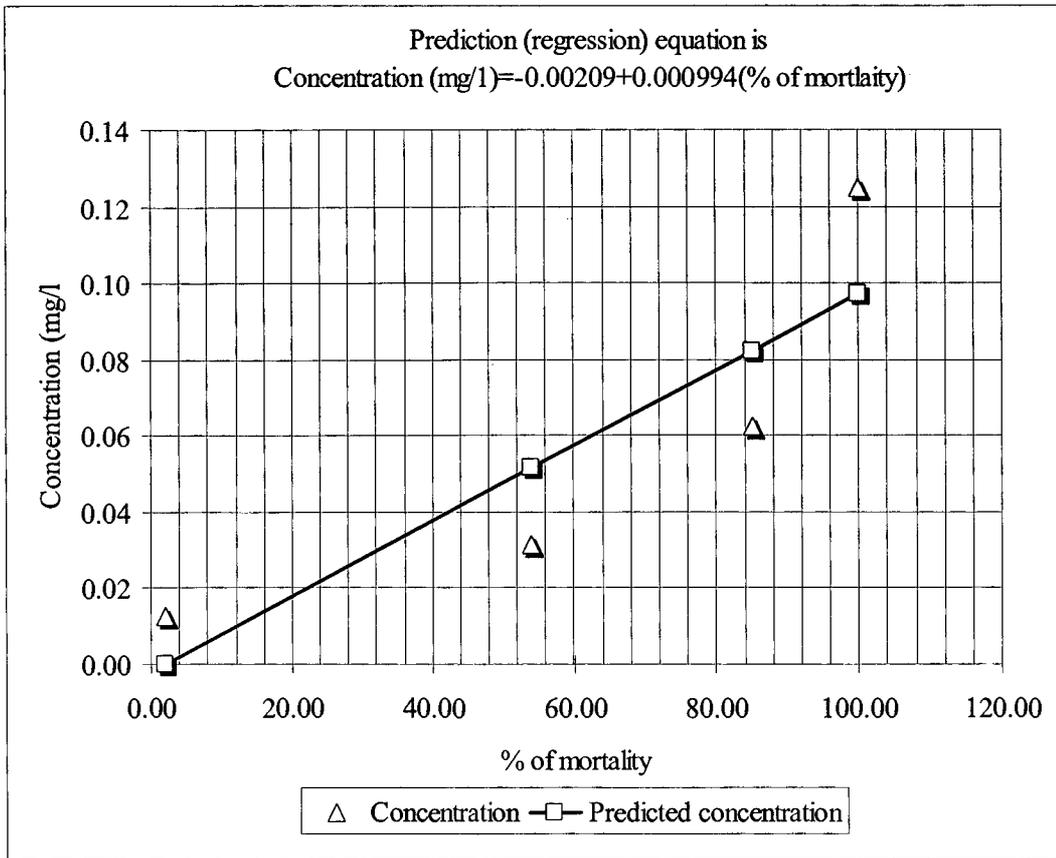
Fig.36:Relationship between concentration and % of mortality in Fenthion Insecticide



LC₅₀ values = 0.0453 mg/l.

LC₉₀ values = 0.0814 mg/l.

Fig.37: Relationship between concentration and % of mortality in Temephos Insecticide



LC₅₀ values = 0.0476 mg/l.

LC₉₀ values = 0.0874 mg/l.

Adult Susceptibility status of *Cx. quinquefasciatus*

The results of susceptibility tests conducted with the adult females of *Cx. quinquefasciatus* against the diagnostic dosages of DDT, Malathion, Deltamethrin and Permethrin are presented in Table 57. The details of results of tests conducted with five replicates against the diagnostic doses of DDT, Malathion, Deltamethrin and Permethrin are given in Table 5 in Appendix I.

Tests against DDT : The exposure of 100 females of *Cx. quinquefasciatus* in four replicates to the diagnostic dose of 4% DDT resulted only 25.77% mortality. The observed mortality in control sets was only 3%. The results revealed very high degree of resistance to DDT in the adult population.

Tests against Malathion : The exposure of 100 females of *Cx. quinquefasciatus* against 5% Malathion resulted only 47% of mortality in adult females. The mortality in control sets was nil. The vector species has also developed very high degree of resistance to Malathion.

Tests against Permethrin : The exposure of 100 females of *Cx. quinquefasciatus* to 0.25% Permethrin produced high mortality of 96.81%. 6% of mortality was observed in the control sets. The results indicated the development of low degree of tolerance to Permethrin in adult female population of *Cx. quinquefasciatus*.

Table 57: Susceptibility status of *Cx.quinquefasciatus* adult females to diagnostic doses of DDT, Malathion, Permethrin and Deltamethrin insecticides in Panaji, Goa

Insecticide (Conc.)	Number of Replicate	Exposure Period	Control		Experiment		% corrected mortality
			No. Exposed	No. Dead	No. Exposed	No. Dead	
DDT (4%)	5	4 hours	100	03	100	28	25.77
Malathion (5%)	5	1 hour	100	0	100	47	47.0
Permethrin (0.25%)	5	3 hours	100	06	100	97	96.81
Deltamethrin (0.025%)	5	1 hour	100	05	100	98	97.89

Tests against Deltamethrin : The exposure of 100 females of *Cx. quinquefasciatus* to 0.025% Deltamethrin resulted high mortality of 97.89%. The observed mortality in control sets was only 5%. The vector species showed low degree of tolerance to Deltamethrin also. The response of vector species to both the synthetic pyrethroid insecticides was found to be similar.

Chapter 5 - DISCUSSION

Breeding Behavior of *Cx. quinquefasciatus*

Mosquitoes are known to breed in several types of breeding habitats. The selection of the breeding places by the mosquito species depends on the location, specific requirement of physico-chemical characteristic of their breeding waters, presence of larval competitors and predators and other biological factors such as fauna and flora in the breeding habitat (Sinha, 1976; Kaul et al. 1977; Blaustein & Kotler, 1993; Shilulu et al. 2003). *Cx. quinquefasciatus* is known to breed in diverse ecological niches (Barraud 1934; Chow and Thevasagayam 1957; Fernando 1963; Mattingly 1969; Muturi et al. 2007 a,b). Bang (1989) stated that urbanization has led to ecological degradation favoring the breeding of *Cx. quinquefasciatus* in many cities throughout the tropical and subtropical areas of Southeast Asia region.

Kaul et al. (1977) found that heavy breeding of *Cx. quinquefasciatus* was associated with the high pollution conditions and the water temperature in the range of 14°C and 30°C and the temperature below and above this range seemed to act as limiting factor. For mosquitoes, water temperature of 35 °C is fatal to the larvae and the lower lethal temperatures are around 7°C to 10°C (Rajagopalan, 1980). Hassan et al. (1993) reported that *Cx. quinquefasciatus* larvae were most abundant in polluted drains and further stated that in most areas of its distribution, the species preferred habitats rich in dissolved matter and such habitats tend to have high total dissolved substance (TDS), which is the sum of all organic, inorganic and suspended solids in water. De Alwis & Munasinghe (1971) and Sarkar et al. (1978) observed that the pH of the water in the breeding habitat is important for *Cx. quinquefasciatus* and the optimum pH range was found between 7 and 8.6 in the polluted drains.

The high breeding of *Cx. quinquefasciatus* in polluted habitats like drains, cesspits, cesspools, septic tanks, ditches etc. have been reported from elsewhere in the country and outside by different workers (Kaul et al., 1977; Rajagopalan et al., 1977; Kumar and Chand 1990; Batra et al., 1995; Urmila et al., 1999, Sukhvir Singh et al., 2000; Murty et al., 2002). In the present study, the observed highest breeding index as well as per dip density of *Cx. quinquefasciatus* in the drains followed by cesspits containing the polluted waters are on the similar lines. However, *Anopheles* and *Aedes* mosquitoes preferred the clean water sources for their breeding.

The breeding of this species has also been reported in a large variety of other habitats from several areas including both urban and rural areas. Ground pools, wells, cisterns, domestic receptacles, curing water collections inside buildings under constructions, water meter chambers, water pits, ponds, metallic drums, cut bamboos, tree holes, cemented tanks, fallen coconut shells, rock pools, paddy fields, stream beds, irrigational channels, seepage areas, marshes, underground tank, overhead tank, water tanks kept both inside and outside, earthen pots, tyres, unused battery cases were found with the breeding of *Cx. quinquefasciatus* from different areas in the country and abroad (Yasuno, 1977; Narayanan and Maruthanayagam, 1985; Malhotra et al., 1987; Srivastava, 1989; Kulkarni and Naik, 1989; Kaliwal, 1991; Ashwani Kumar and Thavaselvam, 1992; Gupta et al., 1992; Muturi et al., 2007 a & b; Muturi et al., 2008). In the present study also, the breeding of *Cx. quinquefasciatus* was encountered in various niches.

As observed in the present study, overall breeding of *Cx. quinquefasciatus* was reduced from the breeding index of 6.54 in the pre-monsoon to 2.95 in the monsoon

period. For the corresponding seasons, the breeding index of *Cx. quinquefasciatus* in drains and cesspits also reduced from 19.05 to 5.65 and 14.35 to 5.52 respectively. Physical impact of heavy rains by flooding and flushing of drains, cesspits and other outdoor breeding foci during monsoon period appeared to be the major limiting factor to reduce the breeding in monsoon months. Similar observations on the influence of rainfall has been reported from Pondicherry by Menon & Rajagopalan (1980) and from Cochin by Eapen & Chandrahas (1994).

Based on the season, the relative importance of the type of breeding sites may vary from one area to another. In Delhi rural area, innumerable irrigation wells were the main breeding habitats in dry months when water conditions were ideal for breeding and during rainy season there was a shift in breeding places when domestic receptacles were important (Yasuno et al., 1977). In the dry season, the drains were more important while during monsoon season the cesspits were equally responsible for adult emergence of *Cx. quinquefasciatus* in Pondicherry (Menon and Rajagopalan, 1980). In the present study also, a tilt in the breeding behavior of the species to the breeding habitats (curing water collections, barrels and water storage tanks kept inside the buildings under constructions) other than the drains and cesspits was noticed in Panaji, probably due to the impact of rains during monsoon season. Therefore, the habitats other than the drains and cesspits played a supporting role by providing the niches for the breeding of *Cx. quinquefasciatus* during rainy season.

The present study enabled to identify the type and relative importance of breeding habitats and seasonal variations in the breeding of *Cx. quinquefasciatus*. With this knowledge, it would be possible to focus antilarval control activities on these sites

selectively in different seasons and help to effectively utilize the available resources and man power which may not be sufficient to deal with every potential breeding site. This approach also provides a basis for devising an appropriate cost-effective vector control strategy for each type of breeding habitat in different seasons. In this context, it is worth mentioning that 'nature' itself limits filarial transmission in a number of ways such as by reducing vector populations due to heavy rainfall, parasitic load and the ecological factors like temperature and humidity and many other bearings (Chandra, 2008). Bio-environmental approach can reduce/eliminate many man-made breeding sources and thus, minimize the use of chemicals to the possible extent.

***Cx. quinquefasciatus*- Development from Egg to Adult.**

The number of eggs laid by the female mosquitoes in the rafts are variable. After oviposition also, the number of eggs hatching to larvae, growing to pupae and finally emerging to adults is also variable due to various factors. The study on these aspects provide information on fecundity, survival/mortality at different stages of development and net emergence of adults. However, there are very few studies on the fecundity of wild mosquito populations, even for some medically important species (Yang et al. 2005). In the field conditions, it is extremely difficult to obtain reliable measurements from field populations due to several factors like fluctuating temperature, food supply, higher density, competition, predators and disease (Service, 1976). The present attempt was made to study the developmental aspects on the wild females of *Cx. quinquefasciatus* in the laboratory.

The positive correlation of egg production with the size of the mosquito and size of the blood meal taken by the mosquito have been reported (Briegel, 1990; Hurd et al.,

1995). Das et al. (1967) observed the positive correlation of insemination with oviposition and viability of eggs in their laboratory experiments and the eggs laid by virgin females were found to be non-viable. Zharov (1980) reported that the number of eggs laid by *A. vexans* ranged between 156 and 198 per female. Chadee and Haeger (1986) reported that *Cx. quinquefasciatus* from a wild population laid 30-350 eggs per raft. Yang et al. (2005) dissected the gravid females and found that un-deposited eggs per gravid female ranged from 80.9 to 163.1 for *Cx. quinquefasciatus* and 29.8 to 71.7 for *Aedes nocturnus* in the wild populations on the island of Kauai, Hawaii.

Panicker and Rajagopalan (1984) studied the biology of *Anopheles subpictus* in the laboratory. The no. of eggs laid by individual *Anopheles subpictus* female ranged from 38 to 286, the mean hatchability of eggs was 64.7%, the rate of pupation ranged from 15.8 to 80.7% and the average male to female sex ratio was 1 : 1.04. The observed mean range of duration of various immature stages in hours were: eggs-40.8±0.75, I instar-33±1.02, II instar-35.5±2.23, III instar-43.6±1.65, IV instar-77.9±3.96, Pupae-27.3±1.32. Salazar and Moncada (2004) found that *Cx. quinquefasciatus* eggs ranged from 152 to 203 in the rafts, the hatch rate was 62.5% and 98.6% adults emerged from the pupal stage at the ambient environmental conditions in Colombia. Almiron & Brewer (1996) while studying the biology of *Cx. quinquefasciatus* in Argentina, found that the highest relative mortality rates occurred during egg and larval stages and total pre-adult mortality varied between 59.1% and 89.1%.

The average number of eggs per raft collected from the villages near Delhi was between 90 and 150 throughout the year, there seemed to be no seasonal variation in the fecundity of the wild population of *Cx. quinquefasciatus* in the area and the duration

lapsed from egg to adult emergence in June ranged from 11 days to 19 days (Yasuno, 1974). The number of eggs laid in each raft of *Cx. quinquefasciatus* ranged from 140-340, eggs hatched to larvae in 1-2 days, development from egg to adult took 8 to 12 days in summer and the duration was temperature dependant (Savage & Miller, 1995). According to Shelton (1973), the time required for *Cx. quinquefasciatus* from egg hatching to adult under 20°-23°C was about 8 days. According to Gomez et al. (1977), at 26±2°C, the duration for development from egg to adult was 10.57 days for females and 10.29 days for males.

In the present study, the percent of eggs (65.27%) hatched to larvae conformed to the observations made by Panicker and Rajagopalan (1984) and Salazar and Moncada (2004). The findings on high mortality at egg stage and larval stage are in line with the results of Almiron & Brewer (1996). The percent of adult emergence from the pupal stage conformed to the observations made at the ambient environmental conditions in Colombia (Salazar and Moncada, 2004). Male to female ratio was almost similar as observed in *An. subpictus* and the duration observed for development from egg to adult emergence in the present study was also closely similar to the earlier findings (Panicker & Rajagopalan, 1984; Savage & Miller, 1995).

In the absence of the predators, the observed pre-adult mortality may be attributed to the non-viable eggs, any infection carried from the wild population to the eggs or any other biotic or abiotic factors. In spite of, the study is made on the wild females of *Cx. quinquefasciatus* in the laboratory at ambient climatic conditions, the present study provided the useful information on the fecundity, survival and the duration required by each stage of immature during the developmental cycle.

Prevalence, Seasonal Distribution, Abdominal status and Resting Behavior of Adult *Cx. quinquefasciatus*.

Transmission of infection through vectors is considered to be a density dependant phenomenon. The density pattern depicted by vector species in any area is influenced by the gross ecology of the terrain and the meteorological variables (Kaul & Wattal, 1968) and as such, the findings on vector populations of one geographic region cannot be applied to the other. It is important to understand the density build up pattern and abundance of the vector species during different seasons which influence the disease transmission in time and space. It is also essential to know about the resting behavior so that the appropriate anti-vector measures could be suggested to prevent/reduce the risk of transmission.

Cx. quinquefasciatus was the most predominant mosquito species in indoor resting collection and contributed 81.99% to the total number of mosquitoes collected indoor. The prevalence of the species was high in all the zones of Panaji. Though, density variation between the zones was statistically not significant, comparatively higher densities of *Cx. quinquefasciatus* encountered in zone IV and V is attributed to the presence of nallah which is running in zigzag fashion and providing breeding ground, as the vector species prefers to breed in polluted waters. Zone-wise prevalence in Panaji was found to be in line with the observations made in Raipur, India where the locality-wise density variations were statistically not significant (Dixit et al., 2002).

During the present study, observed two density peaks of *Cx. quinquefasciatus* were wide apart. The first peak during July appeared to be associated with the pre-monsoon showers in April-May months and the onset of monsoon rains in the month of June in Goa. The subsequent decline in the adult density in August and September

months is attributed to flushing of drains, flooding of other outdoor breeding foci and the mortalities caused due to physical impact of heavy rains. There was 2244.4 mm of rain from July to September months in the year 2005. In urban areas, most of the mating activity in swarms takes place outdoor though some activity has also been observed indoor (Menon & Rajagopalan, 1977). Immediately after emergence, adult mosquitoes rest outside near their breeding places and only after mating, females enter the houses for their blood meals, thus the newly emerged mosquitoes during rainy season are either destroyed or their dispersal is reduced due to the physical impact of rains (Rajagopalan, 1980).

The population of *Cx. quinquefasciatus* started building up again in October due to the cessation of heavy rains and the stagnations in the drains, cesspits etc. during the post-monsoon season and the density gradually attained the second peak in February month. Density variations between the months in Panaji were found to be significant. The impact of rainfall on the density of *Cx. quinquefasciatus* was significant.

The density pattern and the impact of climatic and environmental factors on the prevalence of vector in different seasons have been studied in different areas. In Arthala village near Delhi, the peak density of *Cx. quinquefasciatus* was recorded in April month and density was scarce in January. The population crisis encountered in January was attributed to direct influence of low temperature probably prolonging the development of eggs to adults as well as could be inducing mortality in larval and adult stages (Kaul & Wattal, 1968). In Faridabad, India the peak density was reported in March and April months (Rajagopalan et al., 1977). *Cx. quinquefasciatus* was highly

prevalent during summer and the density gradually decreased during winter and rainy seasons at Kanchrapara, West Bengal, India (De & Chandra, 1994).

On the contrary, in an urban zone of western African Sudan and Savanna, the adults were scarce during hot and dry seasons and the population density increased throughout the rainy season (Subra, 1973). In Pondicherry, India the density declined gradually after February due to drying up of breeding places, later with the first rains of South West monsoon in June-July the density was increased and this trend was reversed in September due to flushing of drains which was marked in November due to very strong North East monsoon (Rajagopalan, 1980). The temperature did not play any role in population regulation in Pondicherry. In Rajahmundry, India the peak density was recorded during the post monsoon season and temperature and rainfall played a vital role in regulating the population of the species (Dhar et al., 1968). The density pattern of *Cx. quinquefasciatus* population in Panaji did not fully conform to any of the above studies. However, it appeared somewhat similar to the observations made in Pondicherry, excepting the impact of North East monsoon seen in Pondicherry.

According to Manoharan et al. (1997) high vector density and the capacity of the vector mosquitoes to pick up mf even from patients with ultra-low levels of microfilaraemia (Southgate, 1992), increase the chances of risk of lymphatic filariasis transmission. In all the months of the year, the observed density of *Cx. quinquefasciatus* females in Panaji was much higher than the estimated level of tolerated density of *Culex quinquefasciatus* up to which there is no risk of filariasis transmission i.e. 34 per ten man hour density (Bhatia & Wattal, 1958; De & Chandra, 1994). Das and Vanamail (2008) reported that both vector density and community microfilaria load (CMFL)

influence the intensity of transmission. Their analysis indicated that there is no risk of transmission as long as CMFL is maintained below 5 microfilaria (mf)/60 mm³ and the vector per man hour density (MHD) is <25, however, the transmission may continue if the vector density is >25 and CMFL is < 5 mf/60 mm³.

In the earlier studies, male to female ratio in indoor resting collection ranged from 1:1.5 to 1:6.5 in different months in Arthala (Kaul & Wattal, 1968) and 1:1.5 to 1:5.8 Rajahmundry (Kanhekar et al., 1994). In a study on the outdoor resting of *Cx. quinquefasciatus* on the shelters, males contributed 76% and females 24% in Sao Paulo, SP (Laporta et al., 2006). Less proportion of males in indoor resting collection is attributed to their exophagic and exophilic behavior and lower life expectancy (Kaul & Wattal, 1968). The present finding on sex ratio is closely similar to earlier observations made in Arthala and Rajahmundry.

The studies on resting behavior have shown mainly endophilic nature of the species in different areas (Barraud, 1934; Kulkarni & Rajaput, 1988; Dixit et al., 2002). In Mysore, India the indoor collection of 46.9% fed, 25% semigravid, 12.5% gravid and only 15.6% unfed *Cx. quinquefasciatus* indicated endophilic nature (Ningegouda & Vijayan, 1992). In the present study also, the indoor collection of 64.7% fed, 12.3% semigravid, 8.2% gravid and only 14.7% unfed confirmed the endophilic behavior of the vector in Panaji. The proportion of fed, semigravid and gravid females together contributing 85.3% to total females not only indicates the high fecundity level in the population but also the chances to translate diseases after feeding on the infected host (Yang et al., 2005).

The various abdominal conditions depict the stage of gonotrophic cycle. The latter is regulated by endogenous rhythms apart from the climatic conditions (Rao, 1984). The frequency of feeding by the mosquito is linked with the digestion of blood, the maturation of eggs and oviposition and these processes proceed simultaneously which is known as the gonotrophical relationship (Rao, 1984). A study on the influence of temperature and relative humidity on the length of the gonotrophic cycle (LGC) of *Cx. quinquefasciatus* within a temperature range of 23.5°C to 31°C and relative humidity range of 54.4% to 84.3% under ambient laboratory conditions demonstrated a significant correlation between the LGC and temperature and relative humidity and observed greater LGC (Median: 20.64) in cooler months (October to March) than in warmer months (Median: 4.69) of April through September in Delhi (Kaul et al., 1984). In the present study, the observed strong correlation between different abdominal conditions of *Cx. quinquefasciatus* females and the meteorological variables and also the significant variations between the different abdominal conditions over different months and the two way interactions of abdominal conditions and months, emphasized the existence of relationship of feeding and gonotrophic cycle with the meteorological variables in the wild populations. Panaji being located on the coastline, without climatic extremities the length of gonotrophic cycle is expected to be shorter, which in turn would influence frequency of feeding.

The highest preferred indoor resting sites were walls and corners in Ghazibad, India (Wattal & Kalra, 1960) and hanging objects in Ernaculum (Pal et al., 1960) and Rajahmundry (Dhar et al., 1968) in India. Resting behavior observed in Panaji city conforms to the findings made in Ernaculum and Rajahmundry, however the

extent of preference for hanging objects in Panaji was higher than in both the places. Spatio-temporal variations in the number of *Cx. quinquefasciatus* both females and males resting on different sites were significant over the months and also in different zones, showing the uniform pattern of resting behavior of the vector species irrespective of the seasons and different geographical zones of Panaji city. Resting behavior of *Cx. quinquefasciatus* showing very high preference for resting on hanging objects, can be exploited for reducing the high densities of vector mosquitoes of filariasis by using long-lasting insecticide nets (LLINs) as personal protective measure under integrated vector management.

Biting Activity of *Cx. quinquefasciatus*.

Mosquitoes bite in order to suck the blood from the host. Biting activity is one of the important aspects of the biology of vectors of human diseases. The intensity of transmission of filarial infection depends upon the high biting density, anthropophily and high survival rates (Wattal, 1976). Many factors influence the number and periodicity of mosquitoes biting man. Haddow (1954) commenting on the biting activity of African mosquitoes, stated that there are different groups within the same species which bite in successive waves and that the factors like wind, 'impulse to bite' and proximity of breeding places influence the numbers biting at a given time. Rajagopalan et al. (1977) reported that biting behavior and the number of mosquitoes biting on human were influenced by many factors, such as physical impact of rains restraining the biting activity, lower density of vector during monsoon and the impact of larvicidal/adulticidal measure.

Cx. quinquefasciatus was the most dominant species among the 13 mosquito species encountered in human bait collection in Panaji. It contributed 78.61% to the total number of mosquito landing collection. Similar observations on the dominance of *Cx. quinquefasciatus* landing on human bait are made from India and abroad (Rajagopalan et al., 1977; Self et al., 1978; Eapen & Chandras, 1994; Chandra, 2001).

Subra (1980) has reported that the biting activity of *Cx. quinquefasciatus* was not uniform in different geographical areas. Peak biting period of *Cx. quinquefasciatus* has been reported variable in different areas. Peak biting activity was observed at midnight hours in Bangkok (Thailand), Rangoon (Burma) and Pondicherry, India (Sasa et al., 1965; De Meillon & Sebastian, 1967; Vanamail & Ramaiah, 1991). In many of the endemic areas of the world biting peak occurred soon after the midnight (Samarawikrema, 1967; Das et al., 1971, Self et al., 1978; Chandra, 2001). Peak biting activity was observed between 2200 h and 2400 h. Khon Kaen city in Thailand (Pipitgool et al., 1998).

Oduola and Awe (2006) observed peak biting during 2200 – 0200 h in Lagos Metropolis Nigeria. Rao et al. (1981) recorded the maximum biting activity in the second quarter of the night (2100 h - 2400 h) during March, June and September months and the maximum biting activity was shifted to the third quarter (0100 h – 0300 h) of the night in December in East Godavari district of Andhra Pradesh. In the present study, hourly peak biting period appeared different from the above findings. The biting in different hours ranged from 6.04% (2400 h-0100 h) to 11.37% (2300 h-2400 h). Two peaks were observed in biting activity, the first higher peak was between 22.00- 23.00 h

and the second comparatively smaller peak between 0500- 0600. The maximum biting occurred in the second quarter of the night.

The biting activity of *Cx. quinquefasciatus* also showed monthly and seasonal variations in different areas. Rajagopalan et al. (1977) recorded the highest biting of *Cx. quinquefasciatus* during hot months (March to June) and the biting was reduced during monsoon with the least biting in July and the number biting in different months did not conform to the seasonal trend recorded in the hand collection of resting mosquitoes in Pondichery. Rao et al. (1981) recorded the highest number (49.8%) of *Cx. quinquefasciatus* biting in March and the lowest number (4.7%) in June in East Godavari district of Andhra Pradesh. Chandra (2001) observed the distinct biting peak of *Cx. quinquefasciatus* in the month of May and seasonally, higher biting activity of *Cx. quinquefasciatus* was in rainy season than in winter and summer in Culcutta.

On the contrary, Eapen and Chandrahas (1994) observed the highest biting activity in post-monsoon and lowest in monsoon season in Cochin. Similarly, in Panaji, maximum biting occurred during post-monsoon months (October to December). The highest per bait per night biting rate recorded in December (183.0) month and the lowest in September (75.0) in the present study conformed to the densities recorded in the indoor resting hand collections made in Panaji.

The highest number of *Cx. quinquefasciatus* females landed on human bait in post-monsoon (40.16%) and the lowest number in monsoon season (28.14%). Seasonally, the average per bait per night landing rate ranged between 108.25 (monsoon) and 154.50 (post-monsoon). The estimated number of *Cx. quinquefasciatus*

biting was 46796.65 / person/annum. The seasonal biting behavior found in Panaji were quite similar to the observations made in Cochin.

Host odour, carbon-dioxide, lactic acid, sebum and sweat are found as attractants for the mosquitoes towards man (Schreck et al. 1990; Knols et al. 1994; Maijerink and Van Loon, 1999). In addition, the environmental factors (odours from other sources, prohibitive wind speeds), physiological conditions of female mosquitoes (circadian phase, gonotrophic stage and nutritional status and the mosquito genotype (olfactory proteins involved in response to external stimuli) have been reported to affect the responsiveness of mosquitoes (Costantini et al., 1998).

The preferred landing sites of *Cx. quinquefasciatus* were legs, hands, body and face in the decreasing order of preference in East Godavary district and Rajahmundry town of Andhra Pradesh (Rao et al., 1981; Kanhekar et al., 1994). In Lagos Metropolis Nigeria, the density of the species preferring the foot region was significantly higher when compared with other different parts of human host such as the ankle, calf and thigh and the strong biting preference for the foot may have been influenced by the characteristic odour of the foot sweat gland output (Oduola and Awe, 2006; Geier et al., 1996). In an experiment involving 8 species of mosquitoes, five species preferred to receive their blood meals at the head, facial region and the upper torso of the human host (De Jong and Knols, 2002). The preference shown by *Cx. quinquefasciatus* for landing on legs and the hands in Panaji was found to be on the similar lines as observed in Andhra Pradesh, India.

De Meillon et al. (1967) observed in Rangoon that the risk of infection was directly proportional to the biting density of the vector. According to Samarawikrema

(1967) the variations in the biting rhythm of different age groups of the mosquito vectors also influence the transmission of filariasis. The estimated numbers of infective bites/person/year were related to filarial endemicity: 400, 179 and 45 infective bites for Sada (Comoro Islands), Moa (Tanzania) and Mambrui (Kenya) respectively; in two villages of Andhra Pradesh (India) 32 infective bites/man/year maintained a microfilariae rate of 16.7% while in a fishing community of Trinidad infective bites as low as 14/man/year has been found enough to maintain a microfilariae rate of 15% (Subra, 1983).

Interestingly, Vanamail and Ramaiah (1991) observed the coincidence of peak biting hour of vector population and the appearance of microfilaria in the peripheral blood at its maximum density in Pondicherry, which will facilitate the optimum infection of vector population. Presently observed high adult density, high biting activity of *Cx. quinquefasciatus* throughout the night and the location of its breeding places in the close vicinity of the human population increase the risk of transmission.

The information on the biting periodicity will be useful in designing the personal protection measures. Human-vector contact could be reduced by a time (peak biting period) and space (while working) targeted approach of adopting personal protective measures such as protective clothing and repellants which can reduce the annual infective biting rate and reduce transmission potentials (Shriram et al.,2005).

The potential opportunities inherent in the use of attractants in the control of mosquito vector have not been fully utilized and the understanding of all the factors involved in mosquito attraction towards human host, will facilitate the development of control options such as the production of mass trapping devices (Oduola & Awe, 2006).

Due to increased problem of resistance to insecticides, it is necessary to exploit other alternative methods of vector control such as screening of doors and windows, use of long lasting insecticide treated nets (LLINs) and mass trapping devices which can form strong components of the integrated vector control management to reduce the man-mosquito contact rates in order to prevent the transmission of the diseases.

Feeding Behavior of *Cx. quinquefasciatus*

Female mosquitoes are known to feed on the blood of wide variety of animals including man, but some vectors exhibit distinct preference in this regard while others are facultative (Rao, 1984). It cannot be safely assumed as postulated by Bruce Chwatt et al. (1966) that the proportion of mosquitoes biting humans will be found to be higher in human dwellings than in other biotopes (Collins et al, 1989). Michael et al. (2001) found that on an average 27% of the mosquitoes caught resting within individual households, had fed on people outside the household and 13% of mosquitoes biting within households contained blood from at least two persons, with the rate of multiple feeding depending on the density of humans in the household. Therefore, it is essential to find out the source of mosquito blood meal to assess its vectorial efficiency in disease transmission. Host feeding or host selection is a product of numerous factors including host preference, host availability and host irritability. Host selection indicates the host actually fed upon and the host preference indicates innate habit of exercising the choice of a host or hosts (Bruce Chwatt, 1966).

In the present study, *Cx. quinquefasciatus* collected from indoor mostly fed on human and showed very high anthropophilic index of 85.36% in Panaji. Several studies

on the host preference of *Cx. quinquefasciatus* have reported that this species is highly anthropophilic in different parts of the world. Service (1963), in northern Nigeria found that 68.6% of blood meals of *Cx. quinquefasciatus* were positive for human blood 25.7% positive for avian blood and 5.7% for others. Ifteara et al. (1994) recorded very high preference for humans from three sites in Bangladesh with 72% to 97% meals positive for human blood. Bogh et al. (1998) also found the anthropophilic index as high as 95.5% in *Cx. quinquefasciatus* collected indoors on the Kenyan coast. Arunachalam (1987) recorded 98.7% anthropophilic index in *Cx. quinquefasciatus* collected from human dwellings in Tamil Nadu, India.

Mahapatra et al. (1995) recorded very high anthropophilic index of 90.7% in Puri district of Orissa. In Raipur, 89.65%, 60% and 63% of blood meals of *Cx. quinquefasciatus* collected from human dwellings, cattlesheds and mixed dwellings respectively, were positive for human blood, thereby showing high preference towards humans irrespective of the type of biotopes (Dixit et al., 2002). Another report from Tucson, Arizona found that 50% *Cx. quinquefasciatus* fed on human blood (Zinser et al., 2004). 74% of the blood meals of *Cx. quinquefasciatus* tested in Kuttanadu (Kerala) were derived from human host (Samuel et al., 2004). High feeding preference of *Cx. quinquefasciatus* strain towards human in Panaji conforms to these observations made elsewhere in India and abroad, suggesting that this species poses high risk of infection of filariasis besides possessing a great nuisance potential.

Elsewhere, the picture was quite different from the above observations. *Cx. quinquefasciatus* showed differential host preference for a variety of animals. Kaushal Kumar et al. (2002) found that *Culex quinquefasciatus* fed on human beings only when

they are in close contact with them otherwise, they were found to be zoophilic in feeding nature. In their study, 41.4% blood meals from human dwelling collection, 19.8% from cattle sheds and 23.8% from mixed dwellings were positive for human blood and overall 23.33% fed on human, 56.16% on bovine, 3.41% on goat, 1.51% on dogs and remaining on pigs and birds in Delhi and nearby villages. Reisen and Boreham (1976) from Pakistan recorded that only 37.6% fed on human blood. Kay et al. (1979) from northern Queensland found only 8.9% fed on human. Molaei et al.(2007) in Harris County, Texas revealed that 39.1% had acquired blood from birds, 52.5% from mammals and 8.3% were mixed mammalian and avian blood meals. Muturi et al.2008 found that only 9.8% of indoor collected populations of *Cx. quinquefasciatus* had human blood.

Although, in many mosquito species there is a genetically determined host preference, the preference shown by a particular species of mosquito for one vertebrate host or the other is likely to be influenced by environmental conditions (Takken, 1991) as well as the ecology and the behavior of both host and vector (Gillies, 1988). Host selection by vectors is thus a result of a combination of intrinsic preferences modulated by extrinsic factors (Mboera and Takken, 1999). Because of close proximity of the breeding places to human habitations and due to the lack of any sizeable numbers of poultry, cattle or other animals, a vast majority of the mosquitoes can be expected to feed on humans in Panaji. The picture of high vector density and high biting activity coupled with the very high anthropophilic index of *Cx. quinquefasciatus* observed in Panaji, increases the risk of transmission of lymphatic filariasis.

Longevity of *Cx. quinquefasciatus*: Parity Rate.

After emergence mosquitoes must live sufficiently long for mating, blood feeding, maturation of eggs and oviposition before there is any new population input from the emerging generation. The ability to determine the age structure and survival of female mosquitoes is of paramount ecological importance as the longevity affects net reproduction rates, dispersal distances and disease transmission. Based on the number of ovipositions made by the females, the approximate age of the mosquitoes is determined. The number of ovipositions by a female depends on a multitude of factors, including the availability of hosts and success in getting the blood meals, intrinsic longevity of the female and survival rates, climatic conditions and intervals between re-feeding (Service, 1976).

Vector parity rate has been studied in several places. DeMeillon et al. (1967) found a lower parous rate in *Cx. quinquefasciatus* collected in a variety of outdoor shelters than in females collected inside houses. The parity rate in Andhra Pradesh (India) was 29.4% with the monthly values ranging between 20.5% and 35.7% and further it was found that the seasonal variations in the climatic conditions did not show marked effect on the daily survival of vector mosquitoes (Rao et al., 1981). Similar phenomenon of climatic conditions on the vector mosquitoes of *Brugia malayi* has been observed by Chandrasekharan et al. (1976) in Kerala.

Nathan (1981) found average parity rate of 28.1% in Trinidad with the monthly values ranging between 16.6% to 37.5%. Self et al. (1978) recorded the parity rate of 23% in Jakarta with the values ranging from 11.07% to 43.0%. In all these places, no seasonal variations in parity rates were evident and it was likely that low parity rate was

partially responsible for the low infectivity rates which have been observed in these areas. High proportions of nulliparous females in these studies was attributed to high number of breeding sites available in big towns and the mosquito catching stations located in the vicinity of these breeding places.

33.28 percent of *Cx. quinquefasciatus* found to be parous mosquitoes in the present study with the values of monthly parity rates varying between 26.11% and 46.60%. The high number of nulliparous mosquitoes may be due to the mosquito catching stations in the present study, located in the vicinity of the breeding places as observed elsewhere (Subra, 1983). Though, the parity rate was highest in September, the percent contribution to total parous mosquitoes by the number of parous mosquitoes during the month was low. It was found in the present study that due to the impact of heavy rains on breeding, the prevalence of *Cx. quinquefasciatus* was very low in August and September months, as such the emergence of adults greatly reduced during monsoon months where there are fewer births than deaths, resulting in the higher parity rate during August to October. In such a situation, the survival rates estimated based on parity will be too high (Service, 1976). On the contrary, due to increase in per man hour densities with the large increases in emergences during post-monsoon months (November to January) and pre-monsoon months (February-March), the percent of parous females in the population was reduced which was indicated by decreased parity rate. However, the post-monsoon season contributed highest (38.69%) to the total number of parous mosquitoes encountered.

The correlation analysis showed the significant negative correlation between parity rate and per man hour density of *Cx. quinquefasciatus*, indicating that higher the density, lower will be the parity rate and vice versa. With the addition of new and young population which increases the density of the species, but decreases the parity rate in the population. In such a situation, in place of parity rate, the percent contribution by the number of parous mosquitoes in each month to the total number of parous mosquitoes encountered in the year will make the epidemiological difference.

The higher rates of parity in *Cx. quinquefasciatus* have also been reported. In three villages of Tanzania, average parity rates were ranging from 46.2% to 57.9% (MacMohan et al., 1981). In Mambui (Kenya), the average parity rate was 63.4% with the highest parity rate of 82.7% in *Cx. quinquefasciatus* occurred during the rainy season which corresponded with the transmission peak (Wijers & Kiilu, 1977). In the same village, considerable differences were observed between the parity rates recorded in three different parts of the village indicating the importance of sampling site selection to get the accurate figures of this parameter. In Mysore, India the observed average parity rate was 42.8% with the monthly values ranging from 36.0% (October) to 67.6% (January), triparous and quadriparous females were encountered during cold season (November and January) indicating the survival of the vector species up to the completion of four gonotrophic cycles (Ninge Gowda & Vijayan, 1992).

The duration required for the development of L1 into L3 stage filariasis parasites inside the mosquito body is 8-10 days (Samarawikrema, 1967). In spite of monthly variations in parity rate, biparous and triparous females were encountered in all the months in the present study and the potential for transmission comes from the female

mosquitoes that survive a minimum of two gonotrophic cycles (Shriram et al., 2005). The period between successive ovipositions which may be longer than the duration of the gonotrophic cycle may vary in the field conditions. In the natural populations of *Anopheles gambiae* the intervals between the successive blood meals were greater than the duration of the gonotrophic cycle. The delays between the repetitive gonotrophic cycles can invalidate the estimation of survival rates based on the parity (Service, 1976). Therefore, the approximate age of the mosquitoes may be higher in the field populations than the age determined based on the parity rate.

The parity rate and age grading are the important parameters which throw light on the changes in the population structure of vector mosquitoes (De Meillon & Khan, 1967). The parity status and different age composition also influence the disease transmission potential and the reproductive capacity of the vector mosquito population. In the present study, cooler climate with higher humidity during monsoon and post-monsoon months appeared to favour the increase in the longevity of the adults and during these months few females survived up to the completion of four gonotrophic cycles, indicating the increased survival and the risk of transmission as the infected *Cx. quinquefasciatus* females were also encountered during both the seasons in the present study.

Infection and Infectivity Rate in *Cx. quinquefasciatus*.

Globally, the majority of lymphatic filariasis caused by *W. bancrofti* is transmitted by *Culex quinquefasciatus* (Curtis & Feachem, 1981). In India, 98% of LF parasite is transmitted by *Cx. quinquefasciatus* (Sunish et al., 2008). Transmission of filariasis in an area would depend on the density of vector species, number of

microfilaria carriers and microfilarial density in their peripheral blood. Interestingly, a decrease in the transmission potentiality of *Cx. quinquefasciatus* was observed when mosquitoes were fed on the high microfilaria count carriers (Das, 1976). In contrast, the mean number of infective larvae per infective mosquito compared well with expected intake of microfilaria when the mosquitoes were fed on low microfilaria count carriers (Krishnaswamy et al., 1959).

The study by Subramanian et al. (1998) on the uptake of *W. bancrofti* microfilariae by *Cx. quinquefasciatus* and their development in relation to human mf density revealed that the L3 yield was related to mf intake indicating that *W. Bancrofti* - *Cx. quinquefasciatus* complex showed 'limitation', i.e. a decreasing yield for an increasing uptake. Mortality in the vector population was observed when the infective microfilaria larvae were present in large numbers (Omori, 1966; Kershaw et al., 1953; Subramanian et al., 1998). Such a situation, would limit the natural transmission of filariasis.

In the present study, both infected and infective *Cx. quinquefasciatus* females were encountered. The infected mosquitoes were encountered during pre-monsoon (February), monsoon (July and September) and post-monsoon months (October and January) and the infection rate ranged from 0.30 to 1.60. *Cx. quinquefasciatus* females with L-3 stage of parasites were found during two months- October and January and the infectivity rate during these months were 0.80% and 0.26% respectively. Both infected and infective mosquitoes were encountered in October and January months of post-monsoon season which coincided with the high density period of *Cx. quinquefasciatus*.

The infection rate and infectivity rate are reported from many areas endemic for lymphatic filariasis. Earlier, Bounsulo (1968) reported the infection rate ranging from 0.7% to 3.3% and infective rate ranging from 0.2% to 0.9% during 1964 to 1966 in Panaji, Goa. In Pondicherry, the infection rate and infectivity rates in different months ranged from 7.5% (October) to 21.5% (April) and 0.0 (June) to 4.5% (September) respectively (Rajagopalan, 1980). The infection rate and infectivity rate ranged between 0 and 4.4 and 0 and 2.2 respectively and the infective mosquitoes were encountered in only three months (February, September and November) in a semi-urban area in Kerala. (Jain et al., 1989). Kanhekar et al. (1994) recorded the infection rate for eight months, ranging from 0.8% (September, 1992) to 8.6% (May, 1991) but, the infective mosquitoes were encountered only once (infectivity rates of 1.1% in November, 1991 and 0.6% in August, 1992) during two years. De and Chandra (1994) found an overall infection rate of 2.3% and infectivity rate of 0.28% at Kanchrapara in West Bengal and stated that both infection rate and infectivity rate were not meager in the study area. The infection rate and infectivity rate are also reported from some other areas in the country and abroad (DeMeillon et al., 1967; Azeez and Chakravorti, 1980; Rath et al., 1984; Kumar and Chand, 1990; Dutta et al., 1995; Adhikari and Haldar, 1995; Rajendran et al., 1997; Murty et al., 2002).

The infectivity rates of *Cx. quinquefasciatus* do not necessarily reflect the prevalence of bancroftian filariasis in the area. Estimated on the mosquitoes collected on the human baits, the infectivity rate was only 0.97% in the small town of Mambrui (Kenya) which had a microfilariae rate of 20% and it was 0.6% only in Moa village (Tanzania) where the microfilariae rate was 24.2%; similarly, the mean number of

infective larvae/infected female was not related to disease prevalence (Subra, 1983). No correlation was found between the adult density of *Cx. quinquefasciatus* and both infection and infectivity rates (De Meillon et al., 1967; Rath et al., 1984). In the present study also, there was no correlation of adult density either with infection rate or infectivity rate. According to Martin et al. (1972) and Rath et al. (1984), man hour density of *Cx. quinquefasciatus*, infection rate and infectivity rate did not show any association with mf rate and mean mf density.

Climatic conditions in different endemic areas determine the transmission period. The temperature below 16°C and above 33°C adversely affect the development of filarial parasites in mosquitoes and due to extreme climatic conditions during most part of the year northwestern states and some eastern states of India are non-endemic for filariasis (Das, 1976). According to Singh et al. (2000), the extreme climatic conditions with low humidity during most part of the year, probably act as a natural check against the transmission of filariasis round the year despite considerable increase in mf rate among the migratory population and also increase in the vector density over the years in the non-endemic areas.

According to Azeez and Chakravorti (1980) both infection and infectivity rates were of higher order during hot and humid months, when people use minimum clothing and sleep outdoors, resulting in more exposure to mosquito bites in Dhanbad, Bihar. De and Chandra (1994) stated that the low infectivity during summer may be due to low survival and higher mortality of mosquitoes as reported from Vellore, India (Laurence, 1963) and Ceylon (Samarawikrema, 1967) where filarial transmission was interrupted during dry hot months of the year. Pondicherry having highly humid climate, the factors

are conducive for the efficient transmission of *W.bancrofti* throughout the year (Rajagopalan, 1980).

Contrasting results have been obtained from big urban settlements of Southeast Asia, where the breeding of *Cx. quinquefasciatus* is more intense, the infectivity rate was usually low (0.3%) but due to the enormous number of biting females, the number of infective bites/person/year was always very high : 1106, 1850 and 647 in Pondicherry, Calcutta (mf rate-14.8%) and Jakarta (mf rate-6%) respectively, nevertheless they are able to maintain microfilariae rates and in several parts of the world, *Cx. quinquefasciatus* is often breeding for the greatest part of the year, as a consequence, *W. bancrofti* transmission is perennial (Subra, 1983).

The infected and infective *Cx. quinquefasciatus* mosquitoes were encountered in October and January months of post-monsoon season. This coincided with the highest seasonal per man hour density of *Cx. quinquefasciatus* females and highest percent contribution to parous mosquitoes during post-monsoon season in Panaji. The vector mosquitoes surviving longer due to cooler climate during this period, it appears that the post-monsoon season is crucial for the transmission of lymphatic transmission and required measures are necessary to prevent/interrupt the active transmission. The warm, humid and moderate climate in Panaji is conducive for the active transmission of filariasis. Very high adult density and biting density, highly anthropophilic index and the infected/infective mosquitoes encountered in the present study clearly indicate the high vulnerability of the area for active transmission of filariasis throughout the year.

Susceptibility status of *Cx. quinquefasciatus* Larvae and Adults

Insecticides belonging to organochlorine, organophosphate, carbamate and synthetic pyrethroid are being used in health and agriculture sectors and the indiscriminate use of these chemicals has resulted in the resistance problem. Deployment of chemical control embraces the whole gamut of strategies which include indoor residual spraying, antilarval application of larvicides, fogging operation, use of insecticide treated bednets (ITBNs) and an ever lengthening list of household insecticide formulations for personal protection measures.

The larvicidal application i.e. use of chemicals against the mosquito larvae is more liable to induce resistance than the adulticides (Brown and Pal 1971), may be due to the fact that the larvae are exposed to the toxicant for a longer period resulting in the elimination of all susceptible individuals and permitting the survival of the resistant ones in the field (Beard 1952).

The recent study by Mukhopadhyay et al. (2006) in Rajahmundry, India revealed very high degree of resistance to malathion and fenitrothion and susceptible to temephos and fenthion. Though, the resistance to temephos and fenthion in *Cx. quinquefasciatus* and other mosquitoes in India has been documented earlier (WHO 1992, Pillai 1996), there is very little information on the susceptibility status in different areas (Mittal et al. 1999). The tests conducted by Mittal et al. (1999) with the larvae of *Cx. quinquefasciatus* collected from different areas in Delhi showed considerable variations in its susceptibility to fenthion indicating the possible resistance to the insecticide in Delhi. Resistance to temephos has been report from Brazil (Andrade, 2003).

Earlier, the susceptibility tests conducted by Thavaselvam et al. (1993) with the larvae of *Cx. quinquefasciatus* against 3.125 mg/l malathion and 0.125 mg/l fenitrothion had produced 76% and 91% mortalities respectively, showing low degree of resistance to these larvicides but, the larvae were fully susceptible to temephos in Panaji. In the present study, 57% mortality against 3.125 mg/l malathion and 89% mortality against 0.125 mg/l fenitrothion indicating the development of higher level of resistance to malathion and increased level of tolerance to fenitrothion. However, the *Cx. quinquefasciatus* larvae were found to be fully susceptible to both fenitrothion and temephos.

Different strains of mosquitoes may react differently to selection for resistance. A study by Das and Rajagopalan (1980) in Pondyichery, revealed highly susceptible status of *Cx. quinquefasciatus* larvae to fenitrothion, probably because of the frequency of fenitrothion resistance gene is so low that it could not be selected for resistance. This aspect may be true for Panaji strain of *Cx. quinquefasciatus* also, as the vector species has remained susceptible to both fenitrothion and temephos though, these chemicals are being used continuously under the Programme for several years.

In India, Brown and Pal (1971) reported for the first time the resistance to DDT in *Cx. quinquefasciatus* adult population in a village near Delhi in 1952. Rao et al. (1989) reported the resistance to DDT in *Cx. quinquefasciatus* and susceptible to malathion and deltamethrin from Rajahmundry town of Andhra Pradesh. Mukhopadhyay et al.(2006) found very high degree of resistance to DDT and nil mortality against Malathion in *Cx. quinquefasciatus* adults from Rajahmundry town. Resistance to DDT, malathion, permethrin, deltamethrin and some other insecticides in

the adult *Cx. quinquefasciatus* populations has also been reported from other areas from India and abroad (Mukhopadhyay et al., 1993; Sunaiyana et al., 2006; Selvi et al., 2007; Pridgeon et al., 2008).

Thavaselvam et al. (1993) had reported resistance to DDT and malathion in the adult populations of *Cx. quinquefasciatus* in Panaji, Goa. The use of DDT insecticide in the form of indoor residual spray and malathion in fogging operation (space spray) has been discontinued under health sector for more than ten years and seven years respectively in Goa. According to Das and Rajagopalan (1981), withdrawal of selection pressure at the appropriate time is an important factor in the reversal of resistance and theoretically it should be done before the vector population becomes homozygous for resistance. In spite of discontinuation of both DDT and malathion for several years, no reversal in the resistance of *Cx. quinquefasciatus* was noticed in the present study. On the contrary, the level of resistance has further increased in Panaji as shown in the present study.

Deltamethrin and permethrin insecticides are not used in health sector in Goa. In the present study, adults of *Cx. quinquefasciatus* showed low degree of tolerance to both the insecticides. The development of resistance to insecticides which have not been used in health sector, generally ascribed to the cross resistance spectrum of the pesticides used in agriculture (Wattal et al., 1981; Georghiou, 1982).

Development of selection pressure for resistance to any chemical is either due to direct exposure to the candidate insecticide or may be due to the cross resistance mechanism developed by the mosquitoes. It is well known that a very high resistance to one insecticide confers certain degree of cross resistance to other insecticides which are

widely dissimilar in nature (Babers & Pratt, 1951). In a study, when DDT resistant strain and susceptible strain of *Cx. quinquefasciatus* were subjected to fenthion selection for 22 generations, a tenfold increase in tolerance to fenthion was noticed in resistant strain whereas only four fold increase in tolerance was noticed in normal strain (Tadano & Brown 1965). Pieris and Hemingway (1990) reported positive cross resistance to organophosphates and carbamates and negative resistance to permithrin in temephos selected *Cx. quinquefasciatus* strain in Sri Lanka.

As per WHO criteria, the susceptibility status should be categorized as susceptible when the mortality will be between 98% and 100%, verification required between 81 to 97% and resistant when the mortality will be < 80%. Accordingly, *Cx. quinquefasciatus* females in Panaji, Goa were found resistant to both DDT and malathion and verification is necessary for permethrin and deltamethrin.

The present study on *Cx. quinquefasciatus* the important vector species, concludes that the larvae are highly resistant to malathion, tolerant to fenitrothion and fully susceptible to both fenthion and temephos. *Cx. quinquefasciatus* adults are highly resistant to DDT and malathion and they have developed low grade of tolerance to both permethrin and deltamethrin. Fenthion and temephos which are being used for several years under National Filariasis Control Programme are highly effective against *Cx. quinquefasciatus*. However, the future chances of development of vector resistance to insecticides can be delayed by alternating insecticides or using them in grid (mosaic) pattern (Muir, 1977; Curtis et al., 1978). Alternative larvicidal agents such as *Bacillus thuringiensis* H 14 or newly developed IGR insecticide may be used in rotation with other larvicides.

Using biodegradable insecticides like organophosphate and carbamate insecticides which do not persist long in the environment unlike organochlorine insecticides, the chances of environmental pollution could be considerably reduced. With the sound knowledge of ecology and bionomics of vector species in the changing environment, the chemicals could be used judiciously as and when the vector reaches epidemiologically significant level instead of using on regular intervals.

Chapter 6: SUMMARY

The present research study provided the vital scientific data on the bio-ecology of *Culex quinquefasciatus* principal vector of lymphatic filariasis in Goa. The aspects related to both larval and adult populations of *Cx. quinquefasciatus* were covered. The prevalence, relative abundance, seasonal distribution, breeding behavior, fecundity, development from egg up to adult emergence, biting behavior, feeding behavior, resting behavior, longevity, infection rate and infectivity rate with *Wuchereria bancrofti*, the susceptibility status of both larval and adult populations to different insecticides and the influence of meteorological variables were studied.

The breeding of *Cx. quinquefasciatus* was detected in drains, cesspits, cesspools, water storage tanks comprising cement tanks, plastic tanks and underground sumps, curing water collections and water stagnations inside the buildings under construction, wells, barrels (iron and plastic), domestic containers, coconut shells and tyres. Among all the habitats, polluted drains (13.71 ± 7.10) followed by the cesspits (10.42 ± 4.50) were the most important breeding habitats for the vector species. Seasonally, the highest breeding was found during the pre-monsoon due to more stagnations of water in the drains and cesspits and the lowest in the monsoon season due to the over-flowing and flushing of the outdoor breeding foci, especially, the drains and the cesspits.

The Study on the development from egg to adult emergence provided the information on the reproductive potential, hatchability of eggs, mortality rate at different stages of development, net survival against the large number of eggs laid and the duration required for the development. The average number of eggs laid by each female was 120.11. Out of total 4564 eggs laid by 38 wild females, 65.27% eggs hatched to first stage larvae, revealing that 34.73% eggs were non-viable. The mortality

was observed at each stage of development. The net emergence of adults was found to be 38.97% against the total number of eggs laid, indicating the total pre-adult mortality rate of 61.03% from egg to adult emergence. The average male to female ratio was 1.02:1.0. The total number of days taken for development from egg up to adult ranged from 9 to 14 days.

The study on the prevalence and relative abundance revealed that *Cx. quinquefasciatus* was the most predominant species (81.99%) among 15 species of mosquitoes encountered in indoor resting collections. The average male to female ratio was 1: 2.1. Longitudinal observations on the density pattern showed two peaks- first peak in July and the second peak in February. The highest per man hour density (PMHD) was in February (48.65) and the lowest in September (6.65). Density variations between the months was significant ($p < 0.05$). Seasonally, the highest PMHD (34.5) was found in premonsoon followed by postmonsoon. PMHD of males ranged between 6.4 in monsoon and 13.6 in premonsoon season. Among the meteorological variables, only rainfall significantly influenced the density pattern of the vector species. The impact of temperature, relative humidity and number of rainy days was non-significant. Density variations between the zones was non-significant ($p > 0.05$), indicating closely similar relative abundance in all six zones of Panaji.

The examination of the abdominal conditions of the indoor resting female *Cx. quinquefasciatus* mosquitoes showed 14.71% unfed, 64.79% fed, 12.26% semi-gravid and 8.22% gravid females. In the present study, the observed strong correlation between different abdominal conditions of *Cx. quinquefasciatus* females and the meteorological variables and also the significant variations between the different abdominal conditions

over different months and the two way interactions of abdominal conditions and months, emphasized the existence of relationship of feeding and gonotrophic cycle with the meteorological variables in the wild populations.

Of the total *Cx. quinquefasciatus* females, 85.28% comprising fed, semi-gravid and gravid females resting indoor confirmed the endophilic resting behavior. 62.39% of females and 65.06% of males rested on the hanging objects showing the preference to rest on these objects as compared to the walls, the objects on floor and the horizontal surfaces. Spatio-temporal variations in the number of *Cx. quinquefasciatus* both females and males resting on different sites were significant over the months and also in different zones, showing an uniform pattern of resting behavior of the vector species irrespective of the seasons and different geographical zones of Panaji city.

Among 13 species of mosquitoes collected during the landing on human bait, *Cx. quinquefasciatus* was the most predominant species both in Mala (77.65%) and Kamrabhat (79.84%) areas. There was no significant variation between the areas indicating more or less similar biting activity in both the areas. Variations in the biting activity between the months were significant ($p < 0.05$) in Panaji. The vector species was found biting throughout the night with peak biting between 2300-2400 h. The maximum biting (75.08 ± 30.52) occurred during the second quarter (2100-2400 h) of the night. The variations within the different quarters were also significant ($p < 0.05$). The highest per bait per night biting rate was recorded in December (183.0) month and the lowest in September (75.0). The leg and hand were the preferred body parts for biting ($p < 0.05$) and seasonally, there was no marked variation in the preference for any body part.

Average per bait per night biting rate ranged between 108.25 (monsoon) and 154.50 (post-monsoon).

The analysis carried out to find out the sources of blood meals showed that the vector species mostly fed on human host and the anthropophilic index was found to be 85.36%. The percent of blood meals reacted with bovine antiserum was small (5.45%). Remaining 9.19% blood meals did not show any reaction either with human or bovine antisera and these mosquitoes might have fed on other animals.

33.28 percent of *Cx. quinquefasciatus* found to be parous with the monthly parity rates ranging between 26.11% (February) and 46.60% (September). The variations in the parity rates between the months were found significant ($p < 0.05$). In spite of monthly variations in parity rate, biparous and triparous females were encountered in all the months in the present study and the potential for transmission comes from the female mosquitoes that survive atleast two gonotrophic cycles. The correlation between the parity rate and per man hour density was significant ($p < 0.05$). The correlation of abdominal conditions of wild females with relative humidity, actual rainfall and the number of rainy days also signify the influence of meteorological variables on the gonotrophic cycle leading to the parity rates. The parity rates in different seasons were; 28.45%, 38.48% and 33.33% during pre-monsoon, monsoon and post-monsoon seasons respectively. However, the post-monsoon season contributed the highest (38.69%) to the total number of parous mosquitoes encountered during July, 2006 to June, 2007.

Infected and infective *Cx. quinquefasciatus* females were encountered in the present study. The infected mosquitoes were found during five months and the infection

rates in these months were: 0.30 in July, 0.59 in September, 1.60 in October, 1.05 in January and 0.44 in February, indicating the presence of infection in the vector mosquitoes during pre-monsoon (February), monsoon (July and September) and post-monsoon months (October and January). Infective mosquitoes were found during two months- October and January and the infectivity rate during these months were 0.80% and 0.26% respectively. Both infected and infective mosquitoes were encountered in October and January months of post-monsoon season. The estimated infective bites during October and January months were 34.84 and 8.20 respectively. There was no definite correlation between the adult density of *Cx. quinquefasciatus* and both infection and infectivity rates. The estimated number of infected bites were 10.64 in July, 13.48 in September, 69.95 in October, 34.33 in January and 17.97 in February months.

The susceptibility tests carried out with both larvae and adults revealed different levels of susceptibility to different insecticides. The larvae were highly resistant to malathion, tolerant to fenitrothion and fully susceptible to both fenthion and temephos. Fenthion and temephos which are being used for several years for larval control by National Vector Borne Diseases Control Programme in Panaji, continue to be highly effective against *Cx. quinquefasciatus* larvae. LC_{50} and LC_{90} values were also determined for all four larvicides. Adult susceptibility tests against the diagnostic dosages of insecticides revealed that *Cx. quinquefasciatus* were highly resistant to DDT and malathion and they have also developed a low grade of tolerance to both permethrin and deltamethrin though, these chemicals are not used in the health sector in Goa.

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Appendix I

Table 1: Larval Suceptibility tests with III-IV stage larvae of *Cx.quinquefasciatus* against different concentrations (mg/l) of Fenitrothion.

Replicate Number	Control		Experiment	
	No.Exposed	No. Dead	No. Exposed	No.Dead
0.025 mg/l				
1	25	0	25	05
2	25	0	25	02
3	25	0	25	02
4	25	0	25	04
0.05 mg/l				
1	25	0	25	15
2	25	0	25	10
3	25	0	25	13
4	25	0	25	08
0.075 mg/l				
1	25	0	25	20
2	25	01	25	18
3	25	03	25	15
4	25	0	25	18
0.125 mg/l				
1	25	0	25	22
2	25	0	25	23
3	25	0	25	21
4	25	0	25	23

Table 2: Larval Suceptibility tests with III-IV stage larvae of *Cx.quinquefasciatus* against different concentrations (mg/l) of Malathion.

Replicate Number	Control		Experiment	
	No.Exposed	No. Dead	No. Exposed	No.Dead
0.0625 mg/l				
1	25	0	25	0
2	25	0	25	0
3	25	0	25	0
4	25	0	25	0
0.125 mg/l				
1	25	0	25	02
2	25	0	25	01
3	25	0	25	0
4	25	0	25	03
0.500 mg/l				
1	25	0	25	04
2	25	02	25	02
3	25	0	25	07
4	25	0	25	04
3.125 mg/l.				
1	25	0	25	15
2	25	0	25	12
3	25	0	25	13
4	25	0	25	17

Table 3: Larval Suceptibility tests with III-IV stage larvae of *Cx.quinquefasciatus* against different concentrations (mg/l) of Fenthion.

Replicate Number	Control		Experiment	
	No.Exposed	No. Dead	No. Exposed	No.Dead
0.0125 mg/l				
1	25	0	25	0
2	25	0	25	01
3	25	0	25	01
4	25	0	25	0
0.025 mg/l				
1	25	0	25	10
2	25	0	25	13
3	25	02	25	11
4	25	0	25	09
0.05 mg/l				
1	25	0	25	24
2	25	0	25	22
3	25	0	25	22
4	25	0	25	23
0.125 mg/l				
1	25	0	25	25
2	25	0	25	25
3	25	0	25	25
4	25	0	25	25

Table 4: Larval Suceptibility tests with III-IV stage larvae of *Cx.quinquefasciatus* against different concentrations (mg/l) of Temephos

Replicate Number	Control		Experiment	
	No.Exposed	No. Dead	No. Exposed	No.Dead
0.0125 mg/l				
1	25	0	25	01
2	25	0	25	0
3	25	0	25	0
4	25	0	25	01
0.03125 mg/l				
1	25	0	25	13
2	25	0	25	11
3	25	0	25	14
4	25	0	25	16
0.0625 mg/l				
1	25	0	25	19
2	25	0	25	23
3	25	0	25	23
4	25	0	25	20
0.125 mg/l				
1	25	0	25	25
2	25	0	25	25
3	25	0	25	25
4	25	0	25	25

Table 5: Adult Suceptibility tests with *Cx.quinquefasciatus* against DDT (4%), Malathion (5%), Permethrin (0.25%) and Deltamethrin (0.025%) insecticides in Panaji, Goa.

Replicate Number	Control		Experiment	
	No.Exposed	No. Dead	No. Exposed	No.Dead
DDT (4%)				
1	20	0	20	06
2	20	02	20	07
3	20	0	20	05
4	20	01	20	07
5	20		20	03
Malathion (5%)				
1	20	0	20	09
2	20	01	20	12
3	20	0	20	10
4	20	01	20	07
5	20		20	09
Permethrin (0.25%)				
1	20	0	20	20
2	20	02	20	20
3	20	01	20	20
4	20	0	20	18
5	20	03	20	19
Deltamethrin (0.025%)				
1	20	0	20	20
2	20	02	20	20
3	20	0	20	19
4	20	03	20	20
5	20	0	20	19

Publication and Presentation concerning the present research work

1. **Mahesh B. Kaliwal, Ashwani Kumar, A.B. Shanbhag, A.P. Dash and S.B. Javali (2009).** Spatio-temporal variations in adult density, abdominal status and indoor resting pattern of *Culex quinquefasciatus* Say in Panaji, Goa, India. *Indian Journal of Medical Research* (In Press).
2. **Mahesh B. Kaliwal, Ashwani Kumar and A.B. Shanbhag (2009).** Susceptibility status of *Culex quinquefasciatus* Say (Diptera: Culicidae) to Insecticides in Panaji city of Goa, India. Communicated.
3. **Mahesh B. Kaliwal, Ashwani Kumar, A.B. Shanbhag and Dipak Kabadi (2009).** Periodicity of Biting and Anthropophilic Index of *Culex quinquefasciatus* Say (Diptera: Culicidae) in Panaji, Goa, India. Abstract. X International Symposium on Vectors and Vector Borne Diseases, 4-6 November, 2009.



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दिनांक/Dated 23/09

Dear Dr. Kumar,

Kindly refer to your letter dated 8.9.09.

Your modified paper entitled "Spatio-temporal variations in adult density, abdominal status and indoor resting pattern of *Culex quinquefasciatus* Say, in Panaji, Goa, India" was considered by the Editorial Board of our Journal and has been found to be suitable in principle for publication subject to editorial corrections and will be scheduled for publication in one of the forthcoming issues of IJMR.

With kind regards,

Yours sincerely,


(ANJU SHARMA)

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10/09
Ofc


23/09