CHAPTER I

INTRODUCTION

Nepal is roughly rectangular in shape. The country landmass stretches 885 km from east to west and has a non-uniform width of 193 km north to south. It lies within the sub-tropical to the mountainous region at 26° 22’ to 30° 27’ N latitudes and 80° 4’ to 88° 12’ E longitudes, with an altitude that ranges from 90m to 8,848 m. the country is landlocked and is bordered by India in the East, West and South, and China in the North. Geographycally, Nepal represents a transitional mountain area between the fertile Gangetic Plain of India and the arid plateau of China.

It has a total land area of 147, 181 sq.km. Topographically, Nepal is divided into three ecological regions terai, hill and mountain. The narrow strip of flat alluvial terrin along the southern border, known as the terai, is an extension of the Gangetic Plain and comprises about 23% of the country, including most of the fertile and forest areas.The terai the Churia and Mahabharat Ranges punctuate the terai plains and constitutes the fertile with alluvial soil, with a good water holding capacity. Its northern edge is the Bhabar. The broad flat valleys or Duns found between successive hill ranges.The first elevation next to the terai is the Siwaliks (also known as Churia Range). There are a number of terai-like valleys lying between the Siwaliks and the Mahabharat range, commonly called the Dun Valleys (inner terai plains), such as Chitwan and Dang.

Running parallel with the Churia range is the middle mountain zone, also known as the middle hills or the mahabharat range.This zone also includes the so-called "middle hills" which extend northwards in a somewhat confused maze of ridges and valleys to the foot of the great Himalayas. It has great rivers such as the Karnali, Narayani and Saptakoshi flow through this area into the broad plains of the terai.The high mountain zone, located north of the middle mountain region.This differs from the previous type in its northward, on the long, straight and steep slopes, and narrow valleys which are sensitive to erosion.and spurs of the great Himalayas.The high mountain zone, located north of the Middle Mountain Region.The high himal zone occupies about 23 per cent of the kingdom and is mostly snow covered. The snow line is at 5,000 m in the east and 4,000 m in the west. This zone is an area of rocky, ice-covered massifs, rolling uplands, snow-fields, valley glaciers, and sweeping meadow lands.

The terai area receives heavy precipitation, ranging between 180 and 225 cm. The relative humidity varies between 80 percent and 90 percent during the monsoon but declines in the other months. The climatic conditions in the different parts of the country especially in terai region is favorable for the breeding of Culex mosquitoes, the proven vectors of Japanese Encephalitis and Filariasis and for the availability of the various amplifying hosts of the disease.

In Nepal, four mosquito-borne diseases are prevalent and cause much morbidity and mortality. They are Malaria (Peters et al., 1955, Brydon et al., 1961, Johnson 1966, Pradhan et al., 1970, Shrestha and Parajuli 1980, Parajuli et al., 1981, Shrestha et al., 1988), Japanese Encephalitis (JE) (Pradhan 1981, Khatri et al., 1983, Henderson et al., 1983), Filariasis (Kessel 1966, Jung 1973)and Dengue Fever.

1.1 Culex species as vector

Japanese Encephalitis (JE) and Filariasis are principally transmitted by Culex mosquitoes, although anophelines do play a secondary role in the transmission. *Culex tritaeniorynchus* is a major vector of JE virus in many part of the Oriental Region, including Nepal (Hammon et al., 1949, Hale et al., 1957, Buescher et al., 1959, Reuben et al., 1971a, 1971b; Leake et al., 1986). JE has been isolated from *Culex fuscocephala* in Thailand (Gould et al., 1974) and is a suspected vector in Nepal. *Culex gelidus* is a suspected vector of JE and was the most abundant mosquitoes collected in Sunsari district during September-October 1985 (Leake et al., unpublished data). This virus has been isolated from *Cx. gelidus* in Thailand (Gould et al., 1962). *Cx. quinquefasciatus* is the principle vector of *Wuchereria bancrofti* in Nepal, which is within the endemic zone of Filariasis (Jung 1973). *Culex thelieri* has been found naturally infected with Sindbis and West Nile Viruses (Mcintosh et al., 1967).

Studies of encephalitis before and after World War II, several groups of Japanese and foreign workers supported the view that the mosquito *Culex* *tritaeniorhynchus* plays an important role as the vector of JEV in Northern Asia and wherever JEV occurs.In Japan and Okinawa *Culex tritaeniorhynchus* was the only mosquito consistently infected and was showed to be the principal vector of JE Studies of encephalitis before and after World War II several groups of Japanese and foreign workers supported the view that the mosquito *Culex tritaeniorhynchus* plays an important role as the vector of JEV in northern Asia and wherever JEV occurs.

In Japan and Okinawa *Culex tritaeniorhynchus* was the only mosquito consistently infected and was showed to be the principal vector of JE In India *Culex vishnui* and *Culex bitaeniorhynchus* and *Culex annulorostris* were the probable vector of JEV Furthermore, in Japan Okinawa, Korea and China, JEV has been frequently recorded from other Culex such as *Culex pipiens, Culex quinquefaciatus*, *Culex tritaeniorhynchus. Aedes onicus* and *Aedes togoi.* Isolation from *Aedes vaxans nippoli* has also been reported from Japan and from *Anopheles vagus, Anopheles annularis* were isolated virus in India.

Culex tritaeniorhynchus and other possible vectors of JEV have been recorded from almost all the problem areas of Nepal, which are proven vectors of Indian subcontinent. Historically encephalitis cases were recorded in the hospitals located in the southern plain terai belt of Nepal bordering India. However, these cases were never investigated, and the etiology of the disease was not known until the first epidemics of Japanese encephalitis occurred in a few districts of the western region in July 1978. At that time, a few cases were serologically confirmed as Japanese encephalitis virus (JEV).

Since the first epidemics of Japanese Encephalitis (JE) in 1978, epidemic is occurring every alternate year, and the case incidence is increasing in regular increments up. Until now, the prevalence of three different structures of JEV (Nep. 1/90, B-2524, and B-9548) has been reported. Now 24 districts of terai and inner terai of the region are affected. However, no detailed study of the vector species has been carried out in Nepal.

The total 8874 cases and 1264 death have been reported in Nepal with an average case fatality rate of 14.2 percent in aggregate since 1998. During six years period of time, highest number of cases were reported in 1999 (2924 cases) and the second highest were in 2001 (1888 cases). The lowest number of cases (330 cases) with highest mortality (CFR 20.9 percent) has been reported during the year 2003. The overall mortality of JE varies from 9.77 percent during the year 2000 to 20.9 percent during the year 2003. Comparative assessment of disease in different regions showed that Far western and Mid-western Development Regions have reported the highest number of JE cases during the years 1998 to 2003. The reporting districts based on the abundance of number of cases per 100,000 populations are in the order of Banke, Kailali, Kanchanpur, Parsa, Rupandehi and Morang. Cases started to appear in April-May and reached their peak during late August to early September. Cases started to decline from October EDCD (2001).

Filariasis has been known to be endemic in different areas of Nepal since a long time. It is a public health problem in Nepal. Filariasis is much more prevalent in Nepal due to the presence of suitable environmental condition and mosquito breeding sites. In Nepal, very few researches on Lymphatic filariasis have been undertaken. Out of three species of lymphatic parasites; *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori,* only one species i.e. *Wuchereria bancrofti* is recorded in Nepal. The previous works on filariasis were done by Jung (1973), Pradhan et al., (2003) studied mapping of filarial infection in 37 districts of total 75 districts of Nepal. The study showed that 33 districts of them were endemic for lymphatic filariasis. Of the 33 districts 11 districts were having above 20% prevalence rate, 15 districts between 6-19% and 7 districts between 1-5%. The average 13% of the whole population of Nepal was found to be affected (Sherchand, 2003).

The achievement of MDA in the country by the year 2007 was found to be covered 11 millions of population (DOHS, 2006) and followings are the rounds of MDA conducted in the different districts of Nepal: 5th Round-1 district (Parsa), 3rd Round-4 districts (Makwanpur, Chitwan, Nawalparasi and Rupandehi) and 1st Round-15 districts (Nuwakot, Dhading, Gorkha, Tanahun, Shyangja, Palpa, Kapilbastu, Kavrepalanchock, Ramechhap, Sindhuli, Dhanusa, Mahottari, Sarlahi Rautahat and Bara) (DoHS, 2006) .

Dengue fever is commonly referred to as breakbone fever. This is another viral disease transmitted by mosquitoes. *Aedes aegypti, Ae. albopictus, Ae. Sculellaris* are suitable vectors, *Ae. albopictus* is prevalent in Japan, New Guinea, Norther Australia, Malagasy Republic, the Philippines, and Hawaii.

Ae.albopictus is distinguishable by a silvery stripe on its mesonotum and whitish irregular patches on the lateral aspects of its thorax. Adult are aggresively anthropophagic and are important vectors of dengue viruses (Gould et.al., 1968, Huang 1972). Larvae occur in tree holes in forested areas, also rain barrels and artificial containers. *Aedes sculellaris* is a closely related species characterized by whitish wavy lines composed of scales down each side of the thoracic pleuron.

The Government of Nepal has identified Japanese encephalitis as a priority program. In this relation, government has shown its commitment to implement different disease prevention and control activities and strengthen the diagnostic capacity for different established JE diagnostic laboratories. Moreover, national protocol has been developed for JE diagnosis and government is committed for better clinical management and treatment of disease. For this, government has planned to immunize 250 thousand populations of different JE endemic areas in current fiscal year.

A sharp rise in the JE and Filaria mortality rate has been observed in Chitwan district. JE and Filaria are transmitted by Culex mosquito vectors. JE and Filaria control strategies can be more successful if the distribution and abundance of mosquito vectors is predicted. This study aims to provide a better understanding of the distribution and abundance of Culex mosquitoes in Chitwan district including vectors.

1.2 Breeding site of vector species

Immature stages of *Culex fuscocehala* have been collected in rice fields, grounds pools, foot prints and marshes.in Nepal larvae have been dipped from shallow pools in swampy ground and irrigation ditches.Immatures *Culex gelidus* live in puddles, pools, rice fields and marshy depressions having abundant vegetation. Iammature stages of *Culex quinquifasciatus* are found in any type of habitat from fresh and clear to brackish, turbid and polluted waters. It is common inground pools, ditches, drains, sewage, latrines, septic tanks and artificial containers. *Culex theileri* breed in permanent and temporary bodies of fresh or foul water.They are commonly found in slow moving streams, irrigation ditches, ground pools, ponds, springs and cisterns. *Culex tritaeniorhynchus* is a common rural species in rice fields, shallow marshes, pools, ponds and ditches containing fresh or polluted water with grass or aquatic vegetation in partial shade or full sun.This species becomes dominant in rice paddies when plants reach 0.3m in height (Darsie and Pradhan 1990).

1.3 Previously Reported Culex species in Chitwan District

Culex bitaeniorhynchus was reported in Chitwan, Rapti Valley (Peters and Dewar, 1956). *Culex tritaeniorynchus* was reported in Chitwan, Rapti Valley, VI-63, 6♀.

1.4 Aims and Objectives

The aims and objectives of this study are as follows:

1.4.1 To determine the distribution of different Culex mosquito species on selected villages of Chitwan district;

1.4.2 To determine the indoor and outdoor species present in the study area and their relative density;

1.4.3 To assess seasonal changes;

1.4.4 To determine vector abundance and species composition in the study area;

1.4.5 To determine resting habit of mosquito species in the study area.

1.5 Formulation of Hypothesis

The expected results of this study are as follows:

1.5.1 The number of mosquitoes depends on temperature i.e. there is positive relationship between the number of mosquitoes and temperature.

1.5.2 The number of mosquitoes collected in post-monsoon survey and pre-monsoon survey is independent.

CHAPTER II

LITERATURE REVIEW

Moore et al., (1978) states that Mosquito density was positively correlated with rainfall, the relationship being more marked in the dry, south-coastal part of the island in Puerto Rico.

Takashima et al., (1989) reported at the time of the out breaks of abortion, *Culex pipiens*, *Anopheles* species, *Aedes vexans hipponii*, and *Aedes japonicus* were predominant over *Culex tritaeniorhynchus. Culex tritaeniorhynchus,* almost a solve vector species of JE virus in the Southern part of Japan, is probably not a vector of the virus in Hokkaido.

Gingrich et al., (1992) states that, vector abundance was high in monsoon (May-October), moderate in transition (March-April and November-December) and low in dry (January-February) seasons in Bangkok. More pigs sero-converted in monsoon and transition seasons than in dry seasons. Indices of JE transmission activity (vector abundance, pig sero-conversions, and MIRS) increased proportionately with rainfall. The risk of human infection appeared greatest at the site with moderate vector abundance because of its greatest human population density.

Geevarghese et al., (1994) reported very high incidence of JE cases were in extensively irrigated areas and a low incidence in some of the taluks with less or no irrigation system in Mandya District, Karnataka, India. Mosquito populations were peak during March-April and September.

Gajanana et al; (1997) reported vector abundance was lowest in the hot and dry season (April-June) and highest in the cool and wet season (October-December) in the South Arwt district in Tamil Nadu. The probability of a child receiving an infective bite was 0.53 per JE transmission season.

Prakash et al., (1997) studied the indoor biting behaviour of *Anopheles dirus* Peyton and Harrison, 1979 in upper Assam, India and recorded a high level of endophagy of *Anopheles dirus* with a mean biting rate of 8.7 $\pm$ 9.0. Indoor biting of *An. dirus* 5% started after 19.00hr, increased gradually, was more pronounced between 21.00 and 04.00hr, and ceased after 05.00hr.

Mwandawiro et al; (1999) studied on the host preference of Japanese encephalitis vectors in chiangmai, North Thailand and states that, there was no difference in the proportions of fed females *of Culex tritaeniorhynchus* and *Cx. vishnui* between the cowshed and pigsty collections, and *Cx. gelidus* fed significantly more on the cows than the pigs.

Hanna et al; (1999) reported JE virus activity was more widespread in North Queensland in the 1998 wet season than in the three previous wet seasons, but ecological circumstances (e.g., less intensive pig husbandry, fewer mosquitoes) appear to have limited transmission on the mainland.

Das et al; (2000) studied on the prevalence of *Aedes aegypti* at the international part and airport, Kolkata (West Bengal), India

Guimaraes et al., (2000) reported *Anopheles cruzii* and *Aedes scapularis* feeding on blood inside and around the residence in areas of Serra do mar state park state of Sao Paulo, Brazil. Both species were vectors of malaria and arbovirus respectively, may be involved in the transmission of such disease in rural areas.

According to Tandon and Ray (2000), a high bovine blood index of *Aedes aegypti* and *Aedes albopictus* in the cattle sheds and a high human blood index in the human dwelling in the highly congested residential areas of the city, and from outdoor situations in urban garden in kolkata India. A majority of members of both the species had fed on one host and a small percentage on more than one host, and that both the species were mainly anthropophilic in nature.

Johansen et al., (2001) conducted an entomological investigation of an outbreak of JEV in the Torres strait, Australia, in 1998, and recovered 43 isolates of JE virus from adult mosquitoes (42 isolates from *Culex sitiens* and one from *Ochlerotatus vigilax*). They also reported two confirmed human JE cases in that area and Cape York Perinsula, in Northern Queenland.

Maier (2002) studied the effect of environmental changes on vectors of disease in Germany and neighboring countries with special reference to mosquitoes

According to Myrty et al., (2002) Ninty-three cases of Japanese encephalitis (27 confirmed serologically) were reported in an endemic district of Andhra-Pradesh, India, Mainly during the monsoon months of November and December. A significant positive correlation between densities of mosquitoes of the *Culex vishnui* subgroup and occurrence of Japanese encephalitis cases ($r=0.765$, $P<0.01$) was observed.

In Delhi and environs the feeding behaviour of Anopheline and Culicine mosquitoes was found to be highly influenced by biotypes. Village and areas, different climatic sleeping behaviour of human beings and availability of alternative hosts particularly animals population. Overall anthrophilic index was found to be 24.66% for *Anopheles culicifacies*, 20.96% for *An. annularis*, 26.33% for *Culex quinquefasciatus* and 36.39% for *Cx. vishnui* (Kumar et al., 2002).

Apperson et al., (2002) studied on Host feeding habits of *Culex* and other mosquitoes in the Brough of Queens in New York City and reported, *Culex pipiens* L. and *Cx. restuans* Theobald fed primarily on birds, and their feeding habits support their implication as enzootic vectors West Nile Virus. *Culex salinarius coquilletl* and *Coquilletlidia perturbans* (walker) fed mainly on mammals with fewer blood meals taken from birds, and these two species are potential bridge vectors of West Nile Virus.

Eighteen mosquito species recorded by Ronald Ross since 1898, all of them are vector in mature, was noted within next 50 years in Kolkata. After 1950, 19 mosquito species, 8 recorded prior to and 11 arrived after 1950, b are regular visitors of human dwellings and cattle sheds of Kolkata 10 vector and 9 non vector mosquito species were sucking blood from man and cattle in

Kolkata. *Culex quinquefasciatus* was dominant (62.96%) on average of 492 mosquitoes (Pramanik and Raut, 2002).

Fakoorziba et al., (2003) reported *Cx. tritaeniorhynchus* from different breeding places in and around Mysore city in South India can be different varieties. One variety comparatively has shorter siphon index ratio and longer basal hair tufts on siphon than the one. Mating behaviour of these varieties show that, variety from the city pools was moderate eurygamous and the one form mainly paddy fields moderate stenogamous.

Kanojia et al., (2003) studied on long-term vector abundance and seasonal prevalence in relation to the occurrence of Japanese encephalitis in Gorakhpur district, and reported that *Cx. tritaeniorhynchus* the most likely vector of JE together with other known vector species remained more active during the period of paddy cultivation.

Dutta et al., (2003) conducted a survey on medically important mosquitoes found in Mizoram and recorded forty-seven species in the monsoon season and 48 in the post-monsoon season. Both the primary vectors of malaria, viz, *Anopheles dirus* and *An. minimus* were recorded. Potential vectors of dengue and Japanese encephalitis were also detected; fifteen species reported earlier were seen mising in the present survey.

In Paraiba valley region, state of Sao Paulo Brazil, *Aedes albopictus* commonly found to be fed on a wide range of host, and *Cx. quinquefasciatus* presented similar behaviour but humans and dogs were the most common (Gomes et al., 2003).

Barbosa et al., (2003) studied on the culicidae activity in a restrict forest insi Curitiba urban area (Parana, Brazil) & reported that 15 species were found out of 312 Culicidae specimens. The predominant species belong to *Culex (Culex)* coronation Dyer and knob, 1906 group. Others species found with potential epidemiological importance were: *Haemagogus (Conopostegus) leucocelaenus* (dyar and Shannon, 1924), *Ochlerotatus (Ochlerotatus) flaviatilis* (lUTZ, 1904) *Anopheles (Nyssorhynchus)* (Strodei, 1926).

Rajendran et al., (2003) reported average vector abundance per man hour for *Culex tritaeniorhynchus* was 324.5per month for the period June 1998- May 2000 in south Indian villages. The average minimum infection rate (MIR) per month in *Cx. tritaeniorhynchus* was 1.4 (range 0.0-5.6). Seroconversions (SCs) were recorded in 14 goats (70%) in the first year and 23 goats (74%) in the second year.

Taipe-Lagos et al., (2003) reported twenty-five species or genetic groups were identified among 53,496 specimens collected in a preserved metropolitan area. *Ochlerotatus scapularis, Culex quinquefasciatus* and *Culex declarator* were the most frequent and abundant species have been implicated in disease transmission to man in other regions.

Jeong et al., (2003) studied on prevalence and seasonal abundance of the dominant mosquito species in a large marsh near coast of Ulsan and reported that, *Anopheles sinensis* was most abundant (53.4% in species ratio), followed by *Culex tritaeniorhynchus* (43.0%), *Cx. inatomii* (1.6%) *Ochleratatus dorsalis* (1.3%) and *Cx. pipiens pallens* (0.5%). A malaria vector *An. sinensis* and a Japanese encephalitis vector. *Cx. tritaeniorhynchus* were collected 3,663.3 females and 3,142.5 females per trap night from June to September for the years, respectively. According to the biweekly population changes at the area, *Cx. inatomii* which was dominant species in 1997 was the most abundant in the early July during 1999-2001.

According to Zhou et al., (2004), in western Kenya, *Anopheles gambiae* Giles was the predominant malaria vector species, constituting 84.6% of the specimens, whereas *Anopheles funestus* Giles constituted 15.4% of the vector populations. *An. gambiae* abundance increased by six to eight-folds in the long rainy season over the dry seasons, but *An*. *funestus* abundance peaked 3mo after the long rainy season. *An. gambiae* distribution was more aggregated in the wet seasons than in the dry seasons. Degree of aggregation of *An. funestus* was similar in all four seasons.

Kumar et al., (2004) conducted a study on mosquito diversity in Rajiv Gandhi National Park (Nagarahole), Karnataka state, India and recorded a total of 60 species belonging to 10 genera. Genus *Culex* was predominant. Maximum number of species was found to breed in ponds of the total number of species recorded, 14 are known to be vectors of different mosquito-borne disease in India.

Fettene et al., (2004) studied the behaviour of *Anopheles arabiensis* and *An. quadriannulatus sp.B* mosquitoes and malaria transmission in south western Ethiopia and reported majority of indoor-resting *An. arabiensis* (79.6%) and *An. quadriannulatus sp.B* (94.8%) were caught in cattle sheds. The human blood index of *An. arabiensis* was 7.3% and only one specimen was positive for *Plasmodium* parasites, having both *P. falciparum* and *P. vivax* sporozoites and giving a sporozoite rate of 0.24%. The human blood index of *An. quadriannulatus sp. B* was 1.1% which was significantly different from that of *An. arabiensis*, positive reaction for *Plasmodium* circumsporozoite proteins were not observed in the *An. quadriannulatus sp. B* that were tested.

Dos et al., (2004) reported the density of *Anopheles albitarsis* females showed a marked seasonal variation, with peaks as high as 629 individuals per human per night in the wet season. The low daily survival probability of 0.61 is an important factor limiting the vectorial capacity of *An. albitarsis s.l.* in Southeastern Brazil.

Enrih and Boca (2004) conducted a study on seasonal dynamics of the *Anopheles maculipennis* complex in Osijek, Croatia. During an eight-year investigation of *Anopheles maculipennis,* using CDC traps with Co_2 as an attractant, a total of 3,508 mosquitoes were collected. Long term high water level created better conditions for continuous breeding of mosquitoes, which contributed to a significant increase in abundance rates of all species in the complex.

Sharma et al., (2005) studied on seasonal fluctuation of dengue fever vector, *Aedes aegypti* in Delhi, India and reported water cooler and tires were preferred breeding habitats of *Aedes* mosquitoes in the city out of 103,778 houses surveyed, 20,513 houses and 3,547 containers were reported positive for *Aedes aegypti.* The house container and Breteau indices were very high during the post-monsoon season. The container indices was very high (17.7%) in the defence area in September 2000. The house index for *Aedes aegypti* ranged from 0.1 to 7.4, 0.1 to 11.3 and 0.1 to 11.1 in the municipal corporation of Delhi (MCD), New Delhi Municipal committee (NDMC) and defence areas, respectively.

Arunachalam et al., (2005) conducted observation on the multiple feeding behavior *of Culex tritaeniorhynchus* in Eerala in southern India and states that, *Culex tritaeniorhynchus* had fed mainly (56.6%) on cattle. Pig feeding accounted 6.3% of the total samples. Some samples (n=980, 38.3%) were of serologic mixed origin. Mixed blood meals were mostly (96.7%) from cattle and goats.

Russell (2005) studied on the species diversity of mosquito on Florida. Most of the mosquito species were collected during "high season" month (June to September) than in low season (January to March).

Ulloa et al., (2006) studies on host selection and gonotrophic cycle length of *Anopheles punctimacula* in southern Mexico and states that, *Anopheles punctimacula* was most abundant during the dry season and preferred animals to humans. The daily survival rate in mosquitoes collected in animal traps was 0.96 (Parity rate=0.80; gonotrophic cycle=4 days). The minimum time estimated for developing mature eggs after blood feeding was 72lt. The proportion of mosquitoes living enough to transmit *Plasmodium vivax* malaria during the dry season was 0.35.

Richards et al., (2006) studied the host feeding patterns of *Aedes albopictus* in relation to availability of human and domestical animals in Suburban Landscapes of central north, Carolina and states that, *Aedes albopictus* fed predominately on mammalian hosts (83%), common mammalian hosts included humans (24%), cats (21%) and dogs (14%), about 7% of blood meals was also taken from avian host (chickens and a northern cardinal). These mosquitoes took mixed blood meals from avian and non avian hosts.

Harbison et al., (2006) studied on sampling method for indoor resting malaria mosquitoes in Africa and reported that more mosquitoes were collected when a resting box and a ceiling net used together than a single collector using a hand-held aspirator but only one-third the number collected by pyrethrum spray catches (PSCS). At sites where PSCS are impractical, a resting box and ceiling net can effectively used as an alternative to hand catches in malaria surveillance.

Quiroga-Elizondo et al., (2006) state that, mosquitoes feed on humans with less frequency in comparison with chickens horses and pigs in Monterrey, Northeastern Mexico. The FRS for chickens were the highest of all available hosts (1.7 and 3.2) and they were the most abundant hosts in Eswbedo, and the second most abundant in Guadalupe, indicating a selective bias of *Cx. quinquefasciatus* for chickens i.e. Ornithophagic.

Parida et al., (2006) studies host feeding patterns of malaria vectors of Orissa, India, and reported 0.2% *Anopheles culicifacies* blood meals contained blood from humans and cattle. *An. flaviatilis* and *An. culicifacies* revealed seasonality in their anthropophilic index (Al). *An. fluviatilis* showed a human forage ratio of more than 1 where as *An. culicifacies, An. annularis*, and *An. varuna* had forage ratios of 2.6, 2.5 and 2.4 respectively, for bovine. There was a correlation between the Al of *An. fluviatilis* and malaria slide positively rate.

Kent et al., (2007) reported transmission of *Plasmodium falciparum* was nearly zero during the 2004-2005 rainy seasons because of widespread drought. During 2005-2006, the estimated Entomologic inoculation rate values were 1.6 and 18.3 infective bite per person per transmission season in each of the two village areas, respectively. *An. arabiensis* throughout Africa was the primary vector responsible for transmission of *P. falciparum*.

Kanojia Pc. (2007) reported *Cx. tritaeniorhynchus* have a mojor role in the transmission of JE virus in Bellary district Karnataka, India Risk of transmission of JEV to humans can be reduced by house spraying with residual insecticides and intermittent paddy irrigation.

Howell et al., (2007) studied the influence of house construction on the indoor abundance of mosquito and reported the mean number *of Culex quinquefasciatus* mosquitoes was greater in cement homes than in either wood or other poorer quality homes [Trinidad (TT) cement 17.43, others 14.43; Dominican Republic (DR) cement 4.24, others 3.41]. High abundance of mosquitoes resting indoors with painted interiors than without painted. But a painted exterior was not significant. Indoor resting mosquitoes in the TT (interior OR 2.90, CL 1.09-8.72, exterior OR 2.14, CL 0.89-6.67) and DR (interior OR 3.13, CL 1.41-6.92, exterior OR 1.97 Cl.91-4.26).

Molaei et al; (2007) conducted a study on host feeding pattern of *Culex quinquefasciatus* and its role in transmission of west Nile virus in Harris country, Texas. About 672 blood-engorged *Culex quinquefasciatus*, collected during 2005, were identified by nucleotide sequencing PCR product of the cytochrome b gene 39.1% had required blood from birds, 52.5% from mammals, and 8.3% were mixed avian and mammalian blood meals. Most frequent vertebrate hosts were dog (41.0%) mourning dove (18.3%), domestic cat (8.8%) white-winged dove (4.3%), house sparrow (3.2%), house finch (3.0%), gray cat bird (3.0%) and American robin (2.5%) *Culex quinquefasciatus* is an opportunistic feeder and principal mosquito vector of WNV in this Metropolitan area; however, transmission by other mosquito species or by other modes of infection, such as ingestion, must account for the high WNV infection rates among local blue jays and American crows.

In Nepal, Pradhan (1988) reported seven new country records which include: *Aedes pulchriventer, Ae. subalbopictus, An. fragilis. An. dravidicus, Armigeres durhami, Culex infula* and *Culex pseudovishnui.*

Burgess (1990) found out that in Nepal the study of mosquitoes begins only recently, he recorded 130 species and subspecies in 14 genera of mosquitoes known from Nepal.

Darsie and Pradhan (1990) reported the *taxon Anopheles (Anopheles) gigas* Glies (Complex), as *Anopheles gigas* var bailey. *Anopheles (Celiia)* *filipinae* Manalang. This species was also reported by Pradhan and Brydon (1960) from Lamjung district in the North Central Nepal.

Peters and Dewar (1956) were the first to record certain culicine species occurring in Nepal. Joshi et al., (1965) made a major contribution by reporting 59 species of Culicine, including 28 new country records. Shrestha (1966) reported 97 species, including 36 anophelines and 61culicines.

Darsie and Pradhan (1994) reported 167species and subspecies in16 genera including 54 species of *Aedes*, 44 species of *Anopheles, 31 species of Culex,12 species of Armigeres,12 species of Uranotaenia, ,4 species Mimomyia, 3 species of Mansonia and Heizmannia, 2 species of Malaya and Tripteroides mosquitoes* in Nepal. Out of 44 species of Anopheles mosquitoes five species has been identified incriminated as vector of primary importance. These are *Anopheles mimimus, A. fluviatilis, A. maculates, A. willmori* and *A. anullaris.*

Darsie (1994) found out that since 1987, a study of species of mosquitoes occurring in Nepal has been under way. No pervious work has been understood to record the total mosquitoes of this Himalayan Kingdom of the Nepal.

Gautam et. al., (2009) reported first record of *Aedes aegypti* (L.) vector of Dengue Virus from Kathmandu,Nepal.

Parajuli et al., (1992) carried out an epidemiological study of JE in all epidemic districts of Nepal during the year 1989 and reported that of 868 total JE cases, 227 died. All ages and both sex groups were affected from the disease.

Joshi et al., (1995) carried out an epidemiological survey of JE in all endemic areas of Nepal through National Zoonoses and Food Hygiene Research Centre (NZFHRC) from 1990 to 1993. The CFR were 36.0%, 38.0%, 35.2% and 31.7% in 1990, 1991, 1992 and 1993, respectively.

Zimmerman et al., (1997) reported a first outbreak of JE in Kathmandu Valley, Nepal during September and October, 1995 and treated 15 patients with meningo-encephalitis.

Akiba et al., (2001) conducted an epidemiological study of JE outbreak in the South-Western part of Nepal in 1997 and reported a high density of JE infections. It was estimated that 27.9% of the total population were infected with JE virus in the study area.

Gurung and Singh (2003) studied on factors associated with JE in Nepal. Total of 142 numbers of confirmed JE cases and 142 controls from Banke, Bardia and Dang district were interviewed. They concluded that non-immunization status, presence of households pigs, non-use of mosquito-net and out-door sleeping are the risk factors related with the occurrence of JE.

CHAPTER III

METHODOLOGY

3.1 Description of Study Area

Nepal is a land of extremes and its topography varies from 60m to highest peak in the world. The country is a landlocked mountainous lying between China and India with an area of 1,47,181 sq. Km. About 38 percent of the population lives below the absolute poverty line and lack of Arable Land indicates that the population pressure is high in Nepal. On an average, seven persons are depending on each hectare of arable land for their livelihood (APP, 1997-2017).

Nepal's topographical and Socio-ecological diversification helps to promote periodic epidemics of infectious diseases, epizootics and natural disasters. Millions of people are at risk of infection and hundreds of them die every year due to communicable diseases, malnutrition and other health related events. As also majority of the population is rural area based and illiterate, economic and demographic changes coupled with sudden occurrence of epizootics might possibly have contributed to the disease out breaks. Japanese Encephalitis (JE), Filaria, Malaria, Kala-azar etc. have been affecting large number of people in successive years.

Chitwan District, a western part of Narayani Zone, is one of the seventy-five districts of Nepal, a landlocked country of South Asia. Bharatpur (seventh largest city of Nepal) is the district headquarters of Chitwan District. Bharatpur is the commercial and service center of central South Nepal; it is the merger destination for higher education, health and transportation of the region.

It is an inner Terai district widened from Bhavor region of Terai to Mahabharat hill of north, bordered with Makawanpur and Parsa to the east, Nawalparasi and Tanahun to the west, Dhading and Gorkha to the north and Bihar of India to the south. It covers an area of 2,218 sq. Km and has a population (2001) of 472,048. Ninety percent of population is concentrated at plane valley, while the rest 10.00% in Mahabharat Hill.

It is situated at $27^{0}35'$ N latitude and $84^{0}30'$ E longitude and 144m to 1947m altitude range from sea level. The area enjoys two types of climate: Sub-tropical at Mahabharat range and tropical at foothill of Churia range and at valley areas. Temperature ranges from minimum 7^{0}c to maximum 42^{0}c.

Narayangadh, on the bank of Narayani River is the main town with numerous shopping Zones. At the foot of the Himalayas, Chitwan is one of the few remaining undisturbed vestiges of the Terai region which formerly extended over the foothills of Nepal.

Now there are 40 village development committees (each of which has nine wards or villages) and municipality Bharatpur and Ratnanagar each of which has more than nine wards or urban areas. Chitwan is very rich in beautiful natural scenes, lakes, forest, and river with well-known Chitwan National Park.

3.2 Study area and sampling sites (Number of sites)

The study was conducted on the ten villages among five VDC of Chitwan district. Gitanagar, Kesherbag, Parasnagar, Champanagar village of Gitanagar VDC; Bijayanagar, Rampur villages of Mangalpur VDC; Bramhapuri village of Patihani VDC of Western Chitwan and Suryapur, Kholesimal village of Jutpani VDC; Dobato Village of Pithuwa VDC of Eastern Chitwan.

3.3 Map of Chitwan District Showing Mosquitoes Sampling Sites

Figure 1 Map of Chitwan district

3.4 Agriculture Sector of Chitwan

Chitwan is very famous in Nepal because of its dominant production of mustard from which mustard oil is produced. This popularity of the mustard plantation in Chitwan is attributed to the predominant soil type of the place. The soil in Chitwan is mostly of the silt type. The silt nature of the soil is in turn attributed to the flooding over the ages in the past from the rivers like Gandaki. Chitwan is also profusely spotted with lands with soil type clay which are very good for use as paddy fields. The paddy crop is available in two period, pre-monsoon (March-April) and post-monsoon (August-September).

Maize and wheat is the second and third most cultivated crop of Chitwan. Wheat is cultivated in winter season but Maize is cultivated in all season in Chitwan. Though in some leisure time, farmers used to grow some varieties of grains and pulses such as millet, buck wheat etc., millet is planted in Shrawan (July-August) but buck-wheat is winter crop of Chitwan.

The soil is also very good for growing various types of vegetables such as cabbage, cauliflower, radish, potato, broccoli, carrot, capsicum (winter vegetable) cucumbers, pumpkins, lady's finger, (summer vegetable).

Gitanagar's rice and potatoes of Pithuwa are most popular in Chitwan. Chitwan product 80% of the country poultry industry, and it is also famous for floriculture, Mushrooms cultivation, Bee forms and Honey production etc.

3.5 House structure

Most of the human dwellings were made by bricks with mud; the roof was made by Zinc. Only few were made by mud with hedge roof, and some were cemented house.

3.6 Cattle-Shed

Majority of animal shelter was open type and made up of wooden block with hedge roof and some are closed type made by brick with wooden block with zinc roof.

3.7 Sampling Techniques

3.7.1 Study Design

The study was conducted from August-September 2007 (Post monsoon/rainy season). A unique house code (Like G, P, K and so on) was given on the front of each house. Households were surveyed in sequence daily along black from the start house between 6:00 am to10:00 am. Un-occupied or closed houses and houses where resident did not provide permission for the survey, offices and schools were not sampled. Continuing surveys of un-sampled households an attempt was made to inspect houses that were previously closed or where access had been refused. About four houses were sampled per day per village and total of forty houses were sampled per season. Mosquitoes were not collected on the rainy and windy day. The number of human occupants as well as cattle occupants during the previous night was recorded for each survey house. The indoor collection (human and cattle shed) was completed in 15 / 15 minute and outdoor collection in 30 minute.

The temperature of indoor collection (human and cattle shed) and outdoor collection of each house was noted separately using thermometer.The appeared specimen was instantly sucked by aspirator and they were transported to the plastic cup. The collected mosquitoes were identified using identification key by Darsie and Pradhan (1990).

Immediately after termination of first survey, the sampling procedure was repeated. The second survey was completed on March-April 2008. To differentiate data associated with the different collections, the two survey were referred as ’a’ and ’b’.

3.7.2 Entomological Surveys

Briefly, after asking permission to survey the household, a demographic survey designed to determine the number of occupants, dimension of the property, house construction materials, method of cooking, water use patterns type of sewage disposal, and insecticide use etc. were administered. Indoor collections of adult mosquitoes were attempted in all rooms of the house (when permitted) including walls, under furniture and inside closets and other likely adult mosquito resting sites (cattle sheds).

Outdoor collections similarly attempted outside the house from outside wall, under eaves, vegetation and bushes around cattle sheds and around outdoor-stored material etc.

3.8 Requirements

3.8.1 Materials Required

1. Aspirator
2. Ocular Micrscope
3. Plastic cup
4. Ivory paper

5. Entomological pin
6. Thermocol
7. Torch light
8. Mosquito net
9. Cotton
10. Forceps
11. Plastic bag

3.8.2 Chemical required

1 Nepthalene ball

2 Ethylacetate

3 Nail polish (colourless)

3.9 Killing Method

The collected mosquitoes were killed by using the ethylacetate. For this, a plastic cup was mounted with mosquito net with hole on the center. The hole was covered with a piece of cotton. The mosquitoes were transferred into the cup from the aspitator through this hole. A small piece of cotton was socked with one or two drops of ethylacetate and placed into this cup and the cup was made airtight, after few second the mosquitoes were killed.

3.10 Fixing method

Only *Culex* mosquitoes were fixed on the Ivory paper. Ivory paper was cut into triangular (one end pointed) card. A drop of colorless nail polish was placed at the apex of the triangular card. Each collected specimen was fixed with the help of colorless nail polish; the apex of the card is slightly bent at an angle to make the specimen up right. The bent tip was attached to the pleuron of thorax. The other end of the card was picked with Entomological pin after giving the code number, such specimen were fixed in thermocol kept in box. Such stag specimens were kept in special care to prevent the damage of wings, legs, maxillary palps, proboscis and abdomen. Nepthalene ball was kept inside the box by attaching with glue. Complete information about collected locality, time, site, number of such stag specimens were noted in a questionnaire paper.

3.11 Identification

Each are ever-stag specimen i.e., *Culex* group were identified in the lab to the species level using the identification key by Pradhan and Darsie (1990).

3.12 Tools for Analysis and Presentation:

After identifying these collected mosquitoes, it was processed, arranged and analyzed. The analysis has been made more effective with the help of tables, bar diagrams, graphs wherever necessary. But for testing of hypothesis, Correlation and Hypothesis testing model have been used.

3.13 Analytical Framework:

As far as possible, the analysis has been made quantitative. For Correlation of number of mosquitoes on temperature, the following variables have been defined.

I. Number of Mosquitoes: These are total number of mosquitoes collected in each village in one season. This variable; say (X).

II. Temperature: The temperature recorded in field i.e. average temperature of each village in one season (Post Monsoon and Pre Monsoon season). This variable; say (Y).

‘n’ is the total number of observation.

After having done this, simple Correlation Coefficient is calculated. The formula for calculating simple Correlation Coefficient is

$$Correlation\,Coeficient(r) = \frac{n\sum XY - \sum X \sum Y}{\sqrt{\left[n\sum X^2 - \left(\sum X\right)^2\right]\left[n\sum Y^2 - \left(\sum Y\right)^2\right]}} \quad \ldots\ldots \textbf{Annex-1}$$

Again, for testing, the second hypothesis, that is, the number of mosquitoes collected in Post-Monsoon and Pre-Monsoon survey is independent. The following concepts and method have been followed:

The data has been arranged in to (r×c) manifold contingency table where ‘r’ represents the number of rows and ‘c’ represents the number of columns. The season divided into two groups Post Monsoon season and Pre

Monsoon season and there is 12 species of culex mosquitoes. Now for testing hypothesis χ^2 test is used as a test of independence of attributes at 5% level of significance. Symbolically,

$$\chi^2 = \sum \frac{(O - E)^2}{E} \qquad \text{…………………………… } \textbf{Annex-2}$$

Where,

O= Observed Frequency

E= Expected Frequency

E can be calculated as:

$$E_{ij} = \frac{Total\ of\ i^{th}\ row \times Total\ of\ j^{th}\ column}{Grand\ total}$$

CHAPTER IV

RESULTS

A total of 1197 Culex mosquitoes belonging to 12 species were collected during 4 months period (August and September in post-monsoon and March and April in pre-monsoon) all total of 20 days (80 man-hours) period.

5.1 Number and Density of Mosquitoes collected in Indoor and Outdoor Collection

5.1.1 Post-monsoon survey

The total of 718 Culex Mosquitoes belong to 12 species (12 species in cattle shed, 11 species in human dwelling and outdoor collection) were recorded during ten day period in August and September. *Culex quinquefasciatus* is the predominant species about 121/121 samples were recorded from human dwelling and cattle shed and 80 from Outdoor collection. *Culex mimulus* is the least dominant species only one sample was collected from cattleshed. The density of *Cx. mimulus w*as highest i.e, 1 in Cattleshed. In total highest density 4.91was recorded in Cattleshed. Most of the samples (n=279) were recorded from human dwelling. See table 1.

Table 1

Number and Density of Mosquitoes

Name of species	INDOOR				OUTDOOR		TOTAL	
	Number of Mosquitoes		Density of Mosquitoes		Number of Mosquitoes	Density of Mosquitoes	Number of Mosquitoes	Density of Mosquitoes
	Human	Cattle	Human	Cattle				
Cx. quinquefasciatus	121	121	0.37	0.37	80	0.24	322	0.98
Cx. fuscocephala	103	87	0.41	0.34	60	0.24	250	0.99
Cx. tritaeniorhynchus	21	21	0.44	0.44	5	0.10	47	0.98
Cx. gelidus	9	7	0.32	0.25	12	0.42	28	0.99
Cx. bitaeniorhynchus	5	9	0.25	0.45	6	0.3	20	1
Cx. whitmorei	6	4	0.5	0.33	2	0.16	12	0.99
Cx. pseudovishnuivishnoi	4	5	0.36	0.45	2	0.18	11	0.99
Cx. whitei	4	3	0.44	0.33	2	0.22	9	0.99
Cx. theileri	2	2	0.28	0.28	3	0.42	7	0.98
Cx. barraudi	2	3	0.28	0.42	2	0.28	7	0.98
Cx. sinensis	2	1	0.5	0.25	1	0.25	4	1
Cx. mimulus	0	1	0	1	0	0	1	1
Grand Total	279	264	4.15	4.91	175	2.81	718	11.87

Figure 2
Number of Mosquitoes collected in indoor and outdoor collection in Post Monsoon

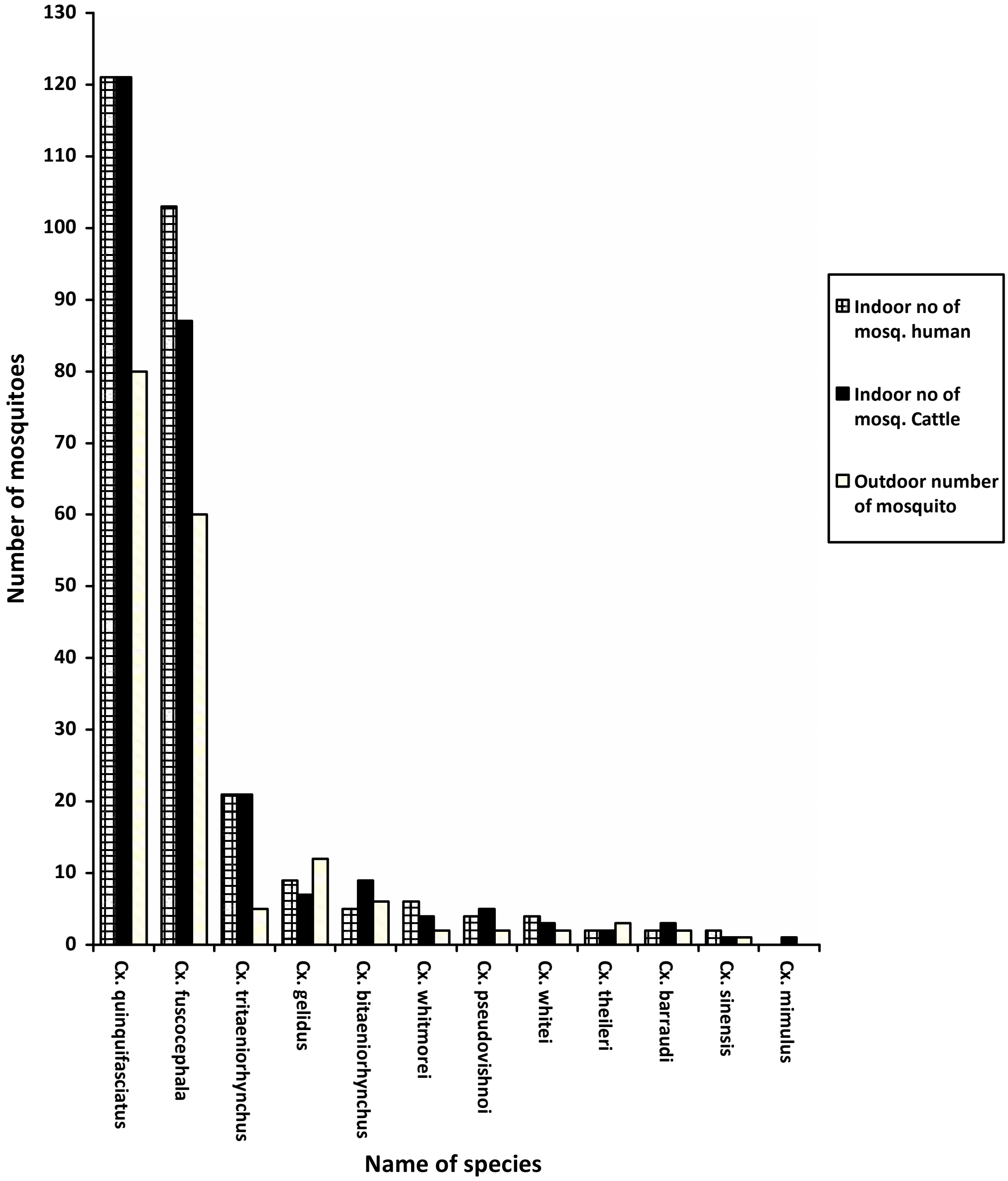
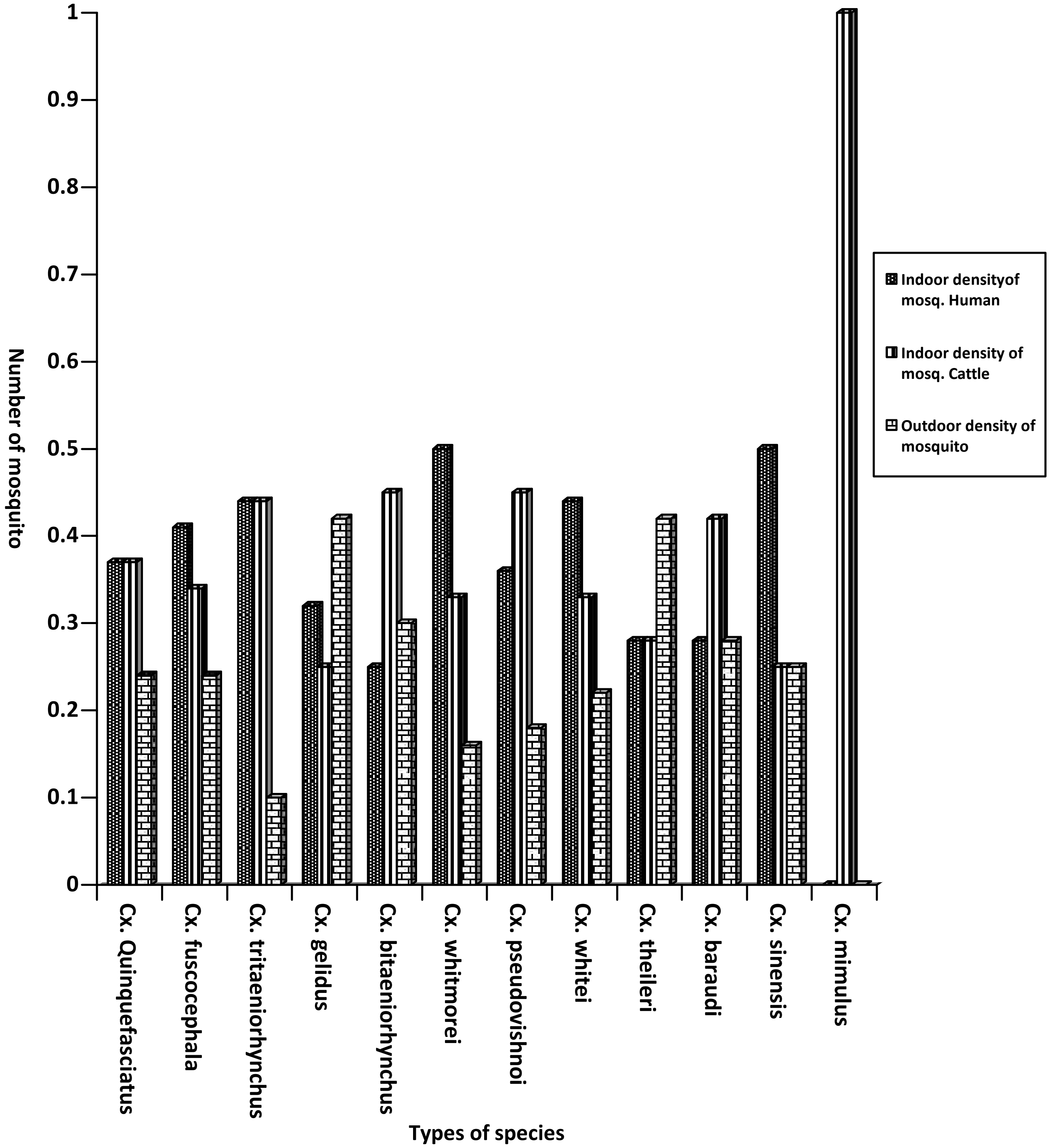


Figure 3
Density of mosquito
indoor and outdoor post monsoon



5.1.2 Pre-monsoon survey

The total of 479 Culex mosquitoes belong to five species (five species in indoor and four species in outdoor collection), were recorded during ten days period in March and April. *Culex quinquefasciatus* was the predominant species about 85,106 and 33 samples were recorded from human dwelling, Cattleshed and outdoor collection respectively. Most of the samples (n=226) were recorded from Cattleshed. *Culex bitaeniorhynchus* was least dominant species. *Cx. whitmorei, Cx. pseudovishnui, Cx. whitei, Cx. theileri, Cx. barraudi, Cx. sinensis, Cx. mimulus* were absent. Density of *Cx. tritaeniorhynchus* (0.42 in human dwelling, 0.57 in cattleshed) was highest. In total highest density 2.38 was recorded in Cattleshed. See table 2.

Table 2

Number and Density of Mosquitoes

Name of species	IN DOOR				OUT DOOR		TOTAL	
	Number of Mosquitoes		Density of Mosquitoes		Number of Mosquitoes	Density of Mosquitoes	Number of Mosquitoes	Density of Mosquitoes
	Human	Cattle	Human	Cattle				
Cx. quinquefasciatus	85	106	0.37	0.47	33	0.14	224	0.98
Cx. fuscocephala	80	92	0.40	0.46	26	0.13	198	0.99
Cx. tritaeniorhynchus	9	12	0.42	0.57	0	0	21	0.99
Cx. gelidus	6	7	0.33	0.38	5	0.27	18	0.98
Cx. bitaeniorhynchus	6	9	0.33	0.5	3	0.16	18	0.99
Grand Total	186	226	1.85	2.38	67	0.7	479	4.93

Figure 4
Pre-monsoon number of mosquitoes

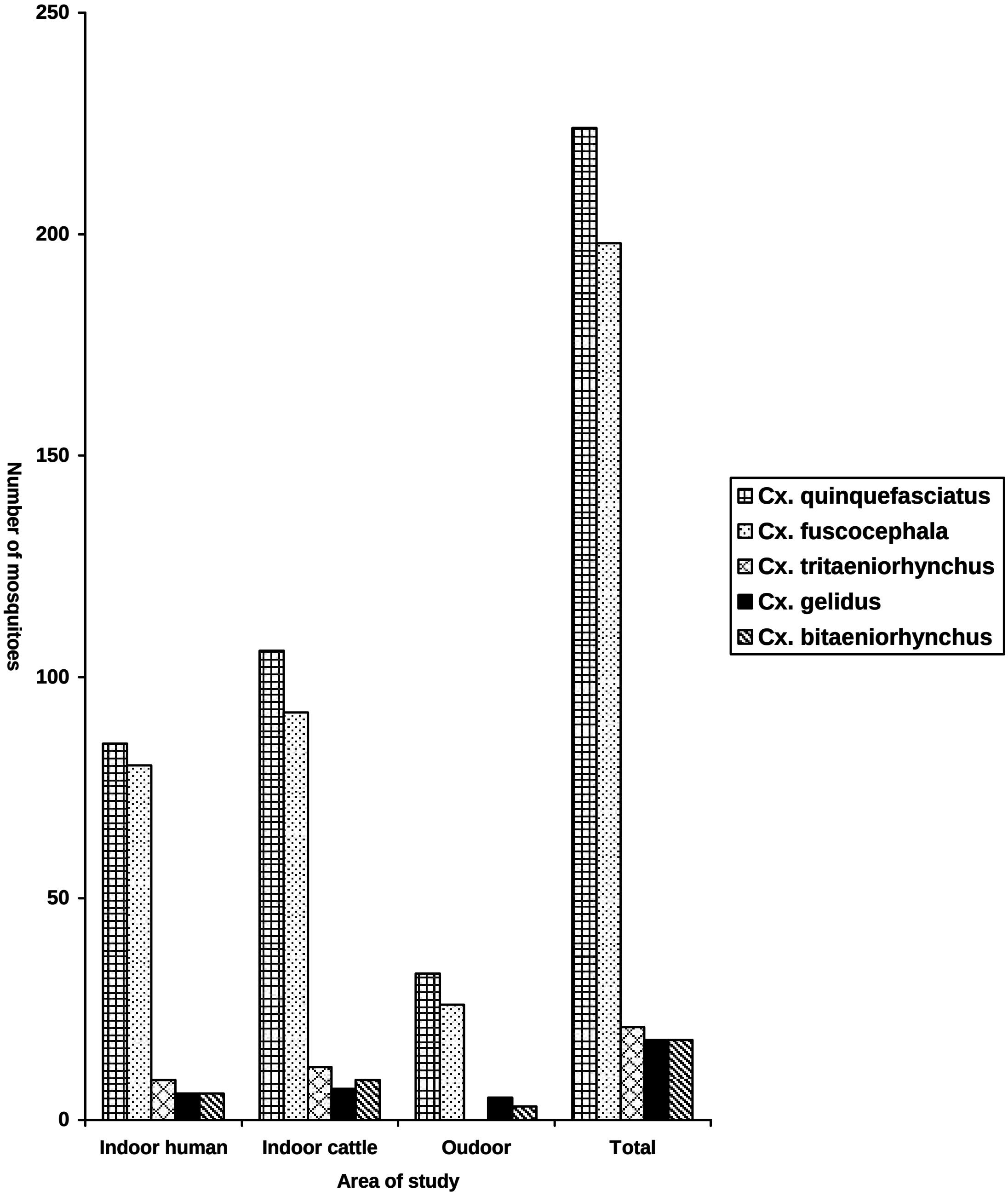
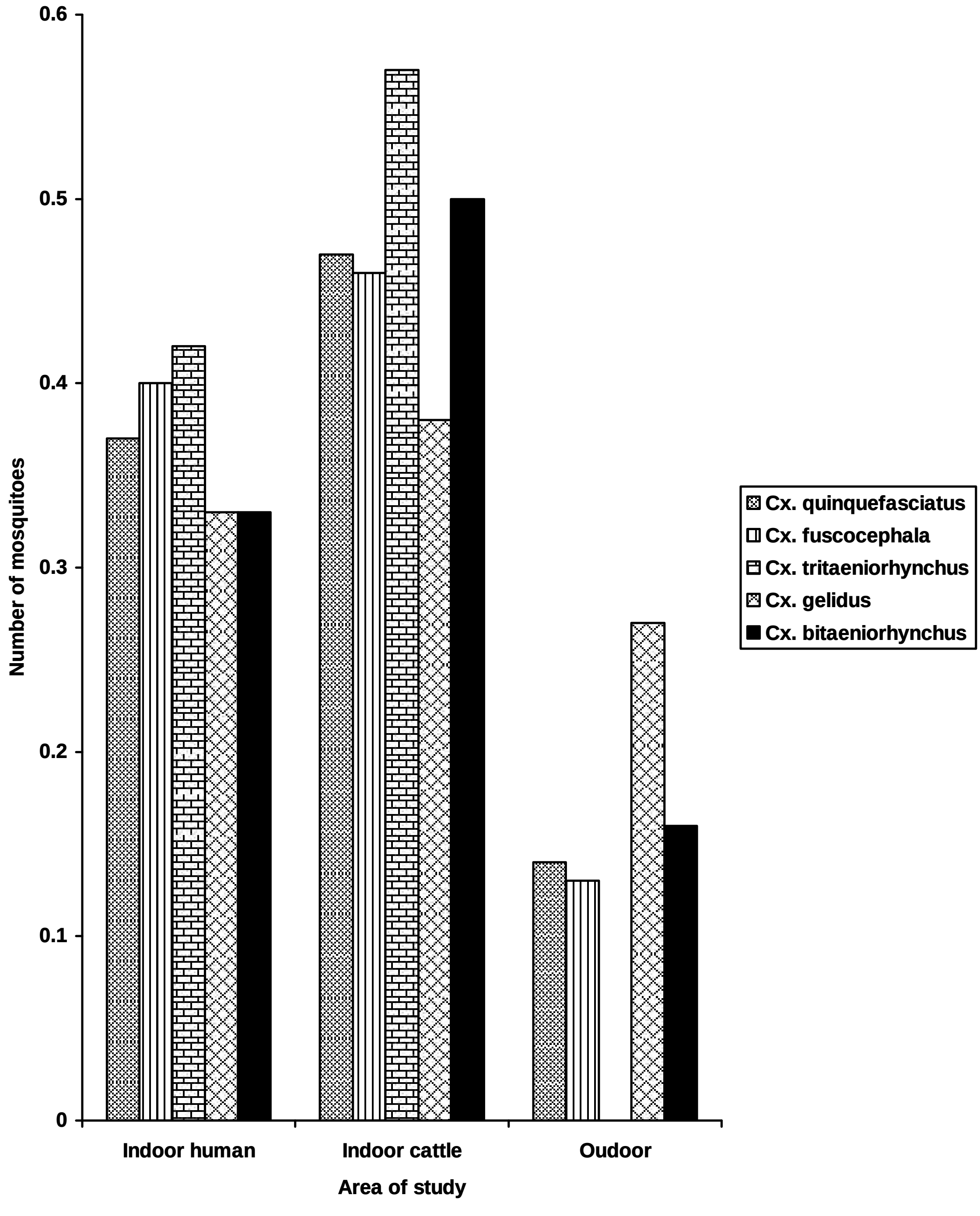


Figure 5
Pre-monsoon density of monsquitoes



5.2 Monthly variations of density of mosquitoes by indoor hand collection and outdoor collections

During the four months, the mosquito samples were highest in September. About 12 species were recorded in September from Bramhapuri village but only two species were recorded in April in same village. *Culex quinquefasciatus* and *Cx. fuscocephala* were widely distributed, they were found in four months of collecting period in both indoor and outdoor hand collection.

Table 3

Density of mosquitoes with Monthly Variation

			IN DOOR				OUT DOOR	
Name of Species	Months	Villages	Human	Cattle	Human	Cattle	Number of Mosquitoes	Density of Mosquitoes
Cx. quinquefasciatus	August	Gitanagar	11	17	0.15	0.23	9	0.12
Cx. fuscocephala			14	9	0.19	0.19	12	0.16
Cx. quinquefasciatus	August	Kesharbag	23	19	0.28	0.23	12	0.14
Cx. fuscocephala			10	6	0.12	0.07	5	0.06
Cx. tritaeniorhynchus			3	1	0.03	0.01	0	0
Cx. bitaeniorhynchus			0	2	0	0.02	0	0
Cx. quinquefasciatus	August	Parashnagar	12	23	0.16	0.31	6	0.08
Cx. fuscocephala			18	11	0.24	0.15	3	0.04
Cx. quinquefasciatus	August	Suryapur	9	10	0.24	0.27	11	0.29
Cx. fuscocephala			9	7	0.24	0.18	6	0.16
Cx. quinquefasciatus	August	Khole- simal	6	7	0.16	0.18	4	0.1
Cx. fuscocephala			8	5	0.21	0.13	7	0.18

Cx. quinquefasciatus	Sept.	Dobato	12	5	0.24	0.1	7	0.14
Cx. fuscocephala			7	7	0.14	0.14	4	0.08
Cx.tritaeniorhynchus			2	2	0.04	0.04	1	0.02
Cx.bitaeniorhynchus			0	2	0	0.04	0	0
Cx. quinquefasciatus	Sept.	Champanagar	26	22	0.22	0.18	10	0.08
Cx. fuscocephala			22	17	0.18	0.14	4	0.03
Cx.tritaeniorhynchus			5	6	0.04	0.05	0	0
Cx. gelidus			3	0	0.02	0	1	0.008
Cx. qinquefasciatus	Sept.	Vijayanagar	7	4	0.12	0.06	6	0.1
Cx. fuscocephala			6	4	0.1	0.06	4	0.06
Cx.tritaeniorhynchus			4	2	0.06	0.03	2	0.03
Cx. gelidus			1	1	0.01	0.01	3	0.05
Cx.bitaeniorhynchus			1	1	0.01	0.01	2	0.03
Cx. whitmorei			1	2	0.01	0.03	1	0.01
Cx. whitei			1	1	0.01	0.01	1	0.01
Cx. pseudovishnui			0	1	0	0.01	1	0.01
Cx. theileri			0	0	0	0	1	0.01
Cx. qinquefasciatus	Sept.	Rampur	6	13	0.09	0.2	7	0.1
Cx. fuscocephala			5	8	0.07	0.12	9	0.14
Cx.tritaeniorhynchus			1	2	0.01	0.03	0	0
Cx. gelidus			2	2	0.03	0.03	4	0.06
Cx.bitaeniorhynchus			1	1	0.01	0.01	0	0
Cx. pseudovishnui			2	1	0.03	0.01	0	0
Cx. qinquefasciatus	Sept.	Bramhapuri	6	9	0.05	0.07	5	0.04
Cx. fuscocephala			7	5	0.06	0.04	9	0.07
Cx.tritaeniorynchus			6	8	0.05	0.06	2	0.01
Cx. gelidus			3	4	0.02	0.03	4	0.03
Cx.bitaeniorhynchus			3	3	0.02	0.02	4	0.03
Cx. whitmorei			5	2	0.04	0.01	1	0.008

Cx. whitei			3	2	0.02	0.01	1	0.008
Cx. pseudovishnui			2	3	0.01	0.02	1	0.008
Cx. barraudi			2	3	0.01	0.02	2	0.01
Cx. theileri			2	2	0.01	0.01	2	0.01
Cx. sinensis			2	1	0.01	0.008	1	0.008
Cx. mimulus			0	1	0	0.008	0	0
Cx. quinquefasciatus	March	Gitanagar	14	8	0.25	0.14	6	0.1
Cx. fuscocephala			11	9	0.19	0.16	3	0.05
Cx. tritaeniorhynchus			1	2	0.01	0.03	0	0
Cx. bitaeniorhynchus			1	1	0.01	0.01	0	0
Cx. quinquefasciatus	March	Champanagar	2	4	0.07	0.15	0	0
Cx. fuscocephala			5	9	0.19	0.34	0	0
Cx. tritaeniorhynchus			1	2	0.03	0.07	0	0
Cx. bitaeniorhynchus			0	3	0	0.11	0	0
Cx. quinquefasciatus	March	Parasnagar	4	9	0.15	0.34	2	0.07
Cx. fuscocephala			4	7	0.15	0.26	0	0
Cx. quinquefasciatus	March	Kesharbag	6	8	0.14	0.19	1	0.02
Cx. fuscocephala			14	11	0.33	0.26	0	0
Cx. tritaeniorhynchus			2	0	0.04	0	0	0
Cx. quinquefasciatus	April	Vijayanagar	2	11	0.03	0.19	6	0.1
Cx. fuscocephala			5	12	0.08	0.21	2	0.03
Cx. gelidus			5	7	0.08	0.12	2	0.03
Cx. bitaeniorhynchus			0	3	0	0.05	1	0.01
Cx.	April	Dobato	12	17	0.19	0.27	4	0.06

quinquefasciatus								
Cx. fuscocephala			8	3	0.13	0.04	6	0.09
Cx. tritaeniorhynchus.			5	3	0.08	0.04	0	0
Cx. bitaeniorhynchus			2	1	0.03	0.01	0	0
Cx. quinquefasciatus	April	Rampur	10	6	0.23	0.14	2	0.04
Cx. fuscocephal			6	9	0.14	0.21	0	0
Cx. gelidus			3	1	0.07	0.02	0	0
Cx.bitaeniorhynchus			3	0	0.07	0	2	0.04
Cx. quinquefasciatus	April	Suryapur	15	27	0.19	0.34	2	0.02
Cx. fuscocephala			10	17	0.12	0.21	1	0.01
Cx. tritaeniorhynchus			0	5	0	0.06	0	0
Cx. bitaeniorhynchus			0	1	0	0.01	0	0
Cx. quinquefasciatus	April	Khole-simal	11	9	0.22	0.18	6	0.12
Cx. fuscocephala			6	7	0.12	0.14	9	0.18
Cx. quinquefasciatus	April	Bramhapuri	9	7	0.2	0.15	4	0.09
Cx. fuscocephala			11	8	0.26	0.18	5	0.11

5.3 Vector Abundance

5.3.1 Species composition: Percent abundance

The percent abundance of Mosquitoes is calculated as,

Percent Abundance: Number of Collected Mosquitoes (SPP) 100%

Total Number of Mosquitoes

(For Vector abundance and species composition)

Culex quinquefasciatus is the most abundant in post and pre monsoon, about 322 i.e, 26.90 percent found in post monsoon and about 224 i.e., 18.71 percent found in pre monsoon. *Cx. mimulus* is the least abundant in both season about one i.e, 0.08 percent found in post monsoon and zero in pre monsoon.

The percent abundance of six species were found less than one percent in post-monsoon and the abundance of seven species is zero in pre monsoon.

The percent abundance of *Cx. tritaeniorhynchus* in post-monsoon and pre monsoon is 3.92 percent and 1.75 percent respectively which is potential vector of JE in Nepal. The percent abundance of *Cx. fuscocephala* in post-monsoon and pre-monsoon is 20.88 percent and 16.54 percent respectively. See table 4.

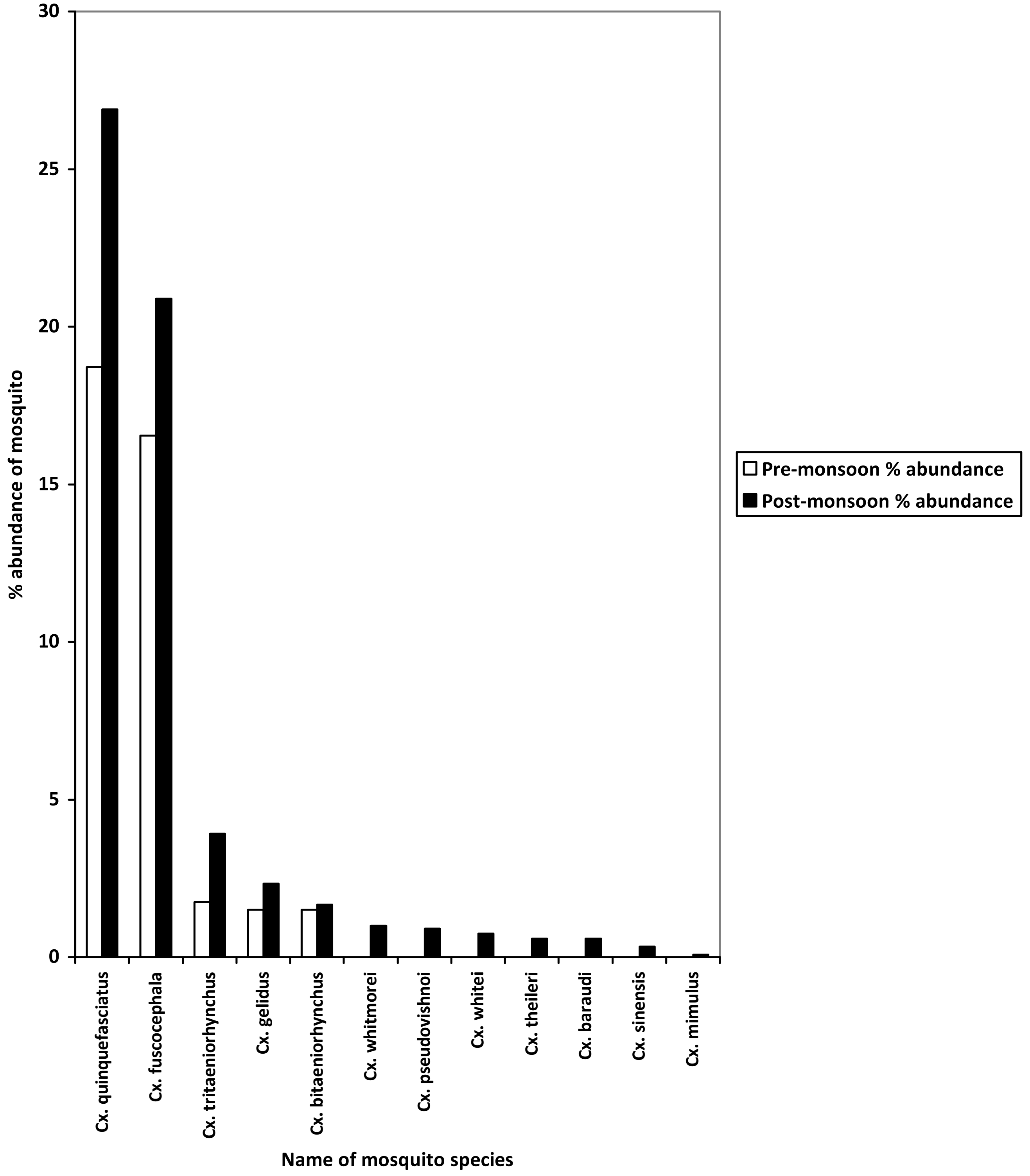
Table 4

Percent Abundance of Mosquito species

Name of Species	Post-Monsoon		Pre-Monsoon	
	Number of Collected Mosquitoes	Percent abundance	Number of Collected Mosquitoes	Percent abundance
Cx. quinquefasciatus	322	26.90	224	18.71
Cx. fuscocephala	250	20.88	198	16.54
Cx. tritaeniorhynchus	47	3.92	21	1.75
Cx. gelidus	28	2.33	18	1.50
Cx. bitaeniorhynchus	20	1.67	18	1.50
Cx. whitimorei	12	1.00	0	0
Cx. pseudovishnui	11	0.91	0	0
Cx. whitei	9	0.75	0	0
Cx. barraudi	7	0.58	0	0
Cx. theileri	7	0.58	0	0
Cx. sinensis	4	0.33	0	0
Cx. mimulus	1	0.08	0	0

Figure 6

Graphical presentation of Percent Abundance of Mosquito Species



5.3.2 Indoor resting Density (Human)

The indoor resting Density of Mosquitoes sample is calculated as,

$$\text{Indoor resting Density} = \frac{\text{Number of Mosquitoes}}{\text{Number of bait (Human)}}$$

Highest indoor resting density was 9.33 at 25.75ºc in post-monsoon and lowest indoor density 0.72 at 21.75ºc in pre-monsoon reported in Champanagar village. See table 5.

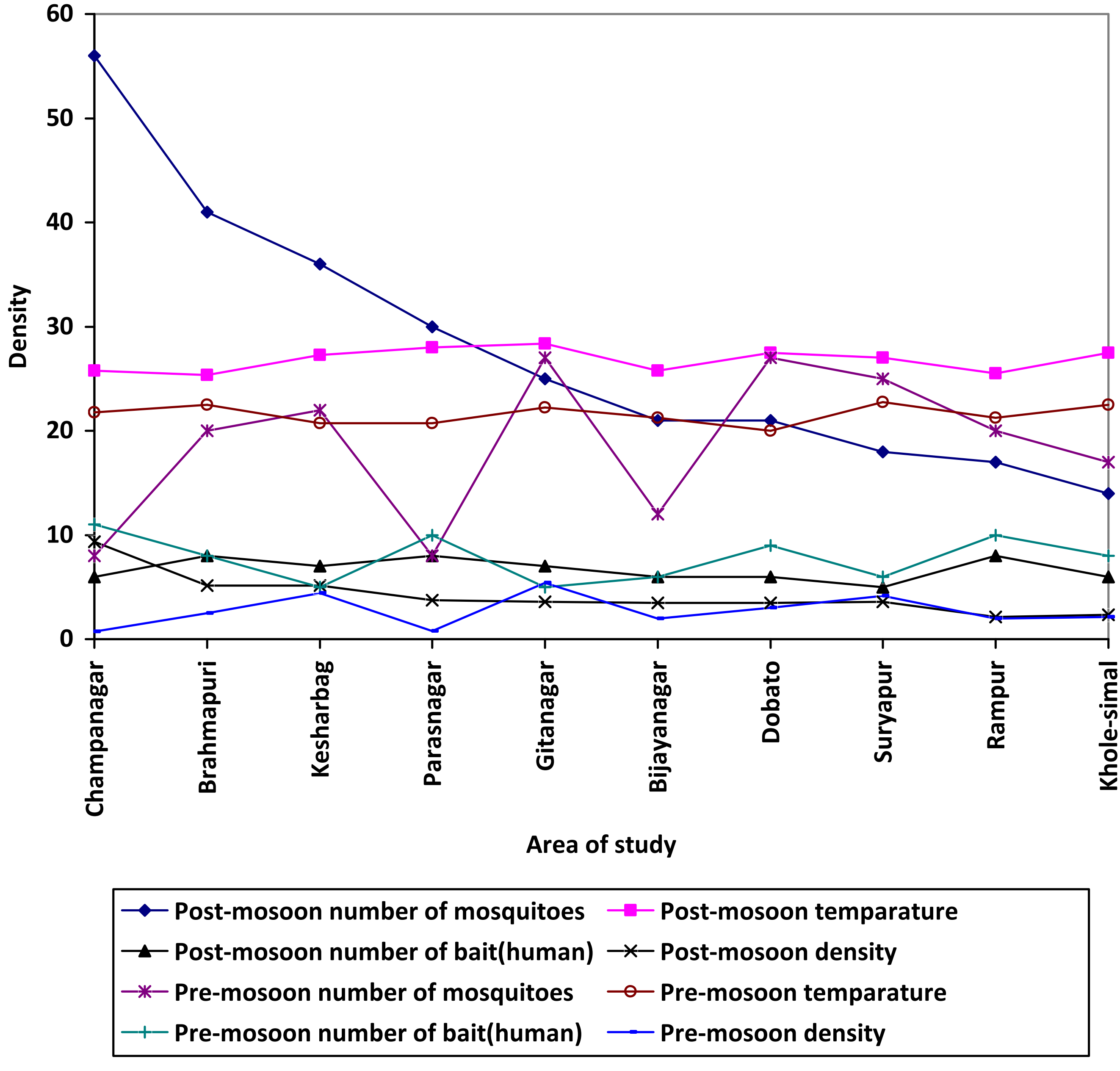
Table 5

Indoor resting Density (Human)

Name of village	Post- Monsoon				Pre -Monsoon			
	Number of Mosquitoes	Temp.^{0}c	Number of bait (human)	Density	Number of Mosquitoes	Temp.^{0}c	Number of bait (human)	Density
Champanagar	56	25.75	6	9.33	8	21.75	11	0.72
Brahmapuri	41	25.37	8	5.12	20	22.5	8	2.5
Kesharbag	36	27.25	7	5.14	22	20.75	5	4.4
Parashnagar	30	28	8	3.75	8	20.75	10	0.8
Gitanagar	25	28.37	7	3.57	27	22.25	5	5.4
Bijayanagar	21	25.75	6	3.5	12	21.25	6	2
Dobato	21	27.5	6	3.5	27	20	9	3
Suryapur	18	27	5	3.6	25	22.75	6	4.16
Rampur	17	25.5	8	2.12	20	21.25	10	2
Khole-simal	14	27.5	6	2.33	17	22.5	8	2.12

Figure 7

Graphical presentation of Indoor Resting Density (Human)



5.3.3 Indoor resting Density (Cattle)

The indoor resting Density of Mosquitoes sample is calculated as,

$$\text{Indoor resting Density} = \frac{\text{Number of Mosquitoes}}{\text{Number of bait (cattle)}}$$

Highest indoor resting density was 5.55 at 22ºc in Suryapur in pre-monsoon and lowest indoor resting density was 0.70 at 25.37ºc in Kholesimal in post-monsoon. See table 6.

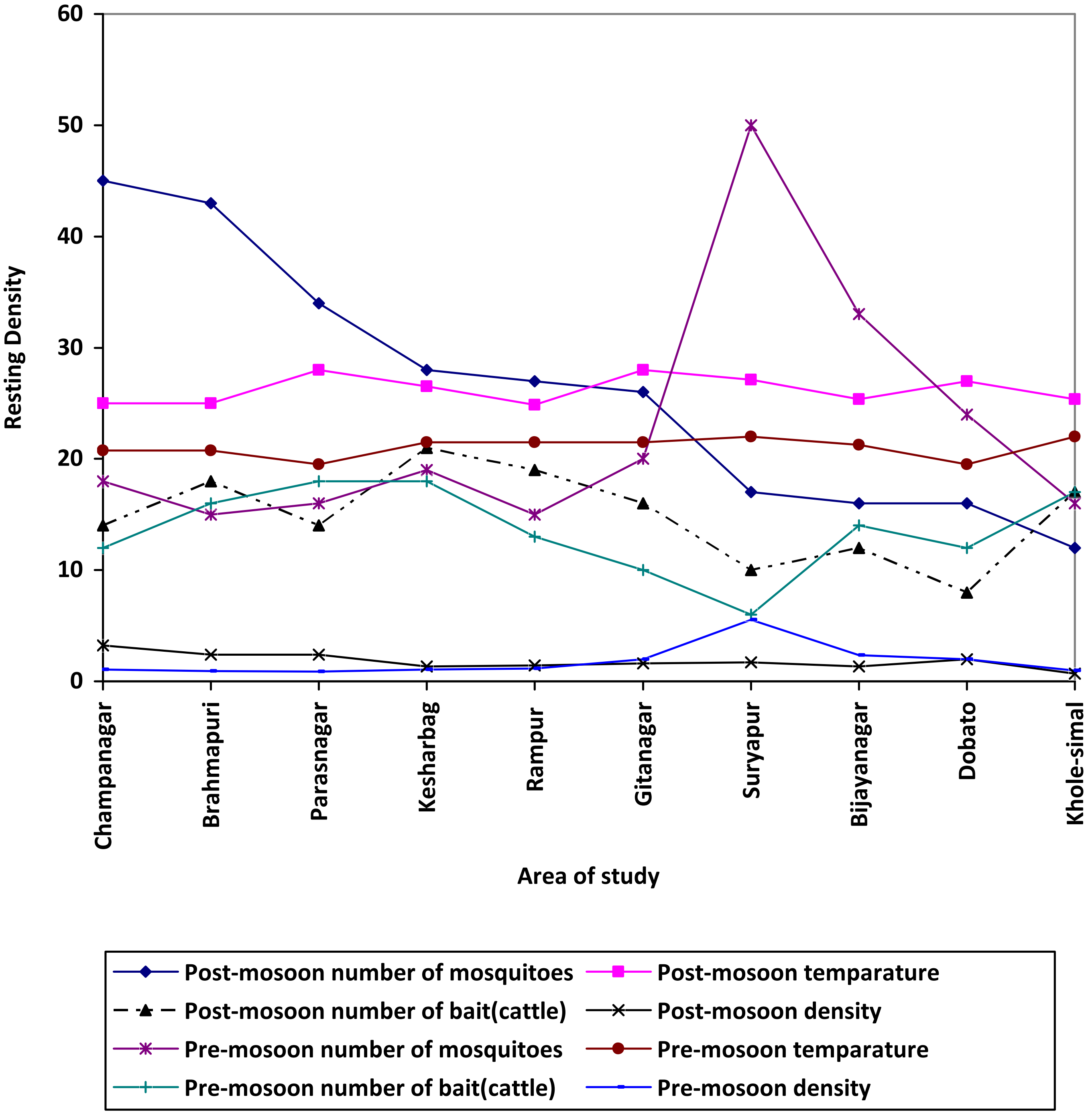
Table 6

Indoor resting Density (Cattle)

Name of village	Post -Monsoon				Pre -Monsoon			
	Number. of Mosquitoes	Temp.^{0}c	Number of bait (Cattle)	Density	Number of Mosquitoes	Temp.^{0}c	Number of bait (Cattle)	Density
Champanagar	45	25	14	3.21	18	20.75	12	105
Brahmapuri	43	25	18	2.38	15	20.75	16	0.93
Parashnagar	34	28	14	2.42	16	19.5	18	0.88
Kesharbag	28	26.5	21	1.33	19	21.5	18	1.05
Rampurr	27	24.87	19	1.42	15	21.5	13	1.15
Gitanagar	26	28	16	1.62	20	21.5	10	2
Suryapur	17	27.12	10	1.7	50	22	9	5.55
Bijayanagar	16	25.37	12	1.33	33	21.25	14	2.35
Dobato	16	27	8	2	24	19.5	12	2
Khole-simal	12	25.37	17	0.70	16	22	17	0.95

Figure 8

Graphical presentation of Indoor Resting Density (Cattle)



5.3.4 Outdoor Density

The Outdoor Density of Mosquitoes samples is calculated as,

$$\text{Outdoor Density} = \frac{\text{Number of Mosquitoes}}{\text{Total number of Mosquitoes}}$$

Both Highest and Lowest outdoor density was found in pre-monsoon, highest 0.22 in Kholesimal at 22.5ºc and lowest 0 at 21.5ºc temperature in Champanagar village. See table 7.

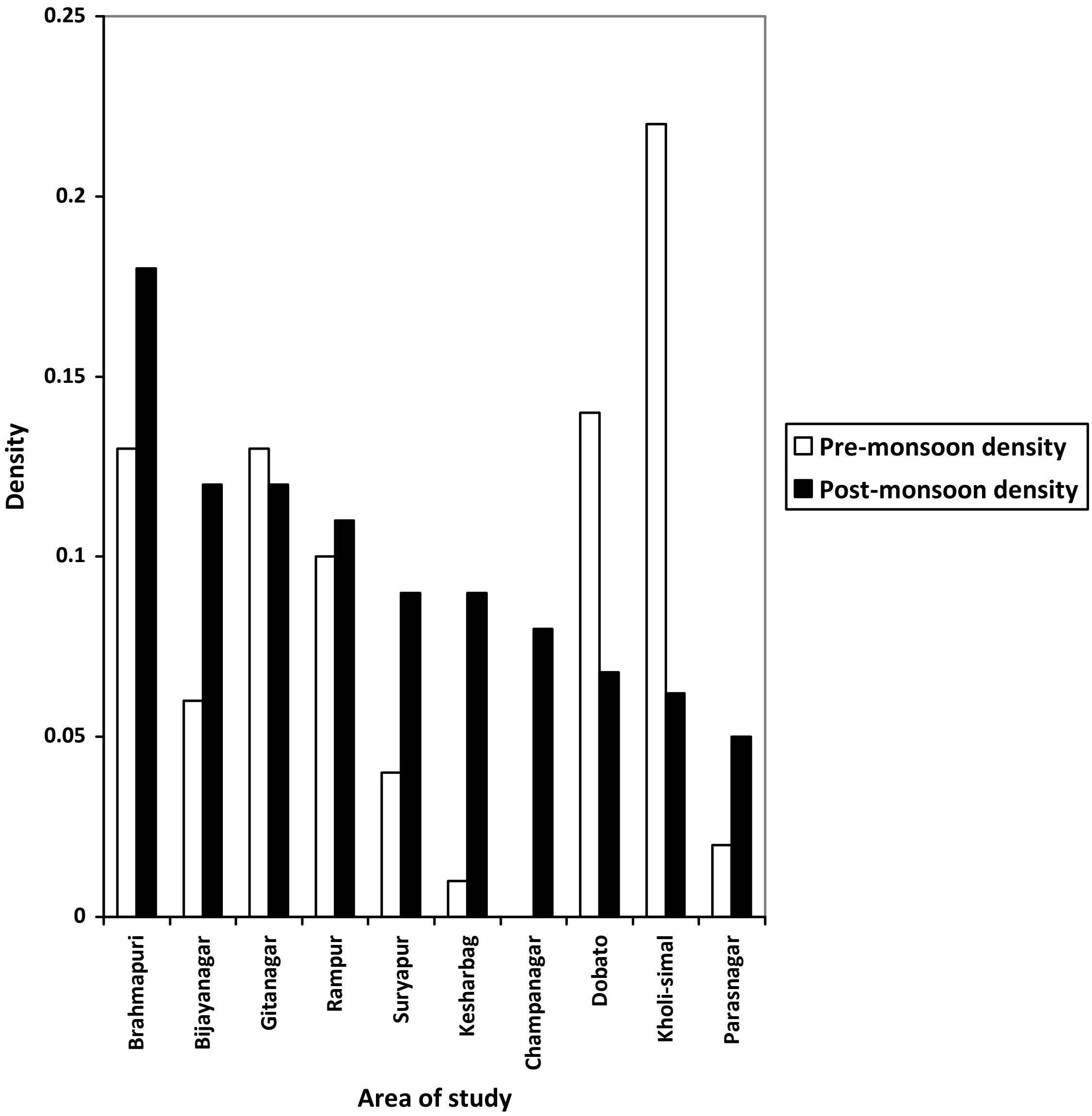
Table 7

Outdoor Density of Mosquitoes

Name of village	Post-Monsoon			Pre-Monsoon		
	Number of Mosquitoes	Temp. ºc	Density	Number of Mosquitoes	Temp. ºc	Density
Bramhapuri	32	25.5	0.18	9	22	0.13
Bijayanagar	21	26.12	0.12	11	22.25	0.06
Gitanagar	21	29	0.12	9	22.25	0.13
Rampur	20	25.37	0.11	7	22.5	0.10
Suryapur	17	28.5	0.09	3	22.25	0.04
Kesharbag	17	26.75	0.09	1	22	0.01
Champanagar	15	25.62	0.08	0	21.5	0
Dobato	12	26.75	0.068	10	20.25	0.14
Khole-simal	11	26.12	0.062	15	22.5	0.22
Parashnagar	9	29	0.05	2	19.75	0.02
Total	175			67		

Figure 9

Graphical Presentation of Outdoor Density of Mosquitoes



5.3.5 Resting Habit

The resting habit of Mosquioes of each specioe is the ratio of number of mosquitioes of each species in indoor collection and number of mosquitioes of each species in outdoor collection.

Resting Habit: Number of Mosquitioes in indoor collection
Number of Mosquitioes in outdoor collection

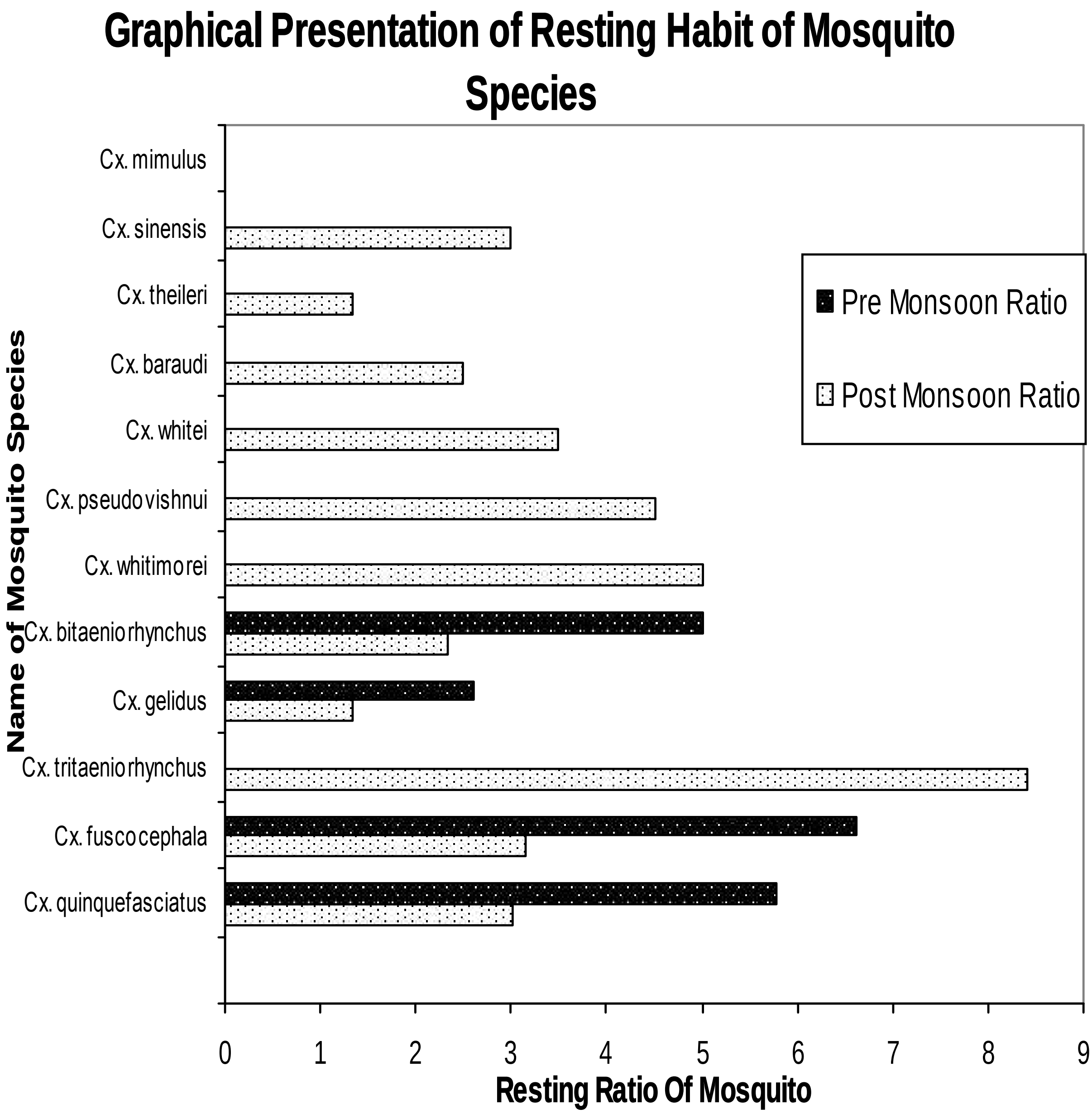
The resting habit of *Culex mimulus* and *Cx. tritaeniorhynchus* was infinitive in post-monsoon and pre-monsoon respectively. The resting habit of *Cx.whitimorei, Cx. pseudovishnui, Cx. whitei, Cx. barraudi, Cx. theileri, Cx. sinensis* and *Cx. mimulus* is zero in pre-monsoon.

Table 8

Resting Habit of Mosquito Species

Name of species	Post-Monsoon			Pre-Monsoon		
	Number of Mosquitoes		Resting Habit	Number of Mosquitoes		Resting Habit
	Indoor	Outdoor		Indoor	Outdoor	
Cx. quinquefasciatus	242	80	3.01	191	33	5.78
Cx. fuscocephala	190	60	3.16	172	26	6.61
Cx. tritaeniorhynchus.	42	5	8.4	21	0	∞
Cx. gelidus	16	12	1.33	13	5	2.6
Cx. bitaeniorhynchus	14	6	2.33	15	3	5
Cx. whitimorei	10	2	5	0	0	0
Cx. pseudovishnui	9	2	4.5	0	0	0
Cx. whitei	7	2	3.5	0	0	0
Cx. barraudi	5	2	2.5	0	0	0
Cx. theileri	4	3	1.33	0	0	0
Cx. sinensis	3	1	3	0	0	0
Cx. mimulus	1	0	∞	0	0	0

Figure 10


Graphical Presentation of Resting Habit of Mosquito Species
Name of Mosquito Species
Cx. mimulus
Cx. sinensis
Cx. theileri
Cx. baraudi
Cx. whitei
Cx. pseudovishnui
Cx. whitimorei
Cx. bitaeniorhynchus
Cx. gelidus
Cx. tritaeniorhynchus
Cx. fuscocephala
Cx. quinquefasciatus
Pre Monsoon Ratio
Post Monsoon Ratio
0
1
2
3
4
5
6
7
8
9
Resting Ratio Of Mosquito

5.3.6 Room Density

The room density of mosquitoes is calculated as,

$$\text{Room Density} = \frac{\text{Number of Mosquitoes (each species)}}{\text{Number of Room}}$$

The highest room density was (i.e. 6.5) of *Culex quinquefasciatus* in Champanagar village. In both season (pre-monsoon and post-monsoon), same (i.e.3.75) room density of *Cx. quinquefasciatus was* in Suryapur village. Room density of *Cx. bitaeniorhynchus* in Dobato and Gitanagar and *Cx. tritaeniorhynchus* in Gitanagar is zero in post-monsoon. In pre-monsoon room density of *Cx.tritaeniorhynchus, Cx. gelidus, Cx. bitaeniorhynchus, Cx. whitimorei, Cx. whitei, Cx. pseudovishnui, Cx. barraudi, Cx. theileri* and *Cx. sinensis* is zero. See table 9.

Table 9

Room Density of Mosquitoes Species

Name of species	Name of village	Post-Monsoon		Pre-Monsoon	
		Number of Mosquitoes	Density	Number of Mosquitoes	Density
Cx. quinquefasciatus	Gitanagar	14	3.5	14	3.5
Cx. fuscocephala		11	2.75	11	2.75
Cx. tritaeniorhynchus		-	-	1	0.25
Cx. bitaeniorhynchus		-	-	1	0.25
Cx. quinquefasciatus	Kesharbag	23	5.75	6	1.5
Cx. fuscocephala		10	2.5	14	3.5
Cx. tritaeniorhynchus		3	0.75	2	0.5

Cx. quinquefasciatus.	Parashnagar	12	3	2	0.5
Cx. fuscocephala		18	4.5	-	-
Cx. quinquefasciatus.	Suryapur	9	2.25	15	3.75
Cx. fuscocephala		9	2.25	10	2.5
Cx. quinquefasciatus.	Khole-simal	6	1.5	11	2.75
Cx. fuscocephala		8	2	6	1.5
Cx. quinquefasciatus	Dobato	12	3	12	3
Cx. fuscocephala		7	1.75	8	2
Cx. tritaeniorhynchus		2	0.5	5	1.25
Cx. bitaeniorhynchus		-	-	2	0.5
Cx. quinquefasciatus.	Champanagar	26	6.5	2	0.5
Cx. fuscocephala		22	5.5	5	1.25
Cx. tritaeniorhynchus		5	0.25	1	0.25
Cx. gelidus		3	0.75	-	-
Cx. quinquefasciatus.	Vijayanagar	7	1.75	2	0.5
Cx. fuscocephala		6	1.5	5	1.25
Cx. tritaeniorhynchus		4	1	-	-
Cx. gelidus		1	0.25	5	1.25
Cx. bitaeniorhynchus		1	0.25	-	-
Cx. whitmorei		1	0.25	-	-
Cx. whitei		1	0.25	-	-
Cx. quinquefasciatus.	Rampur	6	1.5	2	0.5
Cx. fuscocephala		5	1.25	-	-
Cx. tritaeniohrynchus		1	0.25	-	-
Cx. gelidus		2	0.5	-	-
Cx.		1	0.25	2	0.5

bitaeniorhynchus					
Cx.pseudovishnui		2	0.5	-	-
Cx. quinquefasciatus	Bramhapuri	6	1.5	9	0.25
Cx. fuscocephala		7	1.75	11	2.75
Cx. tritaeniorhynchus		6	1.5	-	-
Cx. gelidus		3	0.75	-	-
Cx. bitaeniorhynchus		3	0.75	-	-
Cx. whitimorei		5	1.25	-	-
Cx. whitei		3	0.75	-	-
Cx. pseudovishnui		2	0.5	-	-
Cx. barraudi		2	0.5	-	-
Cx. theileri		2	0.5	-	-
Cx. sinensis		2	0.5	-	-

5.4 Temperature versus Month

The total of 12 species were found at 25.26ºc in September in Bramhapuri village and only two species (i.e. *Culex quinquefasciatus* and *Cx. fuscocephala)* were found at 28.33ºc in August, at 20ºc in March in Parasnagar village and at 26.33ºc in August, at 22.33ºc in April in Kholesimal village. See table 10.

Table 10

Number and Density of Mosquito with respect to Temperature

Name of species	Name of village	Post-Monsoon				Pre-Monsoon			
		Month	Number of Mosq.	Density	Temp. ºc	Month	Number of Mosq.	Density	Temp. ºc
Cx. quinquefasciatus	Gitanagar	August	35	0.48	28.45	March	28	0.5	22
Cx. fuscocephala			37	0.51			23	0.41	
Cx. tritaeniorhynchus			-	-			3	0.05	
Cx. bitaeniorhynchus			-	-			2	0.03	
Cx. quinquefasciatus	Kesharbag	August	54	0.66	26.83		15	0.35	21.41
Cx. fuscocephala			21	0.25		March	25	0.83	
Cx. tritaeniorhynchus			4	0.04			2	0.04	
Cx. bitaeniorhynchus			2	0.02			-	-	
Cx. quinquefasciatus	Parashnagar	August	41	0.65	28.39	March	15	0.57	20
Cx. fuscocephala			32	0.43			11	0.42	
Cx. quinquefasciatus	Suryapur	August	30	0.57	27.54	April	44	0.56	22.33
Cx. fuscocephala			22	0.42			28	0.35	
Cx. tritaeniorhynchus			-	-			5	0.06	
Cx. bitaeniorhynchus			-	-			1	0.01	
Cx. quinquefasciatus	Khole-simal	August	17	0.45	26.33	April	26	0.54	22.33
Cx. fuscocephala			20	0.54			22	0.45	
Cx. quinquefasciatus	Dobato	Sept.	24	0.48	27.08	April	33	0.54	19.91
Cx. fuscocephala			18	0.36			17	0.27	
Cx.			5	0.10			8	0.13	

tritaeniorhynchus									
Cx. bitaeniorhynchus			2	0.04			3	0.04	
Cx. quinquefasciatus	Champanagar	Sept.	58	0.5	25.42	March	6	0.23	21.33
Cx. fuscocephala			43	0.37			14	0.53	
Cx. tritaeniorhynchus			11	0.09			3	0.11	
Cx. gelidus			4	0.03			3	0.11	
Cx. quinquefasciatus	Vijayanagar	Sept.	17	0.29	25.74	April	19	0.33	21.58
Cx. fuscocephala			14	0.24			19	0.33	
Cx. tritaeniorhynchus			8	0.13			-	-	
Cx. gelidus			5	0.08			14	0.25	
Cx. bitaeniorhynchus			4	0.06			4	0.07	
Cx. whitmorei			4	0.06			-	-	
Cx. whitei			3	0.05			-	-	
Cx. pseudovishnui			2	0.03			-	-	
Cx. theileri			1	0.01			-	-	
Cx. quinquefasciatus	Rampur	Sept.	26	0.40	25.24	April	18	0.42	21.75
Cx. fuscocephala			22	0.34			15	0.35	
Cx. tritaeniorhynchush			3	0.04			-	-	
Cx. gelidus			8	0.12			4	0.09	
Cx. bitaeniorhynchus			2	0.03			5	0.11	
Cx. pseudovishnui			3	0.04			-	-	
Cx. quinquefasciatus	Bramhapuri	Sept.	20	0.17	25.26	April	20	0.45	21.75
Cx. fuscocephala			21	0.18			24	0.54	
Cx. tritaeniorhynchus			16	0.13			-	-	

Cx. gelidus			11	0.09			-	-	
Cx. bitaeniorhynchus			10	0.08			-	-	
Cx. whitmorei			8	0.06			-	-	
Cx. whitei			6	0.05			-	-	
Cx. pseudovishnui			6	0.06			-	-	
Cx. barraudi			7	0.07			-	-	
Cx. theileri			6	0.05			-	-	
Cx. sinensis			4	0.03			-	-	
Cx. mimulus			1	0.008			-	-	

CHAPTER VI

DISCUSSION AND COMMENTS

6.1 Discussion

The present study was conducted from August 2007 to September 2007 and March 2008 to April 2008 in Chitwan district of the Country. In this study, an attempt was made to determine the distribution of different Culex mosquito species on ten villages of Chitwan district, the species present in the study area and their relative density in indoor and outdoor, to assess seasonal changes, to determine vector abundance and species composition in the study area and to determine resting habit of mosquito species.The total of 1197 sample was collected during the study period and they were identified in the Lab. About 718 and 479 samplse were recorded from post-monsoon and pre-monsoon collection respectively. In both post-monsoon and pre- monsoon 0*Culex quinquefasciatus* was abundant species. The total of twelve species was reported in post-monsoon and only five species in pre-monsoon. Five species of post-monsoon and four species of pre-monsoon were suspected vector of different mosquito born disease. Three suspected vector of JE i.e. *Culex tritaeniorhynchus, Cx. fuscocephala, Cx. gelidus,* and one principal vector of Filariasis i.e. *Cx. quinquefasciatus* and one suspected vector of West-Nile virus i.e., *Cx. theileri* were recorded.

In the overall present study about twelve Culex species were recorded in cattle shed; eleven species from human dwelling and outdoor collection in post monsoon and in pre monsoon about five species from indoor (Human and Cattle shed) and only four species from outdoor collection. The numbers of species were greater in Cattle shed; this may due to open type of Cattle sheds.

The Correlation Coefficient between number of mosquitoes (X) and Temperature (Y) is 0.0685 (See Appendix 1). The number of mosquitoes depends on temperature. The calculated value of χ^2 (40.781) is greater than tabulated value (19.68) at 5% level of significance and 11 degree of freedom.

So, null hypothesis is rejected. Hence, the number of mosquitoes collected in Post Monsoon Survey and Pre Monsoon Survey is dependent.

In the present study *Culex quinquefasciatus* is most abundant species followed by *Cx. fuscocephala, Cx. tritaeniorhynchus, Cx. gelidus, Cx. bitaenjorhynchus, Cx. whitmorei, Cx. pseudovishnui, Cx. whitei, Cx. theileri, Cx. barraudi, Cx. sinensis and Cx. mimulus.* *Cx. tritaeniorhynchus* was the third most abundant species in indoor collection. The numbers of *Cx. tritaeniorhynchus* in indoor and outdoor collection were 42 and five in post-monsoon season and 21 and zero in pre-monsoon season respectively.

Kanojia (2006) reported *Culex quinquefasciatus* is the fourth most abundant species, one isolate of JE virus has been obtained from this species. *Cx. tritaeniorhynchus, Cx. quinquefasciatus, Anopheles subpictus* and *An. peditaeniatus* were collected throughout the year. *Cx. tritaeniorhynchus* was the most abundant species showed two density peaks, first in February and second in October. Its population dynamics is closely associated with paddy cultivation in Bellary district, Karnataka.

Das et al., (2004) reported *Culex tritaeniorhynchus* is pre dominant in outdoors and playing a main role in JE transmission in Warangal and Karim Nagar district of Andhra Pradesh.

Arunachalam et al., (2004) reported *Culex tritaeniorhynchus* (66.7%) was most abundant species in Kerala, south India with increase in number associated with rice cultivation.

Kumar et al; (2004) recorded a total of 60 species belonging to 10 genera. Genus *Culex* was predominant in Rajiv Gandhi National Park (Nagarahole), Karnataka state, India.

Minakawa et al., (2002) reported *Anopheles gambiae* was the pre dominant species in both larval and adult samples. Livestock and human host availability affect the relative abundance of *An.gambiae* larvae in aquatic habitats, but the distribution of Anopheline adults in houses is determined by the distance from houses to larval habitats.

In the present study, *Culex quinquefasciatus* and *Cx. fuscocephala* were reported in 4 months (August, September, March and April). A total of 12 species were reported in September from Bramhapuri village. *Culex whitemorei, Cx. pseudovishnui, Cx. whitei, Cx. theileri, Cx. barraudi, Cx.*

sinensis and *Cx. mimulus* were reported in August, March and April. Mosquito's population was peak in August-September (post-monsoon).

Geevaryhes et al., (1994) reported that mosquito's population was peak during March-April and September. Very high incidence of JE cases were in extensively irrigated areas and a low incidence in some of taluks with less or no irrigation system.

Muturi et al., (2006) reported *Anopheles arabiensis*, *Culex quinquefasciatus,* and *Anopheles pharoensis* were more abundant in rice agro ecosystem than in the non-irrigated agro ecosystem and in planned than in the unplanned rice agro ecosystems. But An. *funestus* was more abundant in the non-irrigated agro ecosystem.

In the present study, among 1197 Culex sample, 12 species were recorded. *Culex quinquefasciatus* was the most abundant species in both post-monsoon and pre-monsoon season. *Culex mimulus* was the least abundant species. Vector abundance of *Cx. quinquefasciatus* was 26.90% in August-September (post-monsoon) and 18.71% in March-April (pre-monsoon). The vector abundance of *Cx. tritaeniorhynchus* was 3.92% in August-September (post-monsoon) paddy cultivation period and 1.75% in March-April (pre-monsoon) which is the third most abundant species. The percentage abundance of *Culex whitimorei, Cx. pseudovishnui, Cx. whitei, Cx. barraudi, Cx. theileri, Cx. sinensis, and Cx. mimulus* was least in post monsoon and zero in pre-monsoon. All 12 species were more abundant in August-September (post-monsoon).

The vector abundance was least in pre-monsoon season i.e. hot and dry season (March-April) and highest in post-monsoon season i.e. wet or rainy season (August-September), paddy cultivation period.

Gingrich et al; (1992) state that, vector abundance was high in monsoon (May-October), moderate in transition (March-April and November-December) and low in dry (January-February) seasons in Bangkok.

Same to present study, Gajanana et al; (1997) reported vector abundance was lowest in the hot and dry season (April-June) and highest in the cool and wet season (October-December) in the south Arwt district in Tamil Nadu.

Pramanik and Raut (2002) reported *Culex quinquefasciatus* was dominant (62.96%) on average of 492 mosquitoes in Kolkata.

Barboso et al., (2003) reported 15 species among the collected 312 Culicine specimens in a restrict forest insi Curitiba urban area (Parana, Brazil).

Rajendran et al., (2003) reported average vector abundance per man hour for *Culex tritaeniorhynchus* was 324.5 per month for the period June 1998-May 2000 in south Indian villages.

Kanojia et al., (2003) states that *Culex tritaeniorhynchus* the most likely vector of JE together with other known vector species remains more active during the period of paddy cultivation in Gorakhpur district.

Anopheles sinensis was most abundant (53.4%) in species ratio, followed by *Cx. tritaeniorhynchus* (43.0%), *Cx. inatomii* (1.6%), *Ochleratatus dorsalis* (1.3%) and *Cx. pipiens pallens* (0.5%) in a study carried out by Jeong et al., (2003).

Kanojia et al., (2004) reported, *Culex tritaeniorhynchus*, the primary vector of JE in Bellary district, India. More specimens (n=20,966) were found resting in indoor habitats than in outdoor vegetation (n=383), despite the availability of outdoor resting sites. Indoor residual insecticide may provide an effective control method in this area.

Mwangangi et al., (2006) reported that *Culex quinquefasciatus* (65.7%) was the non- anopheline species. Culicine and Aedine species densities were significantly higher during the Post-harvesting period. Transplanting stage is favourable for the growth of immature stages of *Anopheles arabiensis* and provides a narrow window for targeted larval intervention in rice.

Yaw et al., (2006) state that Mosquito survivorship and fecundity may be affected by environmental factors such as temperature and humidity. A Chigher smbient temperature may be facilitate blood meal digestion, reduce the length of the gonotropic cycle, and change the lifetime fecundity of a mosquito.

In present study In Suryapur village rooms of only one house was spread by GHAMBHIB Chloropyriphos 50%+ Cypermethrin 5% EC and in the room of first house of Kesharbag, DHOOM All Night (Alethrin Mat) was used in post monsoon. More specimens were found resting in indoor habitats (n=242). Indoor resting specimens in Post monsoon (n=543) and in pre monsoon (n=412). The specimens in outdoor vegetation in Post monsoon (n=175) and in pre monsoon (n=67).

Odiere et al; (2007) recorded 63% *Anopheles gambiae* S.S. (46% female) and 37% were *An. arabiensis* (66% female) out of total 10,517 mosquitoes samples in outdoor resting. Additionally 617 *An. funestus* (58%female) and 5,232 *Culex spp.* (males and females together) were collected in Kenya.

6.2 Comments

The major Comments of the study are given below:

1. A total of 1197 Culex Mosquitoes (718 in post-monsoon survey and 479 in pre-monsoon survey) were recorded.
2. About 12 species were recorded (12 species in cattle shed, 11 in human dwelling and outdoor collection) in post-monsoon survey and 5 species (5 in indoor-cattle shed and human dwelling, 4 in outdoor collection) in pre-monsoon survey.
3. Highest number of Mosquitoes sample (n=403) was collected in September.
4. *Culex quinquefasciatus* is the most abundant species in both season (26.90 percent in post monsoon survey and 18.71percent in pre-monsoon survey).
5. Indoor resting density (human) 9.33 was highest in post-monsoon survey at 25.75°c in Champanagar village.
6. Indoor resting density (cattle) 5.55 was highest in pre-monsoon survey at 22°c in Suryapur village.
7. Outdoor density, 0.22 was highest in pre-monsoon survey at 22.5°c in Kholesimal village.
8. The resting habit of *Culex mimulus* and *Cx. tritaeniorhynchus* was infinitive in post-monsoon and pre-monsoon survey respectively.
9. The room density of *Culex quinquefasciatus* (6.5) was highest in Champanagar village.
10. About 12 species of Culex Mosquitoes were found at 25.26°c in September in Bramhapuri village.
11. The number of mosquitoes is positively correlated with temperature. The number of mosquitoes is dependent on temperature.
12. The number of mosquitoes collected in post-monsoon survey and pre-monsoon survey is dependent.

CHAPTER VII

RECOMMENDATION

1. To reduce the vector contact, use of insecticide impregnated mosquito (bed) nets is strongly suggested. Moreover reduction of outdoor activities, discouraging to sleep outside the house during summer and rainy season, and un-exposing body parts by wearing long sleeves clothes should be highlighted to protect the people from mosquito bites.
2. Intermittent irrigation and periodic flushing of rice fields during rice cultivation should be followed.
3. Use of biological control methods viz., microbial agents like *Bacillus thuringiensis* and *B. sphaericus,* and introduction of carnivorous fishes like *Gambusia affinalis* etc. are recommended to control JE & Filaria vectors rather than chemical ones.
4. There is lack of general knowledge about Mosquitoes disease (JE & Filaria) in the district. So considerable mass awareness and public health education campaigns are thus conducted to change public perception of communities and VDCs level about the disease problem, its emergence and for its prevention and control.
5. Environmental and entomological studies in relation to the endemicity of JE particularly with respect to vector, larval habitat, temperature, humidity etc. as well as amplifying hosts should be carried out.
6. Public Awareness Program should be applied from the government level (Health Ministry) and related other government and private sector to aware the villagers about the *Culex* mosquitoes (Character, breeding and biting habit) their diseases and prevention from the bite and control these mosquitoes.

ANNEXES

A. Hypothesis Testing

The aim of the present research study is also testing the hypotheses which are mentioned in **CHAPTER I.** The methodology has been also mentioned in **CHAPTER III.** The following principles have been adopted for testing the hypothesis.

Annex - 1

First Hypothesis Testing

The First Hypothesis is “The number of mosquitoes depends on temperature i.e. there is positive relationship between the number of mosquitoes and temperature.”

Procedures

At First, Simple correlation coefficient of number of mosquitoes on temperature has to be found out.

Correlation Analysis

For Calculating the correlation coefficient of number of mosquitoes and temperature, let us denote the variables symbolically, i.e.

Number of Mosquitoes =X

Temperature (°c) =Y

Number of observation = n

The variable X denotes the number of mosquitoes of different villages. The variable Y represents the field temperature.

Table 1

Computation of Correlation Coefficient

Serial Number	X	Y	X²	Y²	XY
1	116	25.74	13456	662.547	2985.84
2	116	25.42	13456	646.176	2948.72
3	81	26.83	6561	719.848	2173.23
4	78	22.33	6084	498.628	1741.74
5	73	28.33	5329	802.588	2068.09
6	72	28.45	5184	809.402	2048.4
7	64	25.24	4096	637.057	1615.36
8	61	19.91	3721	396.408	1214.51
9	58	25.74	3364	662.547	1492.92
10	56	22	3136	484	1232
11	56	21.58	3136	465.696	1208.48
12	52	27.54	2704	758.451	1432.08
13	49	27.08	2401	733.326	1326.92
14	48	22.33	2304	498.628	1071.84
15	44	21.75	1936	473.062	957
16	42	21.75	1764	473.062	913.5
17	42	21.41	1764	458.388	899.22
18	37	26.33	1369	693.268	974.21
19	26	21.33	676	454.968	554.58
20	26	20	676	400	26.2
Total	**∑X=1197**	**∑Y=481.09**	**∑X²=83117**	**∑Y²=11728.05**	**∑XY=28884.84**

$$Correlation\,Coeficient(r) = \frac{n\sum XY - \sum X \sum Y}{\sqrt{\left[n\sum X^2 - \left(\sum X\right)^2\right]\left[n\sum Y^2 - \left(\sum Y\right)^2\right]}}$$

$$r = \frac{20 \times 28884.84 - 1197 \times 481.09}{\sqrt{\left[20 \times 83117 - 1197^2\right]\left[20 \times 11728.05 - 481.09^2\right]}}$$

$$= \frac{577696.8 - 575864.43}{\sqrt{[1662340 - 1432809][234561 - 231447.588]}}$$

$$= \frac{1832.07}{\sqrt{229531 - 3113.412}}$$

$$= \frac{1832.07}{26732.462}$$

$$= 0.0685$$

Hence, there is positive relationship between number of mosquitoes (X) and Temperature (Y). In other words, they are dependent on each other.

Annex - 2

Second hypothesis Testing:

The second hypothesis is “The number of mosquitoes collected in post monsoon survey and pre monsoon survey is independent.”

For the testing of this hypothesis, Chi –Square (¡^{2}) test has been used.

Procedures

1. Setting of Hypothesis:

i. Null Hypothesis (H_o): The number of Mosquitoes collected in post monsoon survey and pre monsoon survey is independent.

ii. Alternative Hypothesis (H_1): The number of Mosquitoes collected in post monsoon survey and pre monsoon survey is dependent.

2. Level of significance: 5 percent

3. Test Statistics: ¡^{2} - test

4. Computation: ¡$^{2} = \sum \frac{(O - E)^2}{E}$

Table 2

Observed Frequency Table

Name of Species	Post-Monsoon	Pre-Monsoon	Total
Cx. quinquefasciatus	322	224	546
Cx. fuscocephala	250	198	448
Cx. tritaeniorhynchus	47	21	68
Cx. gelidus	28	18	46
Cx. bitaeniorhynchus	20	18	38
Cx. whitimorei	12	0	12
Cx. pseudovishnui	11	0	11
Cx. whitei	9	0	9
Cx. barraudi	7	0	7
Cx. theileri	7	0	7
Cx. sinensis	4	0	4
Cx. mimulus	1	0	1
Total	**718**	**479**	**1197**

Table 3

Calculation of Estimated Frequency Table

Name of Species	Post- Monsoon	Pre- Monsoon	Grand Total
Cx. quinquefasciatus	$\frac{546\times718}{1197}=327.50$	$\frac{546\times479}{1197}=218.491$	546
Cx. fuscocephala	$\frac{448\times718}{1197}=268.725$	$\frac{448\times479}{1197}=179.27$	448
Cx. tritaeniorhynchus	$\frac{68\times718}{1197}=40.788$	$\frac{68\times479}{1197}=27.211$	68
Cx. gelidus	$\frac{46\times718}{1197}=27.594$	$\frac{46\times479}{1197}=18.407$	46
Cx. bitaeniorhynchus	$\frac{38\times718}{1197}=22.793$	$\frac{38\times479}{1197}=15.206$	38
Cx. whitimorei	$\frac{12\times718}{1197}=7.197$	$\frac{12\times479}{1197}=4.802$	12
Cx. pseudovishnui	$\frac{11\times718}{1197}=6.598$	$\frac{12\times479}{1197}=4.401$	11
Cx. whitei	$\frac{9\times718}{1197}=5.398$	$\frac{9\times479}{1197}=3.601$	9
Cx. baraudi	$\frac{7\times718}{1197}=4.198$	$\frac{7\times479}{1197}=2.801$	7
Cx. theileric	$\frac{7\times718}{1197}=4.198$	$\frac{7\times479}{1197}=2.801$	7
Cx. sinensis	$\frac{4\times718}{1197}=2.399$	$\frac{4\times479}{1197}=1.600$	4
Cx. mimulus	$\frac{1\times718}{1197}=0.599$	$\frac{1\times479}{1197}=0.4$	1
Total	**718**	**479**	**1197**

Table 4

Calculation of ¡ ^{2}

Observed Frequency (O)	Expected Frequency (E)	$(O-E)$	$(O-E)^2$	$\frac{(O-E)^2}{E}$
322	327.50	-5.5	30.25	0.092
250	268.725	-18.725	350.62	1.304
47	40.788	6.212	38.588	0.946
28	27.59	0.41	0.168	0.006
20	22.793	-2.793	7.8	0.375
12	7.197	4.803	23.068	3.205
11	6.598	4.402	19.377	2.936
9	5.398	3.602	12.974	2.403
7	4.198	2.802	7.851	1.87
7	4.198	2.802	7.851	1.87
4	2.399	1.601	2.563	1.068
1	0.599	0401	0.160	0.268
224	218.491	5.509	30.349	0.138
198	179.27	18.73	350.81	1.956
21	27.211	-6.211	38.576	1.417
18	18.407	-0.407	0.165	0.008
18	15.206	2.794	7.806	0.513
0	4.802	-4.80230.25	23.059	4.802
0	4.401	-4.401350.62	19.368	4.401
0	3.601	-3.60138.588	12.967	3.601
0	2.801	-2.8010.168	7.845	2.801
0	2.801	-2.8017.8	7.845	2.801
0	1.600	-1.60023.068	2.56	1.600
0	0.4	-0.419.377	0.16	0.4
				$\sum\frac{(O-E)^2}{E} = 40.781$

5. Degree of freedom (d. f.) = (r-1) (c-1)

= (2-1) (12-1)

=11

6. Tabulated Value of ¡^{2} at 5 percent level of significance of 11 degree of freedom is 19.68.

7. Decision: Since the calculated value of ¡^{2} is greater than tabulated value, null hypothesis is rejected. Hence the number of mosquitoes collected in post-monsoon survey and pre-monsoon survey is dependent.

B. Recorded Temperature in Post Monsoon and Pre Monsoon

Table 5

Temperature in pre and post monsoon

Village	Number	Temperature in ºc in Post monsoon			Temperature in ºc in Pre monsoon		
		Indoor		Outdoor	Indoor		Outdoor
		Human	Cattle		Human	Cattle	
Gitanagar	1	27	27	28	18	17	14
	2	30	28	29	21	19	18
	3	27	28	30	23	24	28
	4	29	29	29	27	26	29
Kesherbag	1	27	24	25	17	17	16
	2	26	26	27	20	19	19
	3	28	27	27	22	24	25
	4	28	27	28	24	26	28
Parasnagar	1	27	27	26	17	17	16
	2	29	26	26	21	19	18
	3	28	29	28	22	20	21
	4	28	30	31	23	22	24
Suryapur	1	24	25.5	26	19	17	16
	2	27	26	28	23	21	20
	3	28	27	30	24	24	25
	4	29	30	30	25	26	28
Kholesimal	1	26	25	25.5	20	18	17
	2	28	25	26	22	21	19
	3	28	25.5	26	23	23	25
	4	28	26	27	25	26	29
Dobato	1	27	26	25	16	14	14
	2	27	26	26	18	16	16
	3	28	28	27	21	23	24

	4	28	28	29	25	25	27
Champanagar	1	25	25	24	19	18	17
	2	25	25	25.5	21	19	19
	3	25	25	26	22	22	23
	4	26	25.5	27	25	24	27
Vijayanagar	1	25	24	24	18	17	15
	2	26	25	25.5	19	19	18
	3	26	26	27	22	23	27
	4	26	26.5	28	26	27	19
Rampur	1	25	24	24	19	16	18
	2	25	24.5	24.5	20	20	19
	3	26	25	26	23	24	25
	4	26	26	27	24	26	28
Bramhapuri	1	25	24	24	18	16	16
	2	25	25	25	22	19	18
	3	25.5	25	26	24	23	26
	4	26	26	27	26	25	28

Identification key to the genera *Culex*

- Proboscis not only slightly curved, if at all; posterior border of wing evenly rounded or only slightly emarginated
- Scutellum trilobed, with setae in three distinct groups; maxillary palpi shorter than proboscis
- Proboscis only slightly swollen near tip, if at all; with neither flexible long setae apically
- Pre-spiracular area bare
- Mesopostnotum without setae; scutum with another type of scale
- Fore and mid tarsomeres one shorter than other four tarsomeres combined, tarmomeres 4 of fore and mid legs much longer than wide
- Post spiracular setae absent
- Alula fringed with narrow scales
- Pulvilli present; tarsal claws unusually small

Identification key to the *Culex fuscocephala*

- One or two mesepimeral setae present; proboscis without distinct pale-scaled band; tarsomeres without pale bands at joints.
- Anterior surface of midfemur without median longitudinal pale-scaled stripe
- Abdominal terga without basal transverse, pale-scaled band; pleuron with striking pattern of dark and pale integumental stripes

Identification key to the *Culex quinquefasciatus*

- One or two mesepimeral setae present; proboscis without distinct pale-scaled band; tarsomeres without pale bands at joints.
- Anterior surface of midfemur without median longitudinal pale-scaled stripe
- Abdominal terga without basal transverse, pale-scaled band; pleuron with striking pattern of dark and pale integumental stripes
- Integument of thoracic pleuron without dark stripe; scutal integument yellowish or pale brown

Identification key to the *Culex theileri*

- One or two lower mesepimeral setae present; proboscis without distinct pale-scaled band; tarsomeres without pale bands at joints
- Anterior surface of midfemur without median longitudinal pale-scaled stripe
- Post spiracular area and base of prealar knob with distinct pale-scaled patches

Identification key to the *Culex whitei*

- Lower mesepimeral setae absent; proboscis with distinct pale-scaled band; tarsomeres with basal and apical pale bands
- Wing without pattern of pale-scaled or streaks
- Abdominal terga dark-scaled, with or without pale-scaled bands
- Abdominal terga 2-4 entirely dark –scaled, without pale bands or apicolateral pale patches

Identification key to the *Culex sinensis*

- Lower mesepimeral setae absent; proboscis with distinct pale-scaled band; tarsomeres with basal and apical pale bands
- Wing without pattern of pale-scaled or streaks
- Abdominal terga 2-4 with bands or patches or pale scales
- Wing with dark scales on all veins; hind tarsomeres with pale bands at bases longer than those at apices

Identification key to the *Culex barraudi*

- Wing without pattern of pale-scaled or streaks

- Abdominal terga dark-scaled, with or without pale-scaled bands
- Abdominal terga 2-4 with bands or patches or pale scales
- Abdominal terga 2-4 with basal pale-scaled bands only
- Erect scales in center of vertex pale yellow, dingy white or all dark; anterior 0.7 of scutum covered with being yellow, golden or dark scales
- Midfemur with longitudinal stripe of pale scales on anterior surface; post spiracular area with small patch of semi-erect scales on lower anterior aspect
- Longitudinal pale-scaled stripe on anterior surface of midfemur broken into small spots at middle ; costal vein entirely dark –scales

Identification key to the *Culex mimulus*

- Lower mesepimeral setae absent; proboscis with distinct pale-scaled band; tarsomeres with basal and apical pale bands
- Wing without pattern of pale-scaled or streaks on at least 2 areas of costa and 1 area on other veins
- Basalmost pale scaled costal spot involves C, Sc, R, and sometimes R_5
- And Cu; basal pale bands of abdominal terga marrow, usually less than 0.25 length of segment

Identification key to the *Culex gelidus*

- Lower mesepimeral setae absent; proboscis with distinct pale-scaled band; tarsomeres with basal and apical pale bands
- Wing without pattern of pale-scaled spots or streaks

- Abdominal terga dark-scaled, with or without pale-scaled bands
- Abdominal terga 2-4 with bands or patches or pale scales
- Abdominal terga 2-4 with basal pale-scaled bands only
- Erect scales in center of vertex pale yellow, dingy whitish; anterior 0.7 of scutum covered with white scales
- Anterior surf ace of fore and midfemora without speckling of pale scales; white-scaled patch on scutum dense, extending to wing root, dark –scaled posterior to that; wing viens R_1, R_{4+5} and Cu with narrow scales

Identification key to the *Culex whitmorei*

- Lower mesepimeral setae absent; proboscis with distinct pale-scaled band; tarsomeres with basal and apical pale bands
- Wing without pattern of pale-scaled spots or streaks
- Abdominal terga dark-scaled, with or without pale-scaled bands
- Abdominal terga 2-4 with bands or patches or pale scales
- Abdominal terga 2-4 with basal pale-scaled bands only
- Erect scales in center of vertex pale yellow, dingy whitish; anterior 0.7 of scutum covered with white scales
- Anterior surf ace of fore and midfemora extensively speckled with pale scales; paled-scaled patch on scutum thinner, extending posterior to wing root in 4 lines, wing viens R_1, R_{4+5} and Cu with broad scales

Identification key to the *Culex bitaeniorhynchus*

- Lower mesepimeral setae absent; proboscis with distinct pale-scaled band; tarsomeres with basal and apical pale bands

- Wing without pattern of pale-scaled spots or streaks
- Abdominal terga dark-scaled, with or without pale-scaled bands
- Abdominal terga 2-4 with bands or patches or pale scales
- Abdominal terga 2-4 with apical or basal pale-scaled bands only
- Wing with mixed pale and dark scales; hind tarsomeres with apical and basal pale bands about same length
- Abdominal terga 2-4 with broad apical bands of pale scales; abdominal terga heavily speckled with pale scales

Identification key to the *Culex tritaeniorhynchus*

- Lower mesepimeral setae absent; proboscis with distinct pale-scaled band; tarsomeres with basal and apical pale bands
- Wing without pattern of pale-scaled spots or streaks
- Abdominal terga dark-scaled, with or without pale-scaled bands
- Abdominal terga 2-4 with bands or patches or pale scales
- Abdominal terga 2-4 with apical or basal pale-scaled bands only
- Erect scales in center of vertex pale yellow, dingy whitish; anterior 0.7 of scutum covered with beige, yellow, golden or dark scales
- Midfemur entirely dark-scaled or specking of pale scales not forming definite stripe; postspiraular area without scales on lower anterior aspect
- Anterior surface of fore and midfemora entirely dark scaled
- Erect scales on vertex mostly dark anterior surface of hindfemur pale-scaled with narrow black-scaled ring apically; scutum covered with dark coppery gold scales

Identification key to the *Culex pseudovishnui*

- Lower mesepimeral setae absent; proboscis with distinct pale-scaled band; tarsomeres with basal and apical pale bands
- Wing without pattern of pale-scaled spots or streaks
- Abdominal terga dark-scaled, with or without pale-scaled bands
- Abdominal terga 2-4 with bands or patches or pale scales
- Abdominal terga 2-4 with apical or basal pale-scaled bands only
- Erect scales in center of vertex pale yellow, dingy whitish; anterior 0.7 of scutum covered with beige, yellow, golden or dark scales
- Midfemur entirely dark-scaled or specking of pale scales not forming definite stripe; postspiraular area without scales on lower anterior aspect
- Erect scales on vertex pale yellow in center, dark–scaled posterolaterally; hind femur marked otherwise; scutum with scales paler
- Femora and proboscis never speckled with pale scales; scutum with yellow to silvery scales; hind femur with dark band apically constrasting with pale-scaled areas.

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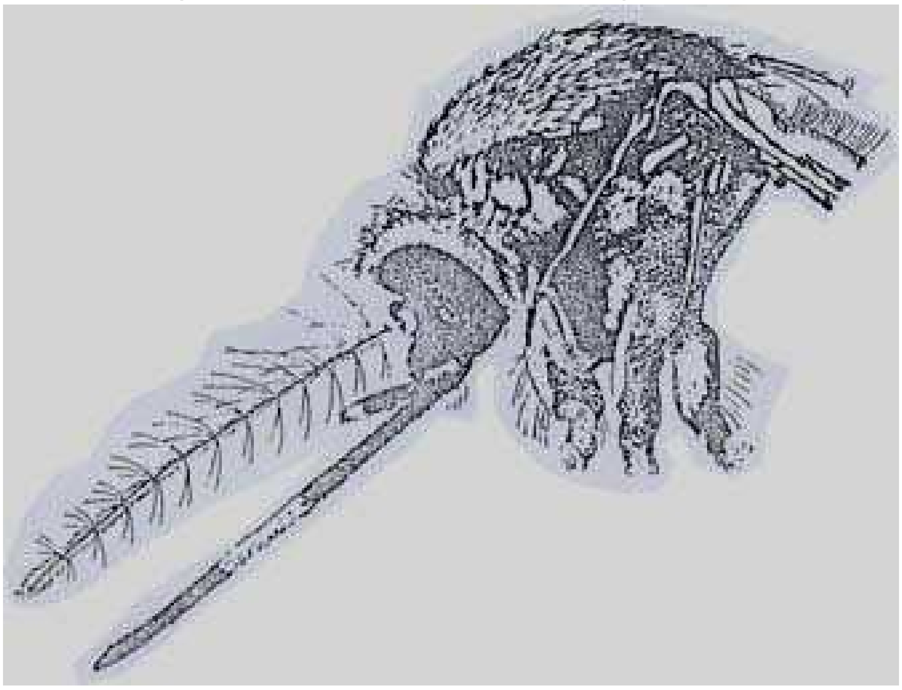
Pictorial of *Culex* (Female)

Figure a

Pleuron with distinct scale patches at least on upper and lower mesokatepisternum and anterior mesanepimeron



Pictorials of the vector i.e. *Culex fuscochephala*

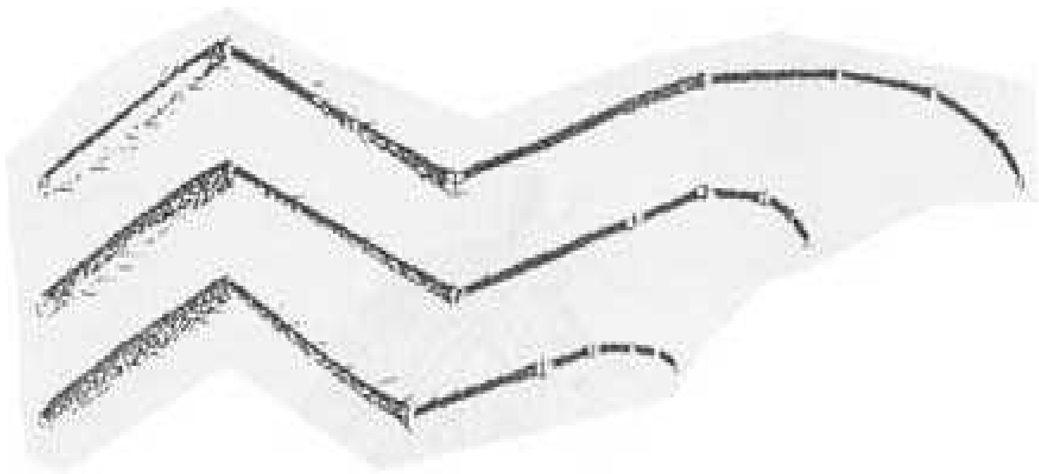


Figure b: Anterior surface of mid-femur without median longitudinal pale-scaled stripe

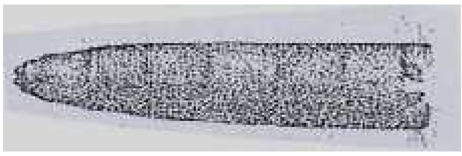


Figure c: Abdominal terga without basal trasverse, pale-scaled bands

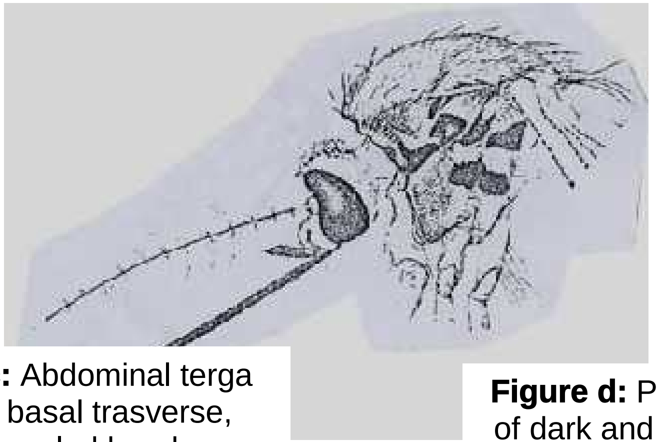


Figure d: Pleur of dark and pal

Pictorials of *Culex whitei*

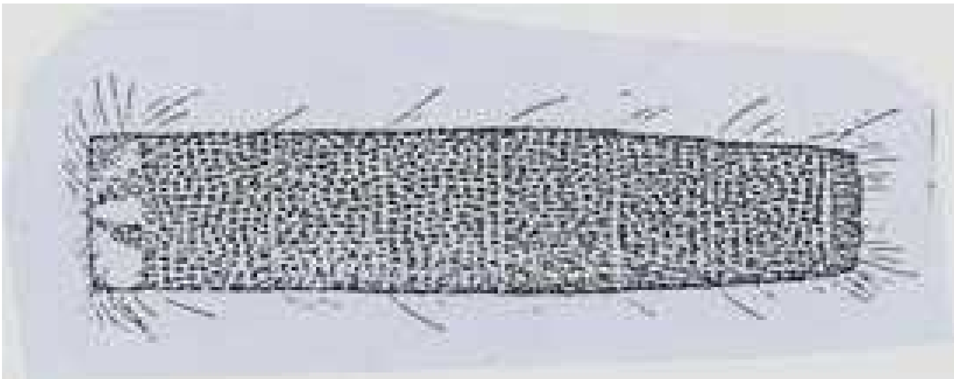


Figure e: Abdominal terga II-VI entirely dark scaled, without pale bands and epicolateral pale patches

Pictorials of *Culex sinensis*

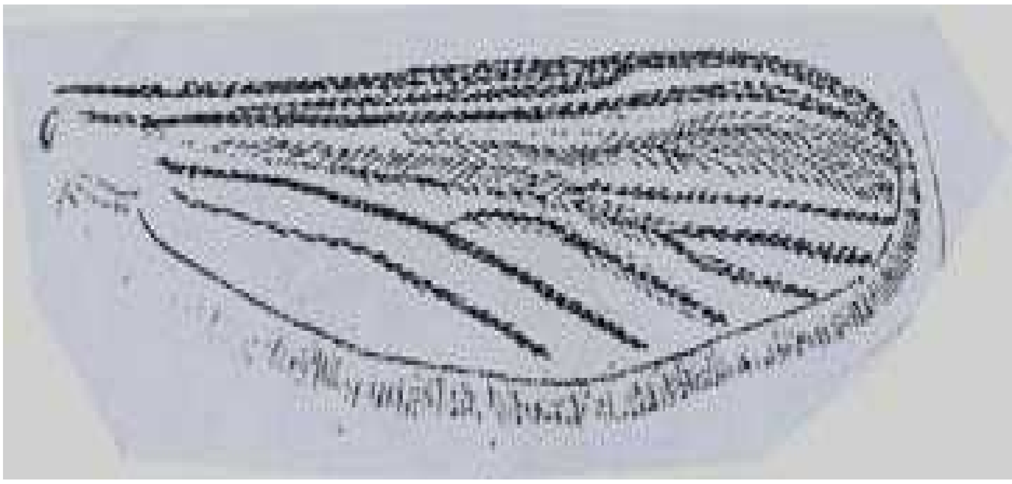


Figure f: wing with dark scales on all veins

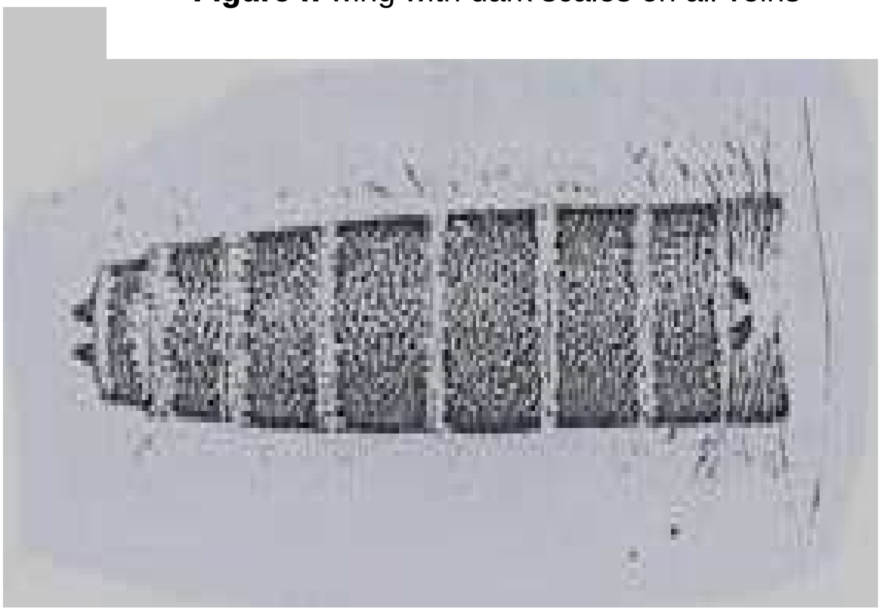


Figure g: Abdominal terga II-VI with dark areas not sprinkled with pale scaled

Pictorials of *Culex bitaeniorhynchus*

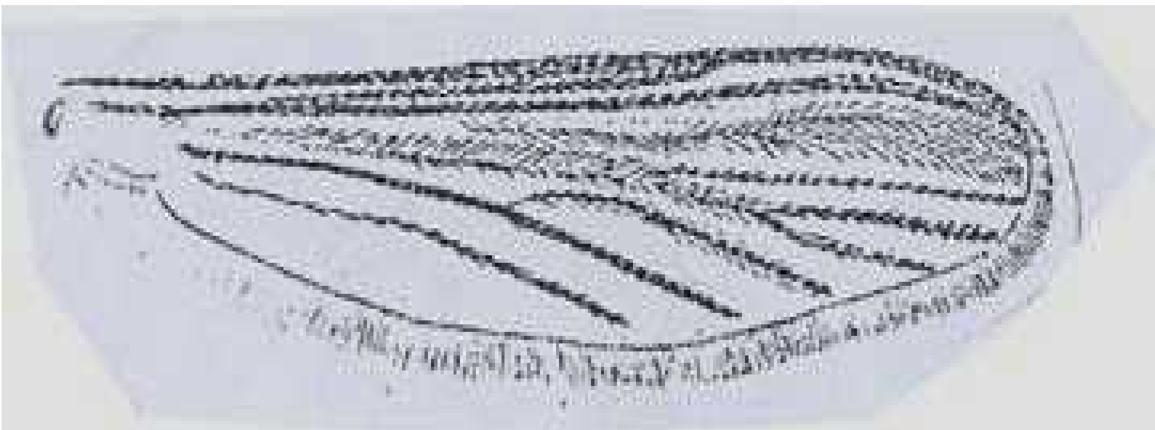


Figure h: wing with dark areas sprinkled with pale scales

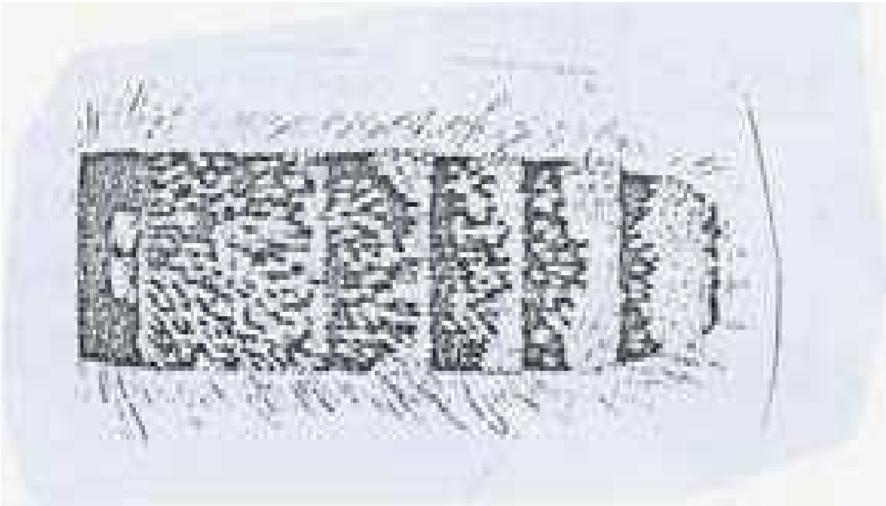


Figure i: Abdominal terga II-VI with dark areas sprinkled pale scaled

Pictorials of the vector i.e. *Culex gelidus*

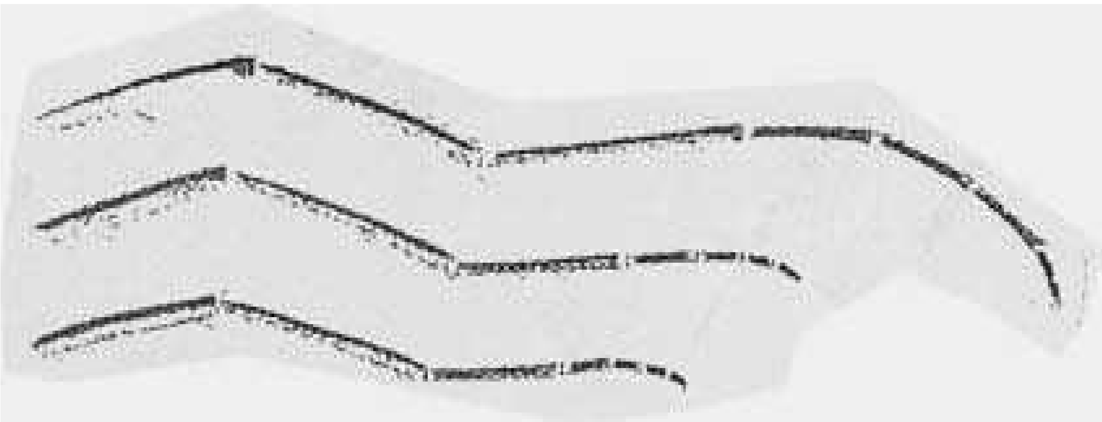


Figure j: Anterior surface of fore and mid femora without speckling of pale scales

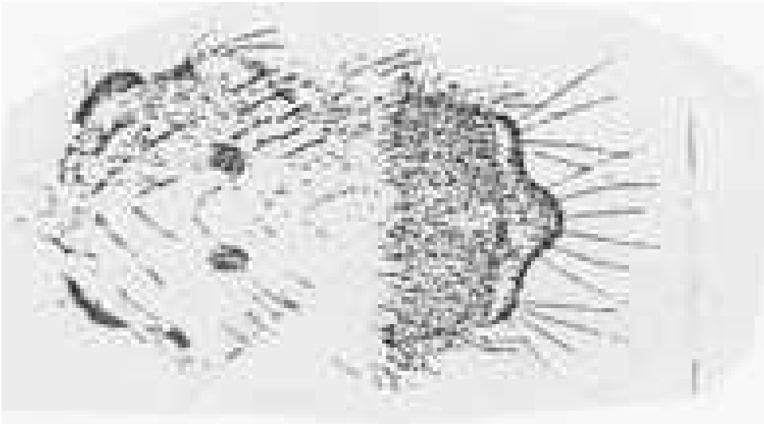


Figure k: Scales on lateral scrutal area behind wing base, prescrutellar area and on scrutellum entirely

Pictorials of *Culex whitmorie*

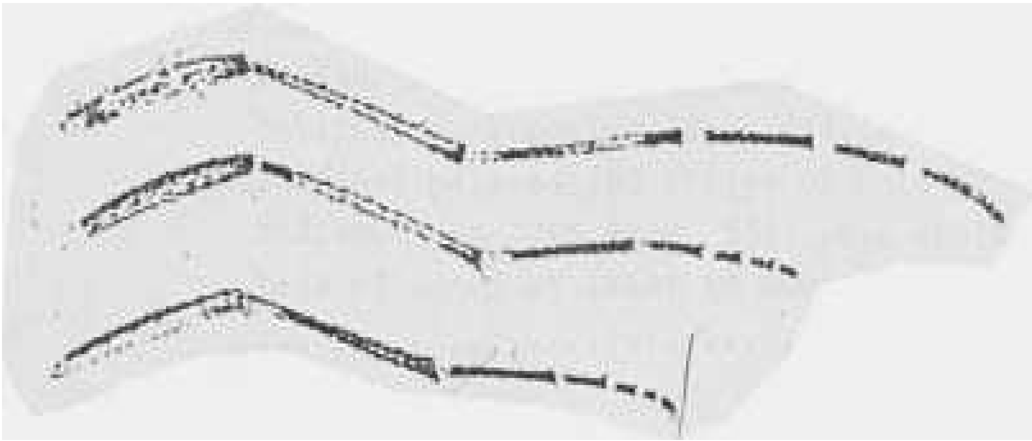


Figure l:
Anterior surface of fore and mid femora extensively speckled with pale scale

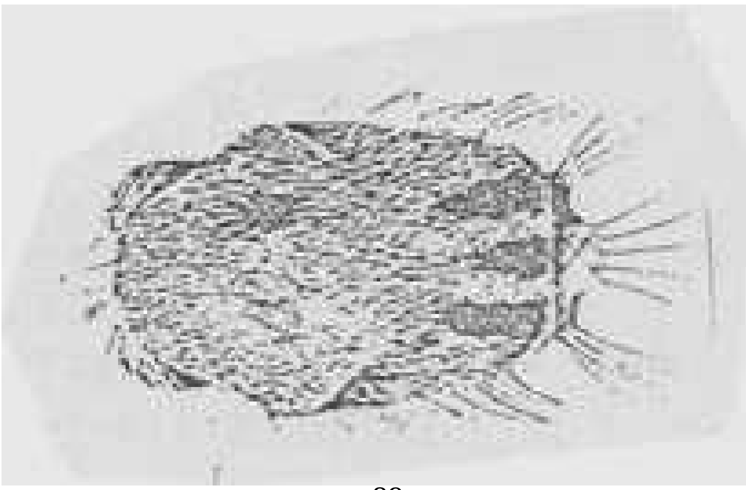


Figure m: Scales on lateral scutal area behind wing base, prescutellar arean and on scutellum predominantly pale

Pictorials of *Culex barraudi*

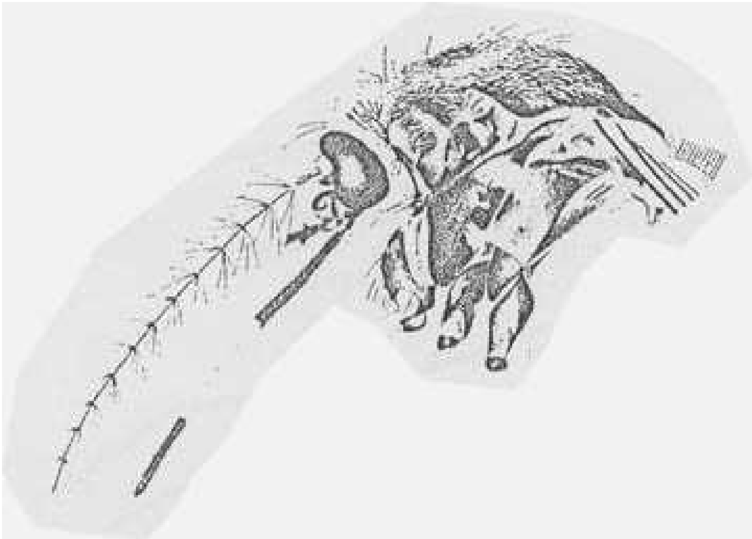


Figure n: Postspiracular area with smooth patch of semi erect scales on lower aspect

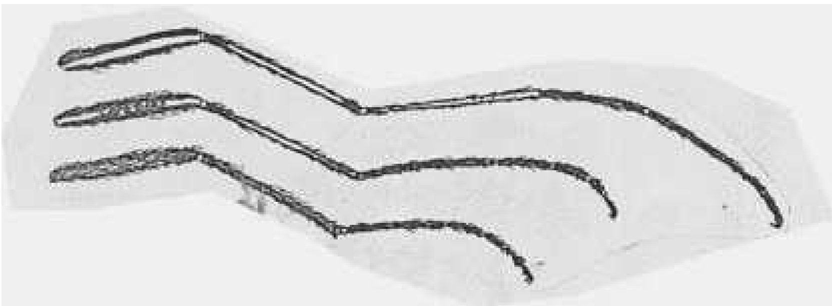


Figure o: Longitudinal pale-scaled stripe on anterior surface of midfemur broken into small spots at middle

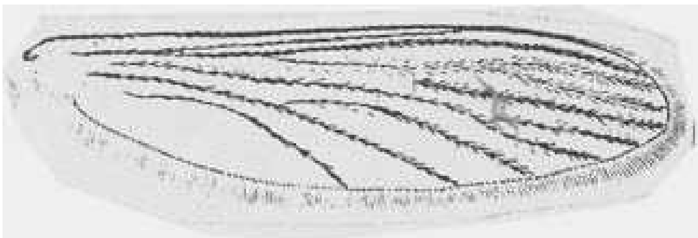


Figure p: Costal vein –scales entirely dark

Pictorials of the vector i.e. *Culex tritaeniorhynchus*

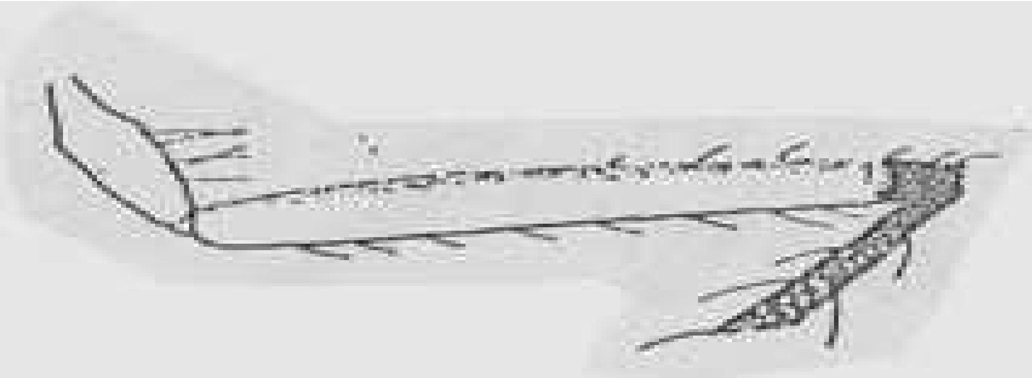


Figure q:
Anterior surface of hind femur pale scaled with narrow black scaled ring apically

Pictorials of the vector i.e. *Culex psuedovishnui*

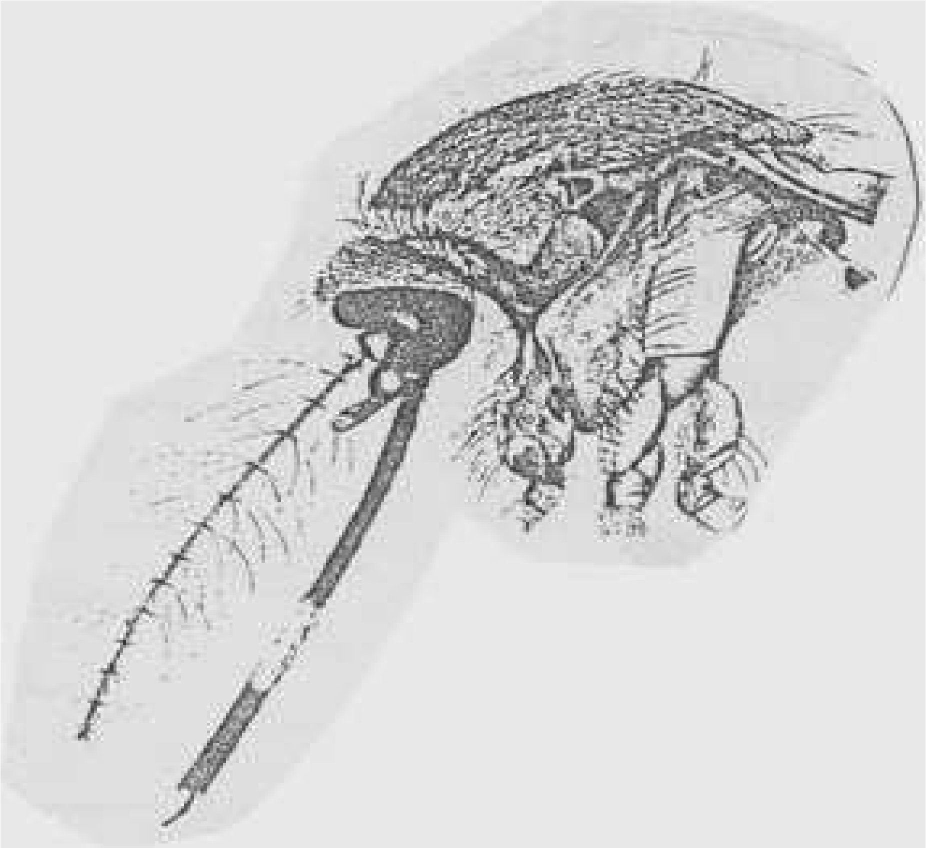


Figure r:
scutum with yellow to silvery scales

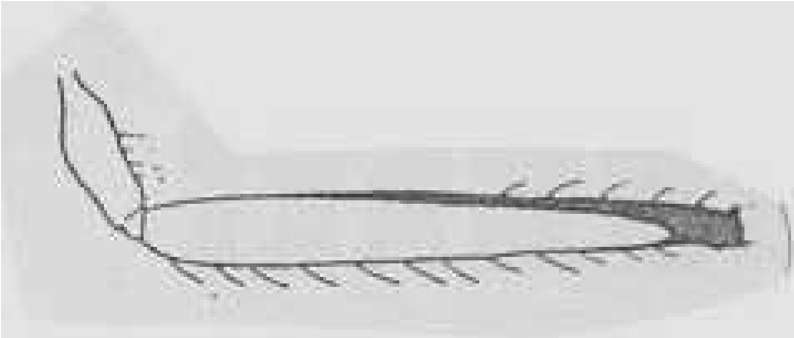


Figure s:
Femora and proboscis never speckled with pale scales

Pictorials of *Culex mimulus*

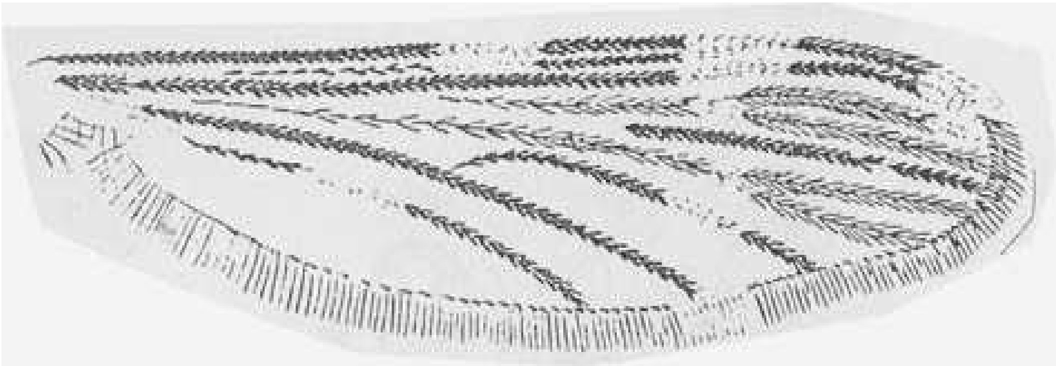


Figure t:
Second pale scaled costa spot involves veins C, Sc, R and sometimes R_s and Cu

Figure u:
Basal pale bands of abdominal terga narrow, usually less than 0.25 length of segment