Seasonal Distribution of *Culex tritaeniorhynchus* Giles (Diptera: Culicidae), the Vector of Japanese encephalitis in Kathmandu valley

Final Project Report

Submitted by

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SUMMARY

Japanese encephalitis (JE) is a common mosquito-borne viral encephalitis found in Asia, and is widespread throughout Asia. It is principally a disease of rural agricultural areas and primarily a zoonotic disease infecting mainly vertebrate animals, e.g. pigs, birds, horses etc. Pigs, wading birds and ducks have been incriminated as important vertebrate amplifying hosts for JE virus due to viremia in them. Man is involved in transmission cycle as an accidental host and plays no role in perpetuating the virus. The disease was first recorded in Nepal in 1978 as an epidemic in Rupandehi district of the Western Development Region (WDR) and Morang of the Eastern region (EDR). At present the disease is endemic in 24 districts. JE was confirmed in 40 residents of the Kathmandu valley, including 30 cases that had no history of travel outside the valley during the incubation period. However, there is little information on the occurrence of this disease in the densely populated Kathmandu valley. The species *Culex tritaeniorhynchus* is suspected to be the principal vector of JE in Nepal as the species is abundantly found in the rice-field ecosystem of the endemic areas during the transmission season and JE virus isolates have been obtained from a pool of *Culex tritaeniorhynchus* females. No study so far has been carried out regarding seasonal distribution of *Culex tritaeniorhynchus* in Kathmandu valley.

Entomological studies were conducted in Kathmandu, Lalitpur and Bhaktapur district of Kathmandu valley, in order to determine the abundance and seasonal distribution of *Culex tritaeniorhynchus.* Emphasis was given to select the villages located on the river side, pond, agro-field ecosystem areas and presence of cattle on the areas. During the study period altogether 4 man hours were spent searching mosquitoes in 16 houses by two collectors in Balkot (Bhaktapur district), Gothatar and Nepaltar (Kathmandu district) and Godavari (Lalitpur district) of Kathmandu valley in indoor and outdoor hand collection to collect adult mosquitoes resting inside the houses. Adult mosquitoes were captured using mouth aspirators, animal baited net trap and CDC light trap. Different breeding places like paddy field, puddles, ponds, river bed, swamps, drain etc. were searched for larvae of *Culex* mosquitoes in Balkot, Gothatar, Nepaltar and Godavari taking dips. Collections were carried out in sequence daily along the block from the start house between 6:00 AM and 10:00 AM. Of the total 37018 adults and 10071, larvae *Culex quinquefaciatus,* the principal filarial vector, was the most commonly captured culicine mosquito (81.83% adult and 83.82% larvae) in Kathmandu valley followed by *Cx. fuscocephala* (adult 6.91% and larvae 6.02 %), *Culex tritaeniorhynchus* (2.24 % adult and 3.27% larvae), *Cx. vishnui* (1.58 % adult), *Cx. pseudovishnui* (1.06 % adult and 1.57% larvae). The least density was found to be for *Cx. gelidus* (0.45% adult) and *Cx. vishnui* (0.23% larvae). Other associated culicine mosquitoes recorded were *Culex hutchinsonie*, *Cx. edwardsii*, *Culex barraudi* and *Armigeres spp.* in different months.

These data provide a better understanding of the density, and seasonal distribution of potential mosquito vectors of disease within the Kathmandu valley and allow for the development of appropriate vector and disease prevention strategies that target vector populations. In addition, this study reports the observation of seasonal fluctuation in densities and resting preference of *Culex tritaeniorhynchus* which would be useful for the possible inclusion of the Kathmandu valley in the national JE prevention and control program. The influence of livestock and human host availability on the distribution and abundance of JE vectors and resting preference provides the effective control method in the area. In addition, findings may provide

important information on larval habitat preference for different *Culex* species, which will be useful in designing and implementation of different larval control operations.

Vector control is largely achieved through the use of chemical pesticides and still playing an important role. Mosquitoes have developed resistance to many pesticides, whereas the predators are still highly susceptible. *Bacillus thuringiensis* Var. Israelensis serotype H 14 as an effective biological control agent against mosquitoes in Israel was successfully tested. Toxicity tests with *Culex pipiens* and *Aedes aegypti* showed that the strain was effective in LD_{50} bioassays (Margalit 1983). The biological insecticide such as *Bacillus thuringiensis* serotype H-14 (B.t. H-14) can be applied through community participation. Rice cultivation in study areas has a marked effect on *Culex* mosquito species diversity. In the periphery of the city, there are a number of ponds, infested with aquatic floating weeds supporting mosquitoes. So the reduction in mosquito densities is to be realized through larval management. Mosquitoes in these ponds can be controlled by physical removal of weeds and fishes, nematode parasite, *Toxorhynchites*, a non-biting predatory mosquito can be used if necessary to control tree hole breeding mosquitoes.The local community can be motivated to remove or empty the receptacles around the premises. *Culex quinqefasciatus* is not only the vector of filariasis but also a serious nuisance. This can be put to good use as providing a strong incentive for community participation in its control. Health education would promote the type of low-cost sanitation that doesnot favour mosquito breeding.

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CHAPTER 1

GENERAL INTRODUCTION

1.0. Introduction

Japanese encephalitis (JE) is a common mosquito-borne viral encephalitis found in Asia. It is an acute infection of the central nervous system caused by flavivirus, related to San Luis Encephalitis (SLE) virus seen in North America, and is widespread throughout Asia; Anderson and Brust, 1995).

JE is principally a disease of rural agricultural areas and primarily a zoonotic disease infecting mainly vertebrate animals, e.g. pigs, birds, horses etc. Pigs, wading birds and ducks have been incriminated as important vertebrate amplifying hosts for JE virus due to viremia in them. Man is involved in transmission cycle as an accidental host and plays no role in perpetuating the virus. The disease was first recorded in Nepal in 1978 as an epidemic in Rupandehi district of the Western Development Region (WDR) and Morang of the Eastern region (EDR). At present the disease is endemic in 24 districts (Bista and Shrestha, 2005).

Japanese encephalitis (JE) is endemic in the Terai region of Nepal. There is little information on the occurrence of this disease in the densely populated Kathmandu valley. JE was confirmed in 40 residents of the Kathmandu valley, including 30 cases that had no history of travel outside the valley during the incubation period. Incidence was 2.1/100,000 and the case fatality was 20% (8/40) **(** Partridge et al**., 2007).** The species *Culex tritaeniorhyncus* is suspected to be the principal vector of JE in Nepal as the species is abundantly found in the rice-field ecosystem of the endemic areas during the transmission season and JE virus isolates have been obtained from a pool of *Culex tritaeniorhyncus* females. It infects vertebrate hosts, primarily birds and swine, in an enzootic cycle (Vaughn and Hoke, 1992; Endy and Nisalak, 2002). Multiple host contacts in a gonotrophic cycle increase the chance of acquiring and transmitting the pathogen (DeFoliart et al., 1987). Multiple feeding within the same gonotrophic cycle increases the potential for human-vector contact bringing them into the proximity of humans. Patterns of disease transmission are influenced by the vector abundance. In order to understand adequately the dynamics of vector-borne disease, one must understand how and why vector populations change over time and environmental factors. It is often hypothesized that the abundance of mosquitoes in a house is associated with the distribution of livestock and humans.

No study so far has been carried out in kathmandu valley regarding seasonal distribution of *Culex tritaeniorhynchus* .

Promlem of Japanese Encephalitis Virus (JEV)

Japanese Encephalitis Virus (JEV) belongs to the genus Flavivirus of Togoviridae family causes Japanese encephalitis (JE) disease in the tropical and subtropical countries. The virus is included into the same group of dengue virus. Due to the wide distribution and serious in nature of the disease caused by the Togoviridae, which multiply in the cytoplasm, show considerable variations in the length of periods for their multiplication cycle. Although the temporal differences are relatively minor within each group. When an infected mosquito bites a prospective host, it infects virus from its salivary glands into the blood stream or lymph of its victim. Successful infection depends upon the presence of sufficient virus in the saliva of the mosquito vector and a capability of neutralizing antibodies in the host. Details of the pathogenesis of infection of human are largely inferred from experimental studies in animals.

The disease was first recorded in Nepal in 1978 as an epidemic in Rupandehi district of the Western Development Region (WDR) and Morang of the Eastern region (EDR). At present the disease is endemic in 24 districts namely Jhapa, Morang, Sunasari, Saptari, Siraha, Udayapur, Dhanusa, Mahottari, Sarlahi, Sindhuli, Rautahat, Bara, Parsa, Makwanpur, Chitwan, Nawalparasi, Rupandehi, Kapilvastu, Palpa, Dang, Banke, Bardiya, Kailali and Kanchanpur.Among them, 10 districts namely Jhapa, Morang, Sunsari, Parsa,
Rupandehi, Dang, Banke, Bardiya, Kailali and Kanchanpur are affected most (Bista and Shrestha, 2005). The mosquito borne mode of JE transmission was elucidated with the isolation of JE virus in 1983 and subsequently in other field studies that also established the role of aquatic birds and pigs in the viral enzootic cycle. (Bista and Shrestha, 2005).
JE is principally a disease of rural agricultural areas and primarily a zoonotic disease infecting mainly vertebrate animals, e.g. pigs, birds, horses etc. Pigs, wading birds and ducks have been incriminated as important vertebrate amplifying hosts for JE virus due to viremia in them. Humans and horses may become ill in transmission cycle. Man is involved in transmission cycle as an accidental host and plays no role in perpetuating the virus. Bovines, ovines and caprines along with humans do not appear to serve as an amplifying and reservoir host. The major vectors of JE feed on bovines and there is serological evidence of bovines being infected with the virus. However, viremia is not found in these animals. They are symptom less and "dead end hosts".

This communicable disease transmissible to human by Culex mosquitoes, primarily *Culex tritaeniorhynchus. Culex tritaeniorhyncus* females feed outdoors beginning at dusk and during evening hours until dawn. Larvae are found in flooded rice fields, marshes, and small stable collections of water around cultivated fields. It infects vertebrate hosts, primarily birds and swine, in an enzootic cycle (Vaughn and Hoke, 1992; Endy and Nisalak, 2002). Multiple host contacts in a gonotrophic cycle increase the chance of acquiring and transmitting the pathogen (DeFoliart et al., 1987). Multiple feeding within the same gonotrophic cycle increases the potential for human-vector contact bringing them into the proximity of humans. Patterns of disease transmission are influenced by the vector abundance. In order to understand adequately the dynamics of vector-borne disease, one must understand how and why vector populations change over time and environmental factors. It is often hypothesized that the abundance of mosquitoes in a house is associated with the distribution of livestock and humans. However, no information is available regarding frequency of multiple feeding among the major JE vector, *Culex tritaeniorhynchus* in Nepal.

1.1 Specific Objectives

Objectives:

General: To report observation on the seasonal distribution of *Culex tritaeniorhynchus* in Kathmandu valley.

Specific:

- to study the seasonal distribution of *Culex tritaeniorhynchus* and other possible vectors of JEV in of Kathmandu, Lalitpur and Bhaktapur districts.

- to determine distribution of *Culex tritaeniorhynchus* among animals.

CHAPTER 2

2.0 LITERATURE REVIEW

Japanese Encephalitis (JE) is a mosquito transmitting disease of vertebrate animals and ids transmissitable to man through the bites of vector mosquitoes. It is primarily a zoonotic disease infecting mainly animals. Man involves as an accidental host and plays no role in perpetuating the virus (Pradhan et al., 1991).

In terms of human morbidity and mortality the JE virus is the most significant and wide spread cause of encephalitis in man. It is an acute infection of the central nervous system, meningo-myelocephalitis. The virus JEV was first isolated in 1933 in Japan and was initially called Japanese 'B' encephalitis (Hayashi, 1934). It is a taxon originally known as arthropod bornr group 'B' virus (Cosals and Brown, 1954).
The occurrence of JEV had beendocumented for more than four decades in Taiwan, Thailand, Malaysia, Japan, Korea, Indonesia and India. However, this disease JE was first found in plain terai of western region of Nepal in 1978 with the outbreak of encephalitis in Rupandehi district. In 1983 the disease encephalitis was first identified as Japanese Encephalitis Virus (Khatri et al., 1983). Since then sporadic cases of JEV have been occurring to the plan terai and inner terai of Nepal; However, epidemics affecting several districts appear only in alternate year (Pradhan, 1984 unpublished).

When JEV was first isolated, there was considerable references of options such as how human infection was occurred. Through transmission of the virus by mosquitoes was among the hypothesis, it was not demonstrated experimentally or by the isolation of virus from naturally infected mosquitoes. Zimmerman et al 1997 **reported** the first proven outbreak of Japanese encephalitis (JE) in the Kathmandu Valley. JE was confirmed in 40 residents of the Kathmandu valley, including 30 cases that had no history of travel outside the valley during the incubation **period (**Partridge,2007)**.**

Multiple feeding was reported in field populations of vectors of malaria, eastern equine encephalitis, St. Louis encephalitis, and western equine encephalitis (Boreham and Garrett-Jones, 1973; Burkot et al., 1988; Mahmood and Crans, 1997; Wekesa et al., 1997; Amerasinghe and

Amerasinghe, 1999). Mosquito gonotrophic cycle and multiple feeding potential: contrasts **between Anopheles and Aedes (Diptera: Culicidae) was studied by Klowden and Briegel (1994).** Arunachalam et al (2005) studied multiple feeding behavior of *Culex tritaeniorhynchus* in Kerala (Southern India)**.**

2.1. JUSTIFICATION

Resting preference and the increase in the number of host contacts as a result of multiple feeding may increase disproportionally the rate of encephalitis virus transmission. Thus, host vector contact is an important parameter in JE epidemiology. However, many epidemiologic models on vector-borne diseases assume that mosquitoes contact one host per gonotrophic cycle (Macdonald, 1957; Garrett-Jones, 1964; Scott et al.,1983). Multiple feeding within a single gonotrophic cycle may result if mosquitoes take small blood meals which are insufficient to terminate host-seeking and increases the potential for human-vector contact. Partial meals and reduced feeding success of mosquitoes can result from defensive host behavior. However, no information is available regarding frequency of multiple feeding among the major JE vector, *Culex tritaeniorhynchus* in Nepal. Currently, JE prevention is focused on the Terai region in Nepal. This study reports the observation of seasonal distribution in densities and resting preference of *Culex tritaeniorhynchus* which would be useful for the possible inclusion of the kathmandu valley in the national JE prevention and control program. The influence of livestock and human host availability on the distribution and abundance of JE vectors and resting preference provides the effective control method in the area. In addition, findings provide important information on larval habitat preference for different *Culex* species, which will be useful in designing and implementation of larval control operations.

CHAPTER 3

3.0. MATERIALS AND METHODS

3.1. Study sites

Mosquito *Culex tritaeniorhynchus* and other possible vectors of Japanese encephalitis virus (JEV) were collected from the different locations of Kathmandu, Lalitpur and Bhaktapur districts of Kathmandu valley. The collection sites selected are as stated below.

S.N.	**District**	**Collection area**	**Selected village**	**Remarks**
1	Kathmandu	Gothatar	Gothatar	
2	Kathmandu	Nepaltaar	Tokha	
3	Lalitpur	Godavari	Hattiban	
4	Bhaktapur	Balkot	Balkot	

3.2. Study design

The field work was carried out at Kathmandu valley from April -September, 2009. Most of the collection villages/localities were selected randomly as far as possible. However, emphasis was given to select the villages located on the river side, agro-field ecosystem areas and present of cattle on the areas. Besides that the areas were selected in the basis of accessibility as well. Most of the villages are situated within 2-3 km from the main road. Households were surveyed in sequence daily along the block from the start house between 6:00 AM and 10:00 AM. Unoccupied or closed houses and houses where residents did not provide permission for the survey, businesses, offices, and schools were not sampled. Each day, prior to continuing surveys of unsampled households, an attempt were made to inspect houses that were previously closed or where access had been refused. Access to houses of each area were attempted a minimum of three times. This process were carried out until all the houses in each neighbourhood had been surveyed or repeated attempts to gain access failed. Immediately after termination of the first survey (pre monsoon), the sampling procedure were repeated. The second survey (post monsoon) were also be carried out. In most of the selected villages *Culex tritaeniorhynchus* and possible vector species. All the mosquitoes were also recorded and brought in the laboratory to identify adequately. Especially samples from the animal baited net traps and light trap were recorded all the samples, trapped in the trap fixed in the villages.

3.3. Ethical clearance

Ethical clearance was obtained from community leaders and the household owners before starting the study especially for indoor hand collection, animal baited net trap collection and light trap collection. The participants were informed in clear, comprehensible terms in the local language about the objectives, study protocol, and advantages and inconveniences. Participants were told they have complete liberty to participate or refuse to participate.

3.4 Entomological surveys

Different collection tools were used to collect *Culex tritaeniorhynchus* and other possible vectors of JEV. The different methods used were as stated below:

1. Indoor hand collection:

Indoor hand collections were carried out inside different shelters in the morning time starting from 6.00- 17.00 in human (4), mixed (2) and animal (2) dwelling spending 15 minutes in each house by one collector. As there were two collectors so they spend 4 man hours in 16 houses in each village on the selected sited of the valley in the month of April to September, 2009. All the collected samples of mosquitoes were brought in the laboratory and properly identified using the identification key prepared by Darsie and Pradhan (1990).

2. Outdoor hand collection

Outdoor collections was similarly attempted outside the house from outside walls, under eaves, vegetation and bushes around cattle sheds and pigeries, and in and around outdoor stored materials etc., for two hours by each collector and transported to the laboratory for identification and enumeration.

3. Larvae collection

In villages different collection sites of the valley different types of breeding places were searched taking at least 10 dips in one collection site, to collect larvae of the *Culex tritaeniorhynchus* and other vector species of JEV and all other *Culex* mosquito larvae in the months of April, May, June , July , August and September, 2009. The collected mosquito larvae were brought to the laboratory and identified adequately with the help of "Mosquito key of Nepal" developed by Darsie and Pradhan (1990).

4. Animal baited net trap collection

One animal baited net trap, the bed net measuring 5m x 5m was used to collect mosquitoes in animal baits tied inside the net whole night. And, in the trap one animal bait was kept whole night. In the morning the mosquitoes trapped in the net trap were collected and brought in the laboratory. All the mosquitoed collected were identified with the help of a mosquito key developed by Darsei and pradhan (1994).

5. Light trap collection

During the study period one light trap was fixed whole night in the outdoor. In the morning all the mosquitoes trapped in light trap were collected and brought in the laboratory. Adult mosquitoes emerged from reared larvae were identified by using the conventional key for mosquito species (Darsie and Pradhan 1990) on the basis of proboscis, occiput, pulvilli, present; tarsal claws, abdominal terga, pleuron, scutal integument, fore- and midfemora, wing veins, vertex etc. If *Culex tritaeniorhynchus* and other possible vectors of JEV were found full fed or half gravid then blood sample would be collected on the filter paper which would be used to determine the types of host preference by means of precipitin tests in due course of time. Mosquitoes were identified, counted by sex and abdominal condition (unfed, fed, semi/halfgravid, and gravid) in the field when possible.

3.5 Data analysis

Entomological data including temperature, humidity and rainfall of the study area were be recorded on the entomology collection sheet. Data were recorded on the entomology collection sheet. Mosquito (only females) abundance were be calculated as number collected per human-hour.

3.6 Limitation of the study

This study describes the JE vector's adult and larval habitats in a Nepali context and as a result of visits to limited numbers of localities and inadequate period (only six months), in each district, the result do not reflect a complete figure regarding the seasonal abundance of the vector species.

If *Culex tritaeniorhynchus* and other possible vectors of JEV were found adequately full fed or half gravid then blood sample would be collected on the filter paper which would be used to determine the types of host preference by means of precipitin tests in due course of time and some *Culex tritaeniorhynchus* and other possible vectors would be preserve for isolation of Japanese encephalitos virus (JEV). Due to unavailability of fed/half gravid *Culex till* September and termination of this project in September, multiple feeding behaviour part of *Culex tritaeniorhynchus* could not be achieved. Therefore, one year complete study is recommended. It must be noted that continuity of this study is necessary.

CHAPTER 4

4.0. Results

During the study period altogether 4 man hours were spent searching mosquitoes in 16 houses by two collectors in Balkot (Bhaktapur district), Gothatar and Nepaltar (Kathmandu district) and Godavari (Lalitpur district) of Kathmandu valley in indoor hand collection to collect adult mosquitoes resting inside the houses. Of the 37018 adults and 10071larvae, *Culex quinquefaciatus*, the principal filarial vector, was the most commonly captured culicine mosquito (81.83% adult and 83.82% larvae) in Kathmandu valley followed by *Cx. fuscocephala* (adult 6.91% and larvae 6.02 %), *Culex tritaeniorhynchus* (2.24 % adult and 3.27% larvae), *Cx. vishnui* (1.58 % adult), *Cx. pseudovishnui* (1.06 % adult and 1.57% larvae). The least density was found to be for *Cx. gelidus* (0.45% adult) and *Cx. vishnui* (0.23% larvae) (Figure 1 & 2). Other associated culicine mosquitoes recorded were *Culex hutchinsonie*, *Cx. edwardsii*, *Culex barraudi* and *Armigeres spp.* in different months.

Indoor/outdoor hand collection

Table 1. Monthwise collection of mosquitoes in indoor hand collections in Godavari area of Lalitpur distric of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	8	14	23	
2	*Cx. vishnui*	0	0	0	11	0	3	
3	*Cx. pseudovishnui*	0	0	0	21	15	0	
4	*Cx. gelidus*	0	0	0	0	0	2	
5	*Cx. quinquifasciatus*	12	10	124	191	176	253	
6	*Cx. fuscocephala*	3	11	12	18	20	23	
7	Other	2	2	5	31	38	38	
	Total	17	23	141	272	249	319	

Table 2. Monthwise collection of mosquitoes in outdoor hand collections in Godavari area of Lalitpur distric of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	2	8	18	
2	*Cx. vishnui*	0	0	0	0	3	3	
3	*Cx. pseudovishnui*	0	0	0	0	2	0	
4	*Cx. gelidus*	0	0	0	0	0	5	
5	*Cx. quinquifasciatus*	17	18	82	75	54	134	
6	*Cx. fuscocephala*	5	7	19	24	33	39	
7	Other	4	7	8	15	26	32	
	Total	26	32	109	114	118	213	

Table 3. Monthwise collection of mosquitoes in indoor hand collections in Balkot areas of Bhaktapur district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	8	21	12	
2	*Cx. vishnui*	0	0	0	11	0	3	
3	*Cx. pseudovishnui*	0	0	0	21	7	0	
4	*Cx. gelidus*	0	0	0	0	0	2	
5	*Cx. quinquifasciatus*	12	32	124	169	33	153	
6	*Cx. fuscocephala*	3	4	12	36	43	13	
7	Other	2	2	5	31	31	3	
	Total	17	36	141	268	114	186	

Table 4. Monthwise collection of mosquitoes in outdoor hand collections in Balkot areas of Bhaktapur district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	10	6	15	
2	*Cx. vishnui*	0	0	0	0	2	0	
3	*Cx. pseudovishnui*	0	0	0	2	3	0	
4	*Cx. gelidus*	0	0	0	0	2	5	
5	*Cx. quinquifasciatus*	17	19	79	34	2	19	
6	*Cx. fuscocephala*	0	0	3	2	12	8	
7	Other	7	5	14	24	24	15	
	Total	24	24	96	62	51	47	

Table 5. Monthwise collection of mosquitoes in indoor hand collections in Gothatar areas of Kathmandu district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	0	9	19	
2	*Cx. vishnui*	0	0	0	0	0	7	
3	*Cx. pseudovishnui*	0	0	0	0	4	0	
4	*Cx. gelidus*	0	0	0	3	3	2	
5	*Cx. quinquifasciatus*	32	31	21	101	44	97	
6	*Cx. fuscocephala*	0	4	4	4	18	6	
7	Other	0	6	6	6	11	3	
	Total	32	41	31	114	80	134	

Table 6. Monthwise collection of mosquitoes in outdoor hand collections in Gothatar areas of Kathmandu district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	0	12	25	
2	*Cx. vishnui*	0	0	0	0	3	13	
3	*Cx. pseudovishnui*	0	0	0	0	11	5	
4	*Cx. gelidus*	0	0	0	5	2	6	
5	*Cx. quinquifasciatus*	34	28	51	69	21	114	
6	*Cx. fuscocephala*	0	12	17	22	4	9	
7	Other	0	5	7	31	18	11	
	Total	34	45	75	127	71	183	

Table 7. Monthwise collection of mosquitoes in indoor hand collections in Nepaltar areas of Kathmandu district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	0	38	23	
2	*Cx. vishnui*	0	0	0	0	0	11	
3	*Cx. pseudovishnui*	0	0	0	3	4	0	
4	*Cx. gelidus*	0	0	0	0	3	0	
5	*Cx. quinquifasciatus*	37	28	126	141	175	86	
6	*Cx. fuscocephala*	6	2	6	20	145	4	
7	Other	4	1	6	26	42	9	
	Total	47	31	138	190	369	133	

Table 8. Monthwise collection of mosquitoes in outdoor hand collections in Nepaltar areas of Kathmandu district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	0	22	44	
2	*Cx. vishnui*	0	0	0	0	0	6	
3	*Cx. pseudovishnui*	0	0	0	7	3	2	
4	*Cx. gelidus*	0	0	0	0	8	0	
5	*Cx. quinquefasciatus*	48	51	33	57	112	53	
6	*Cx. fuscocephala*	2	5	9	19	63	11	
7	Other	6	0	2	25	12	8	
	Total	56	56	44	108	220	124	

A total of 45 *Culex tritaeniorhynchus*, 14 *Cx. vishnui*, 36 *Cx. pseudovishnui*, 2 *Cx. gelidus*, 766 *Cx. quinquefaciatus,* and 87 *Cx. fuscocephala* were recorded in indoor hand collection from Godavari (Lalitpur district) in April-September of 2009 (table 1). 28 *Culex tritaeniorhynchus*, 6 *Cx. vishnui*, 2 *Cx. pseudovishnui*, 5 *Cx. gelidus*, 380 *Cx. quinquefaciatus,* and 127 *Cx. fuscocephala* were recorded in outdoor hand collection from Godavari (Lalitpur district) in April-September of 2009 (table 2).

41 *Culex tritaeniorhynchus*, 14 *Cx. vishnui*, 28 *Cx. pseudovishnui*, 2 *Cx. gelidus*, 523 *Cx. quinquefaciatus,* and 111 *Cx. Fuscocephala* in indoorhand collection and 31 *Culex tritaeniorhynchus*,2 *Cx. vishnui*, 5 *Cx. pseudovishnui*, 7 *Cx. gelidus*, 170 *Cx. quinquefaciatus,* and 25 *Cx. fuscocephala* in outdoor hand collection were recorded from Balkot (Bhaktapur district) in April-September of 2009 (table 3 & 4). 28 *Culex tritaeniorhynchus*, 7 *Cx. vishnui*, 4 *Cx. pseudovishnui*, 8 *Cx. gelidus*, 326 *Cx. quinquefaciatus,* and 36 *Cx. fuscocephala* in

indoorhand collection and 37 *Culex tritaeniorhynchus*, 16 *Cx. vishnui*, 16 *Cx. pseudovishnui*, 13 *Cx. gelidus*, 317 *Cx. quinquefaciatus,* and 64 *Cx. fuscocephala* in outdoorhand collection were recorded from Gothatar (Kathmandu district) in April-September of 2009 (table 5 & 6). 61 *Culex tritaeniorhynchus*, 11 *Cx. vishnui*, 7 *Cx. pseudovishnui*, 3 *Cx. gelidus*, 593 *Cx. quinquefaciatus,* and 183 *Cx. fuscocephala* in indoorhand collection and 66 *Culex tritaeniorhynchus*, 6 *Cx. vishnui*, 12 *Cx. pseudovishnui*, 8 *Cx. gelidus*, 354 *Cx. quinquefaciatus,* and 109 *Cx. fuscocephala* in outdoor hand collection were recorded from Nepaltar (Kathmandu district) in April-September of 2009 (table 7 & 8). Other associated culicine mosquitoes recorded were *Culex Cx. hutchinsonie*, *Cx. edwardsii*, *Culex barraudi* and *Armigeres spp.* etc. in different months.

Animal baited net trap collection

During the study period one animal baited net trap was fixed keeping one animal bait inside the trap in Godavari, Balkot, Gothatar and Nepaltar of Kathmandu valley in the field outdoor to collect adult mosquitoes coming to bite in the trap . 19 *Culex tritaeniorhynchus*, 44 *Cx. vishnui*, 42 *Cx. pseudovishnui*, 2 *Cx. gelidus*, 6149 *Cx. quinquefaciatus,* and 137 *Cx. fuscocephala* were recorded in animal baited net trap collection. Collection from Godavari (Lalitpur district); 9 *Culex tritaeniorhynchus*, 48 *Cx. vishnui*, 32 *Cx. pseudovishnui*, 5 *Cx. gelidus*, 6644 *Cx. quinquefaciatus,* and 261 *Cx. fuscocephala* were recorded in animal baited net trap collection from Balkot (Bhaktapur district); 10 *Culex tritaeniorhynchus*, 66 *Cx. vishnui*, 42 *Cx. pseudovishnui*, 5230 *Cx. quinquefaciatus,* and 302 *Cx. fuscocephala* were recorded in animal baited net trap collection from Gothatar (Kathmandu district); 275 *Culex tritaeniorhynchus*, 214 *Cx. vishnui*, 104 *Cx. pseudovishnui*, 4592 *Cx. quinquefaciatus,* and 280 *Cx. fuscocephala* were recorded in animal baited net trap collection from Nepaltar (Kathmandu district) in April-September of 2009 (table 9-12). Other associated culicine mosquitoes recorded in animal baited net trap were *Cx. Hutchinsonie* and *Armigeres spp.* etc.

Table 9. Monthwise collection of mosquitoes in Animal Baited Net collections in Godavari areas of Lalitpur district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	7	9	3	
2	*Cx. vishnui*	0	0	0	3	0	41	
3	*Cx. pseudovishnui*	0	0	0	8	34	0	
4	*Cx. gelidus*	0	0	0	0	2	0	
5	*Cx. quinquefasciatus*	854	370	887	973	1963	1102	
6	*Cx. fuscocephala*	4	5	7	17	25	79	
7	Other	3	5	29	18	42	74	
	Total	861	380	923	1026	2075	1299	

Table10. Monthwise collection of mosquitoes in Animal Baited Net collections in Balkot areas of Bhaktapur district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	2	0	9	
2	*Cx. vishnui*	0	0	0	3	0	45	
3	*Cx. pseudovishnui*	0	0	0	8	11	13	
4	*Cx. gelidus*	0	0	0	3	0	2	
5	*Cx. quinquefasciatus*	856	137	1027	1181	1963	1480	

6	*Cx. fuscocephala*	0	6	13	36	87	119	
7	Other	0	5	6	32	26	114	
	Total	856	148	1046	1265	2087	1782	

Table 11. Monthwise collection of mosquitoes in Animal Baited Net collections in Gothatar areas of Kathmandu district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	0	4	6	
2	*Cx. vishnui*	0	0	0	0	0	66	
3	*Cx. pseudovishnui*	0	0	0	0	11	31	
4	*Cx. gelidus*	0	0	0	0	0	0	
5	*Cx. quinquifasciatus*	930	176	1039	946	890	1249	
6	*Cx. fuscocephala*	88	3	16	15	37	143	
7	Other	37	5	21	11	38	253	
	Total	1055	184	1076	972	980	1748	

Table 12. Monthwise collection of mosquitoes in Animal baited Net Collection in Nepaltar areas of Kathmandu district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	0	0	275	
2	*Cx. vishnui*	0	0	0	0	0	214	
3	*Cx. pseudovishnui*	0	0	0	2	8	94	
4	*Cx. gelidus*	0	0	0	0	0	0	
5	*Cx. quinquefasciatus*	930	489	1101	838	987	247	
6	*Cx. fuscocephala*	88	13	40	18	37	84	
7	Other	37	22	15	6	46	138	
	Total	1055	524	1056	864	1078	1052	

Light trap collection

Table 13. Monthwise collection of mosquitoes in Light Trap collections in Godavari areas of Lalitpur district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	3	16	34	
2	*Cx. vishnui*	0	0	0	9	18	3	
3	*Cx. pseudovishnui*	0	0	0	2	6	0	
4	*Cx. gelidus*	0	0	0	0	15	17	
5	*Cx. quinquefasciatus*	15	29	837	268	332	379	
6	*Cx. fuscocephala*	3	3	7	55	64	72	
7	Other	5	2	4	21	79	25	
	Total	23	34	848	358	530	530	

Table 14. Monthwise collection of mosquitoes in Light Trap collections in Balkot areas of Bhaktapur district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	0	7	23	
2	*Cx. vishnui*	0	0	0	8	24	9	
3	*Cx. pseudovishnui*	0	0	0	0	5	6	
4	*Cx. gelidus*	0	0	0	0	12	13	
5	*Cx. quinquefasciatus*	5	8	17	223	334	391	
6	*Cx. fuscocephala*	0	1	5	43	48	81	
7	Other	0	0	2	19	76	28	
	Total	5	9	24	293	506	551	

Table 15. Monthwise collection of mosquitoes in Light trap collections in Gothatar areas of Kathmandu district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	20	7	10	
2	*Cx. vishnui*	0	0	0	13	0	18	
3	*Cx. pseudovishnui*	0	0	0	0	4	21	
4	*Cx. gelidus*	0	0	0	2	14	19	
5	*Cx. quinquefasciatus*	230	159	124	196	273	306	
6	*Cx. fuscocephala*	30	1	5	23	69	48	
7	Other	28	7	3	61	41	54	
	Total	288	167	132	315	408	476	

Table 16. Monthwise collection of mosquitoes in Light trap collections in Nepaltar areas of Kathmandu district of Kathmandu valley, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	20	16	24	
2	*Cx. vishnui*	0	0	0	13	9	15	
3	*Cx. pseudovishnui*	0	0	0	0	4	12	
4	*Cx. gelidus*	0	0	0	2	11	9	
5	*Cx. quinquefasciatus*	230	160	124	41	304	363	
6	*Cx. fuscocephala*	30	0	5	58	39	46	
7	Other	28	3	1	43	31	25	
	Total	288	163	130	177	414	494	

During the study period one light trap was fixed outdoor in Godavari, Balkot, Gothatar and Nepaltar to collect adult mosquioes in April-September 2009. 53 *Culex tritaeniorhynchus*, 30 *Cx. vishnui*, 8 *Cx. pseudovishnui*, 32 *Cx. gelidus*, 1860 *Cx. quinquefaciatus,* and 204 *Cx. fuscocephala* from Godavari (Lalitpur district); 30 *Culex tritaeniorhynchus*, 41 *Cx. vishnui*, 11 *Cx. pseudovishnui*, 25 *Cx. gelidus*, 978 *Cx. quinquefaciatus,* and 178 *Cx. fuscocephala from Balkot(Bhaktapur district);* 30 *Culex tritaeniorhynchus*, 41 *Cx. vishnui*, 11 *Cx. pseudovishnui*, 25 *Cx. gelidus*, 978 *Cx. quinquefaciatus,* and 178 *Cx. fuscocephala from Balkot(Bhaktapur district);* 37 *Culex tritaeniorhynchus*, 31 *Cx. vishnui*, 25 *Cx. pseudovishnui*, 35 *Cx. gelidus*, 1288 *Cx. quinquefaciatus,* and 176 *Cx. fuscocephala* from Gothatar (Kathmandu district) and 60 *Culex tritaeniorhynchus*, 37 *Cx. vishnui*, 16 *Cx. pseudovishnui*, 22 *Cx. gelidus*, 1222 *Cx. quinquefaciatus,* and 178 *Cx. fuscocephala* from Nepaltar (Kathmandu district) were recorded in animal baited net trap collection (table 16). Other associated culicine mosquitoes recorded were *Culex Cx. hutchinsonie*, *Cx. Edwardsii*, *Culex barraudi* and *Armigeres spp.* etc. in different months.

Larvae collection

Different breeding places (paddy field, puddles, ponds, river bed, swamps, drain etc.) were searched for larvae of *Culex tritaeniorhynchus* and other *Culex* mosquitoes in Balkot, Gothatar, Nepaltar and Godavari taking dips in the month of April-September. In April, larvae of *Culex tritaeniorhynchus* and other possible vectors of JEV were not found in all locations in April, 2009 (table 17-20). Larvae of other species recorded were *Culex quinquefaciatus* as 1153 and *Cx. fuscocephala* as 5 in April. Searching in different breeding places in the month of May 2009, larvae of *Culex tritaeniorhynchus* and other *Culex* vector species were not recorded in Godavari, Balkot, Gothatar and Nepaltar. But the total number of larvae of *Culex quinquefaciatus* and *Cx. fuscocephala* recorded from Godavari, Balkot, Gothatar and Nepaltar. Similarly in June not any larvae of *Culex tritaeniorhynchus* and other possible vectors of JEV were recorded. But taking dips in different breeding places of Godavari, Balkot, Gothatar and Nepaltar, larvae of *Culex quinquefaciatus* and *Cx. fuscocephala* were recorded. Not any larvae of *Culex tritaeniorhynchus* were recorded but larvae of *Culex pseudovishnui*, *Cx. quinquefaciatus* and *Cx. fuscocephala* were recorded in July (table 17-20). Larvae of *Culex tritaeniorhynchus* in were recorded in August and September from Godavari and Nepaltar and in September also from Godavari, Nepaltar, Balkot and Gothatar. Other associated species were *Cx.vishnui*, *Cx. pseudovishnui*, *Cx. gelidus*, *Culex quinquifaciatus* and *Cx. fuscocephala* recorded in this study during April to September, 2009 (table 17-20).

Table 17. Monthwise collection of mosquito larvae in Godavari areas of Lalitpur district , April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	0	3	103	
2	*Cx. vishnui*	0	0	0	0	0	0	
3	*Cx. pseudovishnui*	0	0	0	6	6	18	
4	*Cx. gelidus*	0	0	0	0	23	30	
5	*Cx. quinquefasciatus*	1153	61	65	304	265	234	
6	*Cx. fuscocephala*	5	0	5	23	31	65	
7	Other	3	0	0	28	16	73	
	Total	1161	61	70	361	344	523	

Table 18. Monthwise collection of mosquito larvae in Balkot areas of Bhaktapur district, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	0	0	137	
2	*Cx. vishnui*	0	0	0	0	0	3	
3	*Cx. pseudovishnui*	0	0	0	0	0	7	
4	*Cx. gelidus*	0	0	0	0	0	0	
5	*Cx. quinquefasciatus*	621	291	41	194	33	973	
6	*Cx. fuscocephala*	0	3	0	26	12	63	

7	Other	0	0	0	0	6	55	
	Total	621	294	41	220	61	1491	

Table 19. Monthwise collection of mosquito larvae in Gothatar areas of Kathmandu district, April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	0	0	17	
2	*Cx. vishnui*	0	0	0	0	0	21	
3	*Cx. pseudovishnui*	0	0	0	0	47	47	
4	*Cx. gelidus*	0	0	0	0	0	0	
5	*Cx. quinquefasciatus*	355	350	32	345	198	703	
6	*Cx. fuscocephala*	33	0	3	31	36	143	
7	Other	33	0	2	9	0	76	
	Total	388	350	37	385	281	1007	

Table 20. . Monthwise collection of mosquito larvae in Nepaltar areas of Kathmandu district , April-September 2009.

S.N.	Species	April	May	June	July	August	September	Remarks
1	*Cx.tritaeniorhynchus*	0	0	0	0	7	63	
2	*Cx. vishnui*	0	0	0	0	0	0	
3	*Cx. pseudovishnui*	0	0	0	9	4	15	
4	*Cx. gelidus*	0	0	0	0	0	38	
5	*Cx. quinquefasciatus*	981	291	164	365	42	381	
6	*Cx. fuscocephala*	13	3	3	14	2	93	
7	Other	3	0	4	15	12	83	
	Total	997	294	171	403	67	780	

Figures 1 & 2 represent percentage of adults and larvae collected from Godavari, Balkot, Gothatar and Nepaltar of Kathmandu valley, April-September 2009. Overall, 37018 adults and 10071 larvae were collected. *Culex quinquefaciatus* was the most commonly captured culicine mosquito (81.83% adult and 83.82% larvae) in Kathmandu valley followed by *Cx. fuscocephala* (adult 6.91% and larvae 6.02 %), *Culex tritaeniorhynchus* (2.24 % adult and 3.27% larvae), *Cx. vishnui* (1.58 % adult), *Cx. pseudovishnui* (1.06 % adult and 1.57% larvae). The least density was found to be for *Cx. gelidus* (0.45% adult) and *Cx. vishnui* (0.23% larvae) (Figure 1 & 2). Other associated culicine mosquitoes recorded were *Culex hutchinsonie*, *Cx. edwardsii*, *Culex barraudi* and *Armigeres spp.* in different months.

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Figure 1. Percentage of adult mosquitoes collected from Kathmandu valley, April-September 2009.

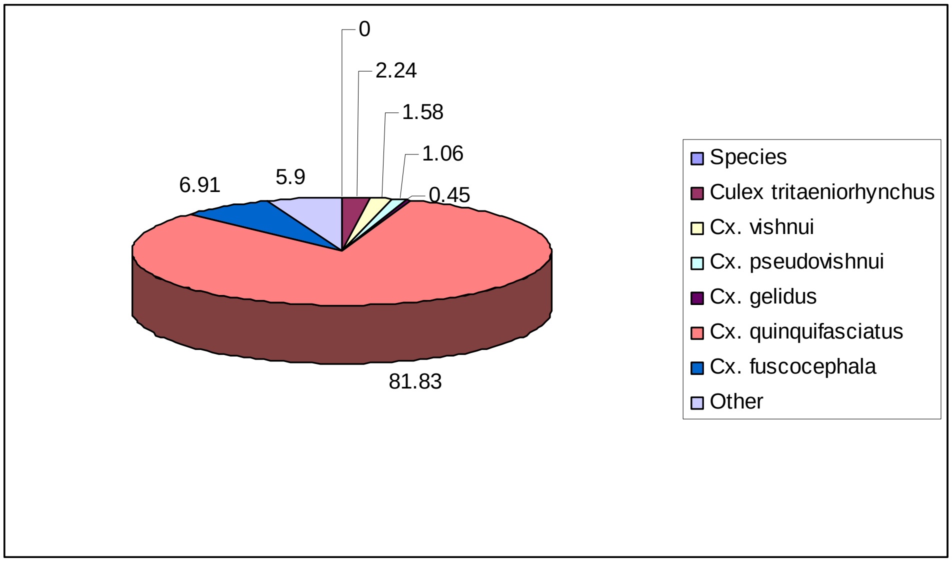
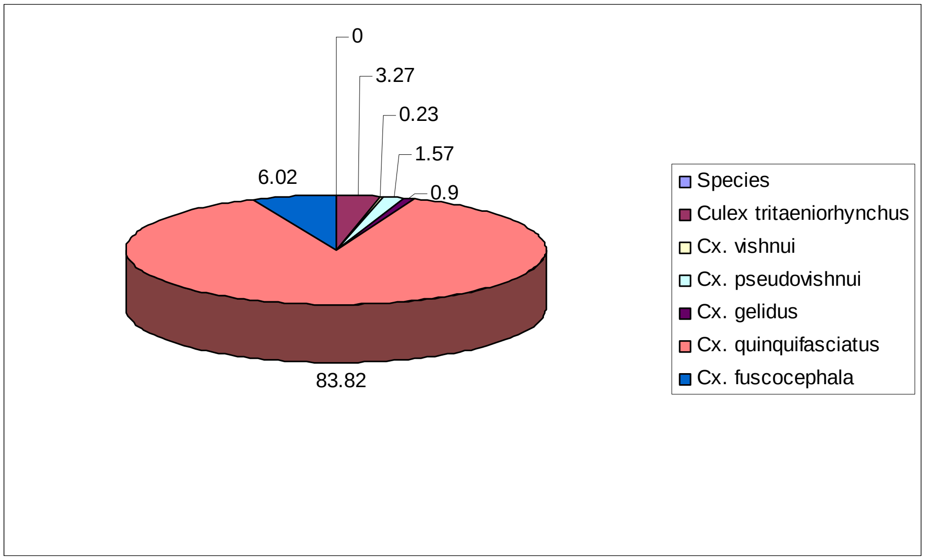


Figure 2. Percentage of larvaecollected from Kathmandu valley, April-September 2009.



CHAPTER 5

5.0. Discussion

This study was carried out in the month of April to September, 2009. April was generally dry month. There was not any rainfall prior to the study period. However, during the study period there were some windy days and very few rain drops were noticed. Due to this type of climatic condition very few breeding sites were found except some perennial river such as Bagmati, Bishnumati, Manahara etc. Most of the river beds were located on the bank of the rivers and most of them were dirty and polluted water collections. Same condition were found in the drains.located in most of the study areas. Hence, there were no records of possible vector JEV i.e. *Culex tritaeniorhynchus* and some other species such as *Culex pseudivishnui*, *Culex vishnui* and so on. Most of the species prefer to breed in the clean water vegetations. It is also well documented in some of the asian countries that *Culex tritaeniorhynchus* prefer to breed in clean water like rice fields, ponds, puddles containing clean water. But during the study period such types of breeding were not found Hence, during the month *Culex tritaeniorhynchus* must have not recorded in most of the collections. But other species recorded were *Culex quinquefaciatus*, *Culex fuscocephala* and *Culex hutchinsonie*. In addition to that some *Culex bauraudi* and *Culex edwardi* were also recorded in some areas of the four collection sites.

A number of variables such as weather conditions especially wind, rainfall and temperature, size of the bait animal and location of the trap (inside or outside) undoubtedly affect the results. Therefore, collections from different sites or collections weree conducted at the same areas on the different months to get adequate information relating the proper use of net traps, light traps, indoor and outdoor hand collection. However, for the breeding places it is not possible to set the fixed places but need to fix the villages. So it might give idea where and when different types of breeding places will be formed. This will definitely help to lay the egg by different species of mosquitoes. This give information of agro-ecological condition, breeding places, seasonal distribution. of the species including *Culex tritaeniorhynchus.* At the same time finding of multiple feeding habit may also be assessed in due course of time.

This study has documented the occurrence of six species of the genus *Culex* in the study areas. However, unable to collect the full fed *Culex tritaeniorhynchus* and *Culex gelidus* for blood meal analysis from outdoor hand collection because only unfed or gravid were represented in these habitats, which made it difficult to carry out serological test. This attempt to collect full fed *Culex tritaeniorhynchus* and *Culex gelidus* to facilitate serological analysis was unsuccessful because of termination of this project in September. Additionally, more mosquitoes from October could not collected, which is known to be ideal breeding month for *Culex tritaeniorhynchus* and *Culex gelidus*.

The obtained results clearly indicated the fluctuation pattern of mosquito densities at each collection site reflected the mosquito abundance, especially *Culex quinquefaciatus* in the study area and the micro condition at each site. The difference in attractiveness between the four collection sites might also have contributed to the different fluctuation pattern in mosquito numbers. Cows in the shed were more abundant and individually much larger. Probably, samples at the cow shed better represented the mosquito population in the study area.

Full fed females observed in this study were lower for these mosquito species *Culex tritaeniorhynchus* and *Cx. gelidus.* Sampling in this study was limited to 2 hours after sunset. Number of full fed *Cx. tritaeniorhynchus* and *Cx. gelidus* might increase towards later part of night. Therefore, a short sampling time limited to evening may be a reason of collection of small number of full fed females. During the study, breeding habitats of the vectors were abundant due to wet rice fields. These habitats probably produced high numbers of new adults, lowering parous rates. Protection of main blood sources with mosquito nets may also had an influence on the low full fed mosquitoes . Increased difficulty in access to blood sources would make feeding success less synchronized, which could render feeding peaks indistinct. Impact of blood sources protection on mosquito populations and JE epidemiology, either desirable or undesirable for humans, deserves further research.

The occurrence of diverse species of genus *Culex*, including important vectors of JE and Bancroftian filariasis and arboviruses, represent a trade off between human health and the benefits accrued to irrigated rice cultivation. Thus, although rice growing is a key source of employment and income generation, urgent consideration must be given to reducing the density of the diverse mosquito species thriving in these areas if these benefits are to be realized. This is especially important in Nepal.

The diversity of habitat types had a marked effect on *Culex* species diversity. Kathmandu valley, which had diverse habitat types, had a rich *Culex* mosquito fauna . Previous findings have reported a close association between adult/larval habitats diversity and mosquito fauna (Darsie and Pradhan, 1990, 1994). The results further demonstrated the possibility of targeting specific habitats at different times of the year depending on the season and site. Some habitats with the highest larval counts such as water reservoirs, ditches, pools, and hoof prints were mainly important during the wet season, and low-count habitats such as paddies and canals were productive throughout the study period. These findings are in agreement with previous observations that the most productive habitats per surface area are not necessarily the most important habitats for vector proliferation over space and time (Fillinger *et al.* 2004), In Venezuela (Grillet 2000), noted that a large number of low density, but continuously productive habitats contribute more to the adult mosquito density than singly high-density larval habitats. Importantly, the current study demonstrated a significant negative association between rainfall and *Culex* larval densities in Murinduko where there was limited rice cultivation. In areas of intense rice cultivation, the rice cropping cycle is considered to impact significantly on mosquito production (Klinkenberg *et al.* 2003), and rainfall is considered to be of insignificant short-term importance (Mukiama 1989). Conversely, in areas of little or no irrigation such as Murinduko, larval production is dependent upon temporary larval development sites (Beier 1990). As rains begin, some time must elapse for water bodies to form to facilitate effective larval breeding (Mukiama 1989). Considering that most of the non paddy habitats in Murinduko were concentrated on stream edges and that the soils and topography of this village could not allow the formation of numerous rain-fed pools, it is logical to assume that productive habitats were rendered less productive after the rains because of flushing out of the larvae, as observed by previous investigators (Martens *et al.* 1995; Russel *et al.* 1963).

Culex quinquefasciatus was most abundant mosquito collected in this study. In urban agglomeration, both man-made and other natural habitats form the mosquitogenic conditions conducive for the transmission of different vector-borne diseases. *Culex quinquefasciatus,* an ubiquitous urban, common domestic species mosquito breeds mainly in a common domestic species abundant in human dwellings and animal shelters, drains, cess pits and cess pools containing domestic effluents and such habitats are extensive and diverse. Immature stages are found in any type of habitat from fresh and clear to brackish, turbid and polluted waters. It is common in ground pools, ditches, drains, sewage la trines, septic tanks and artificial containers. Females feed on man at night indoors and outdoors, which is their preferred hosts in the Indian subcontinent (Sirivanakarn 1976, Reisen and Boreham 1979). The species is the principal vector of *Wuchereria bancrofti* in Nepal, which is within the endemic zone of filariasis (Jung 1973).

Lined drains are constructed on both sides of lane, streets and roads almost in all parts of the city. Domestic sullage water from the residential houses is discharged directly into these drains. In some areas, the sullage water is directly let into the nearby big backwater canal. In a few places, the drains are covered/closed by cement slabs leaving man holes for cleaning and removing any blocks if clogged. Backwaters around the city are favoured as a terminal discharge points of the feeder drains. However, before being drained into the backwaters, the water stagnates in many segments not only due to faulty gradients, but also due to damage or blockage with garbage. Wherever possible, faulty gradients should be rectified with the assistance of the engineering wing in a phased manner depending upon the availability of finances. Until such measures are undertaken, weekly larvicidal spraying needs to be carried out. This should be supplemented by silt/garbage removal. Availability of dustbins at reachable distance needs to be ensured to discourage people from throwing the garbage into the drain. Moreover, the community should be motivated to use the dustbins through appropriate campaigns. While during the rainy season flooding facilitates the free flow of water, it becomes one of the sources of mosquito breeding during non rainy months. During this period larvicidal spraying operations need to be carried out selectively in this habitat also.

Water stagnation in vacant plots and barren lands also supports mosquito breeding particularly when these plots receive sullage water from the houses. Mosquito breeding can be controlled in such situations by spraying larvicides, channeling–water can be drained out into the nearby drains/canals, and as a permanent measure the vacant plots should be filled to avoid water stagnation. The owners of the plots should be directed to fill these plots in public interest. However, findings of this study should be interpreted with caution. It should be noted that the locations selected in Kathmandu valley had different agricultural practices. Therefore, most larval habitats were sampled in those locations may account in part for the differences in adult and larval counts among the four villages.

A preliminary study to determine the adult mosquito species diversity in the four studies sites during the same study period observed a higher density of *Culex quenquefasciatus*. *Culex quinquefasciatus* was abundant in habitats with turbid water, and *Cx. tritaeniorhynchus* was mainly associated with paddy field in which emergent vegetation was present. In most areas of its distribution, *Cx. quinquefasciatus* prefer habitats with turbid water caused by organic matter (Asimeng 1993; Maxwell *et al*. 1999), as was the case in the present study. *Cx. quinquefasciatus* occurred widely in diverse habitat types, but other species were restricted to a few habitat types. This may explain why they were the predominant species, which is probably an indication of its ability to thrive in a variety of ecologic conditions as reported by Hopkins (1952). Females of *Culex fuscocephala* prefer bovines and Pigs as hosts but do attack man. Japanese encephalitis has been isolated f rom this mosquito in Thailand (Gould et al. 1974). Immature stages of *Culex fuscocephala* have been collected in rice fields, ground pools, foot prints and marshes. In Nepal larvae have been dipped from shallow pools in swampy ground and irrigation ditches (Dasie and Pradhan 1990). Larvae of this species was found it to be the most commonly in ponds, river beds etc. This is due to insanitary conditions and environmental degradation. Lack of adequate housing, water supply, sanitation and solid waste management facilities, as well as knowledge, attitudes and practice of the people are the major factors responsible for the proliferation of mosquitoes in the urban environment.

Immatures of *Culex pseudovishnui* were collected from various types of fresh water ground pools, rice fields and stream pools. Reuben (1971) reported that larvae were not encountered in rice fields until the plants were 0.3 m in height. Females occasionally attack man but prefer bovines, birds and pigs (Sirivanakam 1976). Biting rhythm of females showed a peak about 1900-2000 hours with a secondary peak at 0500 hours (Reisen and Aslamkhan 1978). Little is known about the breeding of *Cx. tritaeniorhynchus*, and *Cx.gelidus* in Nepal but records from Darsie and Pradhan (1990) reported *Culex triateniorhynchus* is widely distributed in Oriental Region, west to Middle East, Afrotropical Region, Mediterranean Region, north and east to Maritime USSR, Japan and Korea and south to Indonesia (Darsie and pradhan 1990). It 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 0.3 m in height. Adults are found in cattle sheds and piggeries. They also feed on man and birds (Sirivanakarn 1 976). This species exhibits physiological and ecological plasticity throughout its range for it tolerates extremely variable environmental conditions. *Culex tritaeniorhynchus* is a major vector of Japanese encephalitis virus in many parts of the Oriental Region, including Nepal (Hammon *et al.* 1949, Hale *et al.* 1957, Buescher *et al.* 1959, Reuben *et al.* 1971; Leake *et al.* 1986). *Culex gelidus* species is associated closely with man and his domestic animals. Immatures live in puddles, pools, rice fields and marshy depressions having abundant vegetation. Their preferred hosts are bovines and swine, but they readily attack man (Sirivanakarn 1976). This is a suspected vector of Japanese encephalitis. This study illustrates that larval production is a function of complex interaction of several biotic and abiotic habitat characteristics, many of which were not measured in this study. Moreover, this study was more descriptive than comprehensive. A more quantitative study is warranted, which would include physical, chemical, and biologic habitat characteristics and their impact not only on species diversity but on abundance and seasonal periodicity. Typical larval habitats of *Culex vishnui* are grassy ditches, pools, ponds, animal tracks, swampy g round and fallow rice fields. In growing rice paddies, it is replaced by larvae of *Cx. pseudovishnui* and *Cx. tritaeniothynchus* when rice plants reach a height of 0.3 m. The natural hosts of the females are pigs and birds but man and cattle are readily attacked also. Japanesee ncephalitis virus has been isolated from this species (Reuben 1971; Sirivanakarn 1976).

Water logged marshy lands are common in many parts and support profuse breeding. Due to the vastness of the habitat, spraying with insecticide is neither feasible nor economical. In such conditions, the utility of the nematode parasite *Romanomermis iyengari* can be exploited as a biocontrol agent, which has been proved to be efficacious elsewhere (Paily *et al.* 1994, Reuben *et al.* 1990). Due to rapid urbanization, man-made ponds are being converted into residential plots. However, in the periphery of the city, there are a number of ponds, infested with aquatic floating weeds supporting mosquitoes. Mosquitoes in these ponds can be controlled by physical removal of weeds (Dhanda *et al.* 1994) and stocking the ponds with weedivorous fishes such as *Ctenopharygodon idella* (Chinese grass carp) and *Osphronemus goramy* (Giant gourami) (Rajagopalan *et al.* 1987). An income generating scheme can also be introduced involving the community. The local community can be motivated to remove or empty the receptacles around the premises. *Toxorhynchites*, a non-biting predatory mosquito can be used if necessary to control tree hole breeding mosquitoes. The local community can be motivated to remove or empty the receptacles around the premises. *Toxorhynchites*, a non-biting predatory mosquito can be used if necessary to control tree hole breeding mosquitoes. In addition, *Bacillus thuringiensis* Var. Israelensis serotype H 14 as an effective biological control agent against mosquitoes in Israel was successfully tested. Toxicity tests with *Culex pipiens* and *Aedes aegypti* showed that the strain was effective in LD_{50} bioassays (Margalit 1983). The biological insecticide such as *Bacillus thuringiensis* serotype H-14 (B.t. H-14) can be applied through community participation.

It is necessary to implement separate mosquito control programme linked with sanitation and solid waste disposal, which is carried out by municipalities. While preparing the master plan simple, economic, ecologically sound, reliable, labour intensive and compatible methods both for the organization and the community can be followed. In most parts of Kathmandu valley, the underground water has been found to be contaminated owing to the high watertable and the absence of a proper liquid waste disposal system. Surface drains and canals are mostly used to drain sullage water into the backwaters. Many areas are perennially water logged and thereby prone to mosquitogenic conditions. From the public health point of view, these results indicate that a wide spectrum of *Culex* species thrive in a variety of habitat types whose larval densities vary with space and time depending on the underlying environmental and ecologic conditions. As such, any successful larval control operation, especially one targeting integrated control of diverse mosquito species occurring in a given area, should take into account the spatial-temporal dynamics in larval habitats productivity.

So, this study provides baseline information on *Culex* mosquitoes at three districts of Kathmandu valley. The study suggests that rice cultivation has a effect on *Culex* mosquito species diversity. It also illustrates the importance of how human-made changes could alter species diversity and abundance. The results reaffirm the need to consider peridomestic and natural habitats present in a given area, if significant reduction in mosquito densities is to be realized through larval management. These findings also provide significant information useful in designation of an integrated mosquito control strategy in response to the recent emergence and re-emergence of mosquito-borne diseases in the tropics. An integrated vector control program is advantageous because in addition to reducing the risk of mosquito-borne diseases, it could also result in an overall reduction in densities of nuisance mosquitoes making it more acceptable to the surrounding community.

CHAPTER 6

6.0. Conclusion and Recommendation

6.1. Conclusion

Culex tritaeniorhynchus is a major vector of Japanese encephalitis virus in many parts of the Oriental Region, including Nepal (Hammon et al. 1949, Hale et al. 1957, Buescher et al. 1959, Reuben et al. 1971; Leake et al. 1986). *Culex gelidus* species is associated closely with man and his domestic animals. Immatures live in puddles, pools, rice fields and marshy depressions having abundant vegetation. Their preferred hosts are bovines and swine, but they readily attack man (Sirivanakarn 1976). This is a suspected vector of Japanese encephalitis. This study illustrates that larval production is a function of complex interaction of several biotic and abiotic habitat characteristics, many of which were not measured in this study. There were no records of possible vector JEV i.e. *Culex tritaeniorhynchus* and some other species such as *Culex pseudivishnui*, *Culex vishnui* and so on. It is also well documented in some of the asian countries that *Culex tritaeniorhynchus* prefer to breed in clean water like rice fields, ponds, puddles containing clean water. But during the study period such types of breeding were not found Hence, during the month *Culex tritaeniorhynchus* must have not recorded in most of the collections. But other species recorded were *Culex quinquefaciatus*, *Culex fuscocephala* and *Culex hutchinsonie.* In addition to that some *Culex bauraudi* and *Culex edwardi* were also recorded in some areas of the four collection sites. However, unabled to collect the full fed *Culex tritaeniorhynchus* and *Culex gelidus* for blood meal analysis from outdoor hand collection because only unfed or gravid were represented in these habitats, which made it difficult to carry out serological test. To collect full fed *Culex tritaeniorhynchus* and *Culex gelidus* to facilitate serological analysis was unsuccessful because of termination of this project in September. Additionally, we did not sample more mosquitoes from October, which is known to be ideal breeding month for *Culex tritaeniorhynchus* and *Culex gelidus*. Most of the species prefer to breed in the clean water vegetations.

The obtained results clearly indicated the fluctuation pattern of mosquito densities at each collection site reflected the mosquito abundance in the study area and the micro condition at each site. The difference in attractiveness between the four collection sites might also have contributed to the different fluctuation pattern in mosquito numbers. Cows in the shed were more abundant and individually much larger. Probably, samples at the cow shed better represented the mosquito population in the study area.

Full fed females observed in this study were lower for these mosquito species *Culex tritaeniorhynchus* and *Cx. gelidus.* Sampling in this study was limited to 2 hours after sunset. Number of full fed *Cx. tritaeniorhynchus* and *Cx. gelidus* might increase towards later part of night. Therefore, a short sampling time limited to evening may be a reason of collection of small number of full fed females. During the study, breeding habitats of the vectors were abundant due to wet rice fields. These habitats probably produced high numbers of new adults, lowering parous rates. Protection of main blood sources with mosquito nets may also had an influence on the low full fed mosquitoes . Increased difficulty in access to blood sources would make feeding success less synchronized, which could render feeding peaks indistinct. Impact of blood sources protection on mosquito populations and JE epidemiology, either desirable or undesirable for humans, deserves further research.

The occurrence of diverse species of genus *Culex*, including important vectors of JE and Bancroftian filariasis and arboviruses, represent a trade off between human health and the benefits accrued to irrigated rice cultivation. Thus, although rice growing is a key source of employment and income generation, urgent consideration must be given to reducing the density of the diverse mosquito species thriving in these areas if these benefits are to be realized. This is especially important in Nepal.

The diversity of habitat types had a marked effect on *Culex* species diversity. Kathmandu valley, which had significantly more diverse habitat types, had a rich *Culex* mosquito fauna . Previous findings have reported a close association between adult/larval habitats diversity and mosquito fauna (Darsie and Pradhan, 1990,1994). The results further demonstrated the possibility of targeting specific habitats at different times of the year depending on the season and site. Some habitats with the highest larval counts such as water reservoirs, ditches, pools, and hoof prints were mainly important during the wet season, and low-count habitats such as paddies and canals were productive throughout the study period.

In urban agglomeration, both man-made and other natural habitats form the mosquitogenic conditions conducive for the transmission of different vector-borne diseases. *Culex quinquefasciatus*, the principal vector of *Wuchereria bancrofi* in Nepal, which is within the endemic zone of filariasis (Jung 1973) was abundantly found in this study. *Cx. quinquefasciatus* occurred widely in diverse habitat types, but other species were restricted to a few habitat types. This is due to insanitary conditions and environmental degradation. Lack of adequate housing, water supply, sanitation and solid waste management facilities, as well as knowledge, attitudes and practice of the people are the major factors responsible for the proliferation of mosquitoes in the urban environment. This may explain why they were the predominant species.

This study provides baseline information on *Culex* mosquitoes at three districts of Kathmandu valley. The results have demonstrated a spectrum of *Culex* mosquitoes in relation to breeding habitats. It also illustrates the importance of how human-made changes could alter species diversity and abundance. The results reaffirm the need to consider peridomestic and natural habitats present in a given area, if significant reduction in mosquito densities is to be realized through larval management. Due to rapid urbanization, man-made ponds are being converted into residential plots. However, in the periphery of the city, there are a number of ponds, infested with aquatic floating weeds supporting mosquitoes. Mosquitoes in these ponds can be controlled by physical removal of weeds and fishes, nematode parasite, *Toxorhynchites*, a non-biting predatory mosquito can be used if necessary to control tree hole breeding mosquitoes. *Bacillus thuringiensis* serotype H 14 as an effective biological control agent against mosquitoes in Israel was successfully tested. Toxicity tests with *Culex pipiens* and *Aedes aegypti* showed that the strain was effective in LD_{50} bioassays (Margalit 1983). The biological insecticide such as *Bacillus thuringiensis* serotype H-14 (B.t. H-14) can be applied through community participation.

An income generating scheme can also be introduced involving the community. The local community can be motivated to remove or empty the receptacles around the premises. It is necessary to implement separate mosquito control programme linked with sanitation and solid waste disposal, which is carried out by municipalities. While preparing the master plan simple, economic, ecologically sound, reliable, labour intensive and compatible methods both for the organization and the community can be followed. In most parts of Kathmandu valley, the underground water has been found to be contaminated owing to the high watertable and the absence of a proper liquid waste disposal system. Surface drains and canals are mostly used to drain sullage water into the backwaters. Many areas are perennially water logged and thereby prone to mosquitogenic conditions. From the public health point of view, these results indicate that a wide spectrum of *Culex* species thrive in a variety of habitat types whose larval densities vary with space and time depending on the underlying environmental and ecologic conditions. As such, any successful larval control operation, especially one targeting integrated control of diverse mosquito species occurring in a given area, should take into account the spatial-temporal dynamics in larval habitats productivity.

Overall, these findings also provide significant information useful in designation of an integrated mosquito control strategy in response to the recent emergence and re-emergence of mosquito-borne diseases in the tropics. An integrated vector control program is advantageous because in addition to reducing the risk of mosquito-borne diseases, it could also result in an overall reduction in densities of nuisance mosquitoes making it more acceptable to the surrounding community.

6.2. Recommendations

The results of studies showed that it is possible to contain the mosquito problem in Kathmandu valley with the existing infrastructure. However, it requires concerted efforts with a systematic approach. Hence, the present recommendations are made for tackling the existing situation for effective implementation of the programme are given based on mosquitoes prevalent in the study areas.

(i) Identification and enumeration of all potential mosquito breeding sources and quantify the relative role of different habitats in terms of daily mosquito emergence for prioritizing the areas for control operation is recommended.

(ii) Assessment of the magnitude of the mosquito menace problem through man biting density is necessary.

(iii) Evaluation of the susceptibility status of the most abundant mosquito species against frequently used larvicides and adulticides is important to suggest the choice of insecticides.

(iv) Development of a mosquito control strategy by integrating different control methods including biological control suited to the local needs.

(v) Impart training in control operations to those human resources engaged in this area, design an information, education and communiction system for creating community awareness. Appropriate remedial measures are suggested to correct the defects and implementation of measures to avoid mosquito breeding requires considerable exercise particularly in motivating and mobilizing the community.

(vi) There is an apparent gap between the engineering and health divisions with respect to mosquito control activities. Therefore intersectoral coordination is an important component for the successful implementation of mosquito control programmes. Provision of a sewerage system will be an ideal solution to the problem. In this process, open drains will no longer exist. As a result not only will the expenditure on insecticides and spraying operations be reduced, but also the environmental pollution will be minimized. However, this can be considered as a long-term plan in a phased manner.

(vii) Monitoring the effectiveness of operations and reviewing the programme are of paramount importance to ensure the sustenance of an effective control programme. An action committee can be formed with a senior staff member /reputed senior citizen as the Chairman with local members to review the programme from time to time and to overcome bottlenecks, if any.

CHAPTER 7

7.0. Reference

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Annex

Table 21. Daily Temperature, Rainfall and Relative Humidity During the Study in April, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-04-01	26.8	10.5	5.8	98	48
2009-04-02	27.8	13.0	trace	95	50
2009-04-03	29.2	13.3	0	97	46
2009-04-04	30	7.9	0	96	42
2009-04-05	30	13.7	0	96	47
2009-04-06	29.2	13.6	0	94	50
2009-04-07	29.9	13.4	0	95	27
2009-04-08	30.4	12	0	84	30
2009-04-09	30.6	14.2	0	80	35
2009-04-10	29.3	10.3	0	83	33
2009-04-11	30.3	10.2	0	94	32
2009-04-12	30.9	11.9	0	80	35
2009-04-13	30.8	13.6	0	80	37
2009-04-14	31.8	16.5	0	75	47
2009-04-15	32.4	16	0	86	38
2009-04-16	33.7	16.2	0	95	38
2009-04-17	33.6	14.3	trace	97	26
2009-04-18	32.5	11.7	5.5	89	42
2009-04-19	31.4	13.2	0	80	36

2009-04-20	31.6	16	0	84	40
2009-04-21	32.8	15.8	0	98	38
2009-04-22	33.4	14.2	0	90	24
2009-04-23	33.2	34.4	0	78	26
2009-04-24	35.2	12.4	0	69	20
2009-04-25	33.2	11.5	0	64	23
2009-04-26	32.9	13.3	0	78	34
2009-04-27	33.2	12.5	0	75	28
2009-04-28	32	13.2	0	70	27
2009-04-29	32	13.8	0	74	31
2009-04-30	31.4	16.4	0	89	47
Average	31.38	13.96	0.40	84.64	34.96

Table 22. Daily Temperature, Rainfall and Relative Humidity During the Study in May, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-05-01	31.1	16	trace	86	64
2009-05-02	33	19.3	0	90	49
2009-05-03	29.8	14.3	13	90	44
2009-05-04	30	15	11.5	95	52
2009-05-05	31.4	15	0	96	35
2009-05-06	31.4	13	1.4	93	28
2009-05-07	33	14.2	0	87	30
2009-05-08	33.5	15	0	74	28
2009-05-09	33.1	14	0	85	27
2009-05-10	32.1	15.3	trace	80	40
2009-05-11	26.2	14.7	17.1	96	42
2009-04-12	27.5	14	7.1	98	46
2009-05-13	25.7	12.6	10.2	98	83
2009-05-14	29	12.2	0	98	52
2009-05-15	27.4	14.6	1.6	96	52
2009-05-16	28.2	15.3	0	95	45
2009-04-17	28.6	17.4	trace	95	53
2009-05-18	29.9	17.4	0	89	56
2009-05-19	32.8	18.4	0	90	52
2009-05-20	29	19.9	trace	88	50
2009-05-21	30.4	18	6.5	89	84

2009-05-22	27.5	19	0.5	90	62
2009-05-23	28	18	0	90	48
2009-05-24	29.9	16.6	1	98	65
2009-05-25	23.5	18.6	5.7	95	88
2009-05-26	23.4	17	0	98	79
2009-05-27	27.7	17	0	98	61
2009-05-28	28.2	16.6	1	95	61
2009-04-29	27.6	15.5	0	96	56
2009-05-30	28.8	17.6	1	90	62
2009-05-31	23.6	16.5	1	95	65
Average	29.0	16.06	2.91	92.23	53.16

Table 23. Daily Temperature, Rainfall and Relative Humidity During the Study in June, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-06-01	28.2	16.3	1	98	61
2009-06-02	30.2	16.2	trace	96	59
2009-06-03	28.7	17	4	96	86
2009-06-04	28.8	18.4	trace	96	65
2009-06-05	29	19.9	25.5	93	70
2009-06-06	28.5	18.6	2.7	90	68
2009-06-07	31.5	17	0	97	52
2009-06-08	32.2	17.7	0	92	47
2009-06-09	31.8	16.6	0	90	34
2009-06-10	31.9	15.8	0	90	40
2009-06-11	31.7	18	0	88	47
2009-06-12	31.2	19	5.2	96	62
2009-06-13	30.7	19.0	0	94	53
2009-06-14	31.5	18.5	0	94	48
2009-06-15	32	18.5	0	90	46
2009-06-16	32.6	19.5	0	90	45
2009-06-17	33.6	18.8	0	95	49
2009-04-18	33.1	18	0	94	46
2009-06-19	32.1	19	trace	90	66
2009-06-20	34.2	19.3	0	90	66

2009-06-21	33.5	21.4	0	84	60
2009-04-22	31.7	20.1	20	_	_
2009-06-23	28	20.3	trace	94	80
2009-06-24	29.8	20.3	0	92	62
2009-06-25	31.5	20.2	32.7	94	70
2009-06-26	29.8	21.2	14.6	96	63
2009-06-27	26.4	20.6	11.5	96	76
2009-06-28	25.1	21	9.5	96	77
2009-06-29	30.2	20.4	0	95	71
2009-06-30	27.4	20.4	13	98	88
Average	30.64	19.08	5.37	93.07	60.57

Table 24. Daily Temperature, Rainfall and Relative Humidity During the Study in July, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-07-01	26	20.2	43.3	96	85
2009-07-02	29.5	20	3.9	94	57
2009-07-03	30	20.2	0	94	75
2009-07-04	30.3	20.3	20.3	59	44
2009-07-05	30.3	19.5	7.2	95	79
2009-07-06	30.3	20.5	9.1	96	70
2009-04-07	30.5	21	76.1	93	63
2009-07-08	31.4	21.4	2.8	97	77
2009-07-09	31.6	21	0	96	67
2009-07-10	31	20.6	0	93	52
2009-04-11	29.2	22	4.5	95	90
2009-07-12	25.3	20	5.2	96	83
2009-07-13	30.4	20	18.8	96	64
2009-07-14	31.4	20.6	0	94	78
2009-07-15	31.4	21.3	7.6	98	65
2009-07-16	30.4	21.4	0.2	96	74
2009-07-17	31.2	20.5	0	95	68
2009-07-18	31.4	21.8	trace	91	67
2009-07-19	31.4	20.5	14.4	97	63
2009-07-20	31.8	20.2	8.1	94	74
2009-07-21	31	21	0.6	96	66

2009-07-22	30.2	20.6	2.1	96	66
2009-07-23	30.4	20	1.2	98	66
2009-07-24	29.6	22	trace	98	68
2009-07-25	31.5	20.6	0	96	62
2009-07-26	28.3	21.4	14.9	96	85
2009-07-27	25.1	20.8	99	96	85
2009-07-28	28	19.6	33.8	98	76
2009-07-29	25.6	20.5	3.1	98	86
2009-07-30	29.6	20.6	7.9	96	64
2009-07-31	29.5	20.4	26.5	96	70
Average	29.92	20.67	14.15	94.43	70.13

Table 25. Daily Temperature, Rainfall and Relative Humidity During the Study in August, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-08-01	29.5	20.3	7.7	96	68
2009-08-02	31.1	21.4	0	96	64
2009-08-03	31.6	21.0	0	96	75
2009-08-04	30.2	21.0	0	93	70
2009-08-05	29.6	22.0	3.2	96	75
2009-08-06	27.6	21.5	8.3	89	88
2009-08-07	27.6	20.2	21.8	98	74
2009-08-08	30.5	21.6	0	96	77
2009-08-09	30.9	20.6	0.2	96	68
2009-08-10	28.3	21.5	1.7	96	80
2009-08-11	30.8	21.0	21.3	98	64
2009-08-12	29.6	21.5	14.4	95	67
2009-08-13	29.6	21.7	58.7	94	73
2009-08-14	26.0	21.0	6.9	93	84
2009-08-15	29.3	20.0	5.2	98	76
2009-08-16	27.2	20.5	3.2	98	78
2009-08-17	26.5	20.0	59.6	-	-
2009-08-18	27.8	20.5	16.9	96	90
2009-08-19	30.1	20.0	0.4	96	69
2009-08-20	27.3	20.5	19.1	92	75
2009-08-21	30.2	19.2	6.6	95	65

2009-08-22	31.0	18.8	0	97	58
2009-08-23	27.5	20.2	8.0	95	74
2009-08-24	25.0	20.5	28.8	96	79
2009-08-25	28.5	19.4	0	96	69
2009-08-26	28.6	19.0	0	96	69
2009-08-27	29.8	20.0	28.3	98	68
2009-05-28	29.8	19.7	2.5	97	66
2009-08-29	31.3	20.7	0	95	61
2009-08-30	29.8	20.2	15.5	98	65
2009-08-31	30.3	20.5	0.3	84	64
Average	29.12	20.51	11.43	92.25	69.54

Table 26. Daily Temperature, Rainfall and Relative Humidity During the Study in September, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-09-01	30.4	21.8	15.8	96	63
2009-09-02	29.6	18.5	11.8	96	74
2009-09-03	28.4	19.5	0	98	75
2009-09-04	29.5	19.5	3.0	94	72
2009-09-05	29.0	18.9	2.6	96	62
2009-09-06	26.4	18.4	15.7	96	70
2009-09-07	27.3	19.3	7.0	96	71
2009-09-08	27.7	19.7	0.4	98	73
2009-09-09	26	18.9	1.9	98	73
2009-09-10	26.5	18.6	1.3	95	72
2009-09-11	29.5	17.2	8.4	98	61
2009-09-12	30.2	17.2	0	96	55
2009-09-13	30.6	18.5	0	96	60
2009-09-14	30.0	20.0	traces	96	68
2009-09-15	29.5	20.2	0	94	68
2009-09-16	30.0	18.8	0	96	63
2009-09-17	30.3	18.4	0	96	55
2009-09-18	29.8	20.0	0	96	72
2009-09-19	29.1	17.8	0	94	65
2009-09-20	30.5	17.8	traces	94	65
2009-05-21	28.4	19.0	0	96	68

2009-09-22	30.0	17.9	0	95	53
2009-09-23	29.5	18.1	0	96	58
2009-09-24	29.4	18.3	27.4	96	64
2009-09-25	28.8	18.9	9.8	95	72
2009-09-26	30.5	17.6	0.5	95	60
2009-09-27	31.0	18.5	0	95	63
2009-09-28	31.0	17.6	0	94	63
2009-09-29	30.2	18.0	1.2	98	68
2009-09-30	31.2	16.4	0.7	96	60
Average	29.34	18.64	3.58	95.83	65.16

Table 27. Daily Temperature, Rainfall and Relative Humidity During the Study in October, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-10-01	29.8	17.6	8.0	96	66
2009-10-02	29.6	16.6	0	98	61
2009-10-03	25.7	19.2	0.3	96	88
2009-10-04	28.4	17.4	1.7	99	79
2009-10-05	27.0	18.4	2.5	96	70
2009-10-06	26.0	19.4	3.2	97	84
2009-10-07	24.2	19.3	5.3	98	74
2009-10-08	24.6	17.7	45.1	98	59
2009-10-09	29.0	17.7	0	98	59
2009-10-10	28.5	15.2	0	98	50
2009-10-11	27.5	15.0	0	98	56
2009-10-12	26.0	15.0	traces	92	54
2009-10-13	28.1	13.0	0	98	57
2009-10-14	28.7	14.0	0	98	50
2009-10-15	29.0	13.8	0	95	50
2009-10-16	28.3	13.8	0	95	48
2009-10-17	29.0	14.8	0	98	47
2009-10-18	29.0	13.4	0	98	48
2009-10-19	27.7	13.0	0	96	50
2009-10-20	27.8	12.0	0	96	50
2009-10-21	27.4	12.2	0	98	56

2009-10-22	27.6	13.0	0	99	49
2009-10-23	26.1	11.1	0	98	54
2009-10-24	26.6	10.5	0	96	50
2009-10-25	26.9	11.5	0	98	51
2009-10-26	26.4	12.0	0	98	48
2009-10-27	25.9	11.6	0	98	60
2009-10-28	26.8	13.1	0	94	48
2009-10-29	26.7	12.5	0	98	56
2009-10-30	26.8	11.5	0	97	48
2009-10-31	27.3	11.5	0	98	47
Average	27.36	14.41	2.13	97.06	37.48