CHAPTER ONE

INTRODUCTION

1.1. Background:

Biodiversity is variety in organisms in terms of species or environment for quantification of diversity and comparison of species diversity between different ecosystems in various ecological conditions (Owen, 1966). The mosquito diversity is also the variety of mosquito in the area in terms of genus species or sub-species level.

Mosquitoes (Insecta – Diptera – Culicidae) are the true flies. There are over 2500 different species of mosquitoes throughout the world. (Floore.et.al.2002.)

Mosquitoes are medically important insects. They are the carriers of various diseases. In Nepal three mosquito-borne diseases are prevalent and cause much morbidity and mortality. They are malaria (Shrestha et al. 1988), Japanese encephalitis (Khatri et al. 1983), Filariasis (Kessel 1966).

The mosquitoes are different from the other Dipterans by the presence of scales in the wings. Mosquitoes are the insects having two wings covered by the scales but in some scales are present along the wing veins and a conspicuous fringe of scales along the hind margin of the wings. The venation is very similar in all the species, but the coloration produced by the scales especially in *Anopheles* is useful in the identification of species in that genus. The male mosquitoes are smaller than female. Adult mosquitoes are slender bodied with long legs and delicate wings fringed with scales. Antenna are long, filamentous, each made up of 15 segments. The sexes can usually be distinguished most readily by the antenna; in the female they are long and slender with a whorl of a few short hairs at each joint, whereas in the male they have a feathery appearance due to tufts of long and numerous hairs at the joints. Female mosquitoes possess special elongated, modified mouthparts used for piercing and sucking human and other blood. Most of the Culicid have a long prominent proboscis containing needle like organs for piercing and sucking, but in two sub-families there is no long proboscis. (Barr, A.R.1957)

In many mosquitoes the palpi also furnish a means of distinguishing the sexes, they are usually long in both sexes, and in some mosquitoes, e.g. *Urotaenia*, they are short in both. The proboscis also differ in the sexes, in the male, the proboscis could not pierce flesh. The proboscis appears to be a simple bristle, sometimes curved, consists of a number of needles like organs lying in a groove in the fleshy labium. In the female mosquito there are six of these needle-like organs, the labrum-epipharynx and hypopharynx are the principal piercing organs and act together to form a tube for drawing up blood into the mouth. A tiny tube run down through the hypopharynx, opening at its tip, through which saliva is poured into the wound as through a hypodermic needle to prevent blood from coagulating .The maxillae and mandibles are thin, flat and flexible, the former recognizable by their saw tooth tips. The ensheathing labium bows back as the mosquito bites, the flexible tip or labellum acting as a guide for the piercing organs as they are sunk into the flesh. In the male mosquitoes the maxillae and mandibles are much degenerated, only the remaining part of the apparatus is well developed.

Mosquitoes are most abundant in warm region. They are found throughout the world particularly in tropical climates, from the tropic to the artic circle, from the low land to the peak of the high mountain. They are abundant in marshy lands, near collected waters, stagnant ponds, pools etc.

1.2. Mosquito as a vector:

Because of their variable breeding habits, choice of breeding places, tastes in blood, extent of travels and willingness to come indoors to bite or to rest, different species of mosquitoes have very different relations to human beings. The majorities that are dangerous to man who transmits diseases are as:

1. *Anopheles* mosquitoes: Female *Anopheles* mosquitoes are the sole transmitters of human malaria, which at least until recently has ranked as the most important human disease in the world.

Anopheles is also vectors of malaria of birds and reptiles. (Barr, A.R.1957).

Most *Anopheles* breed in natural waters such as ponds, swamps, edges of streams, rice fields, and grassy ditches.

Malaria is responsible for high infant mortality rates among many populations in the tropical and sub-tropical climates of the world.

Important malaria vector include:

An.culifacies, An.stephensi, An.minimus, An.fluviatilis, An.varuna, An.annularis, An.sundaicus, An.sinensis, An.maculatus, An.aquaslis, An.pseudopunctipennis, An.gambiae, An.funestus, An.punctulatus (Brydon et al. 1970)

***Anopheles* as malaria vector in Nepal:**

An.minimus, An.maculatus complex, An.fluviatilis, An.annularis

(Darsie and Pradhan, 1994)

2. *Aedes* mosquitoes: *Aedes spp* (mainly *Aedes aegypti*) is mainly responsible for the spread of Yellow fever. Yellow fever is an acute, often fatal disease caused by an arbovirus. *Aedes aegypti* mosquito is a member of the sub-genus *Stegomyia*, which contains a group of originally tree-hole breeding mosquitoes, several of which

(E.g. *albopictus, scutelbris, polynesiensis*, as well as *aegypti*) have become more or less domestic and have adopted man made containers as breeding places. *Aedes aegypti* was long known in medical literature as *Stegomyia fasciata. Aedes aegypti* is a pet mosquito as domestic as rat. It is almost never found more than a few hundred feet from human habitations, and feeds readily on human blood. It is a diurnal mosquito, biting principally in the morning and late afternoon, with a siesta in the middle of the day, but it will bite at night when hungry.

Mosquitoes of the subgenus *Stegomyia* is the sole transmitter of the Dengue fever. The causative agents of this disease are:

Ae. aegypti, Ae. albopictus, Ae. scutellaris and *Ae.polynesiensis*.

(Barr, A.R.1957)

3. *Armigeres* along with *Culex, Anopheles, Aedes*, and *Mansonia* harbor Japanese Encephalitis virus (Pradhan, 1981).

In Nepal *Culex tritaeniorhynchus* is suspected to be the principal vector of Japanese Encephalitis. JE is a mosquito borne viral disease caused by Flavivirus.

(Khatri et al.1983)

Mostly the vectors of this disease are prevalent primarily in agricultural regions; they breed in collections of water (typically rice paddies) and are extremely active in the evening and night.

4. *Culex quiquefasciatus* and *Culex pipiens* are the vectors of Filariasis in Nepal. (Kessel.1966) The disease mainly prefers to the Terai region.

1.3. Significance of the study:

The present study focuses on the diversity of mosquitoes in the study area. Mosquitoes are medically important as vector of different diseases such as: Malaria, Japanese Encephalitis, Dengue, Filariasis etc. Information on their

Prevalence/distribution in an area is important for an understanding of the epidemiology of the mosquito borne diseases and their control. The study gives the knowledge of species composition, host predilection, breeding habits, seasonality, longevity and the chance of contact between vector and man and helps in launching of control programmes. The wide spread surveys help in predicting epidemiology and in constructing prognostic map of mosquito borne diseases. The study also helps the people to understand the habitat status of the mosquito species and take action according to it. The study also significances the mosquito species in relation with environmental factors (temperature, rainfall, relative humidity).

Thus work on mosquitoes relating to prevalence, population dynamics, vector potentiality, habitat preference, host preference in domestic animal shelters (cow/cattle/buffalo sheds) and houses of different constructing types and ecology would be foundation step to prognosticate the possible epidemiology of mosquito vectors and mosquito borne diseases. The findings of this study probably would be helpful to precede effective and sustainable measure for mosquito control and to launch the awareness programme for local people from mosquito vectors and mosquito borne diseases.

1.4. Limitation of the study:

The limitation are as follows:

1. Due to lack of sufficient literature in the context of Nepal, it is challenging to conduct study properly.
2. The study was difficult due to constrain budget.
3. The study was done for the partial fulfillment of M.Sc. degree so the study was also limited by the short time duration.

1.5. OBJECTIVES:

The main objectives of the study are as follows:

1. To study the abundance of the mosquitoes in the study area.

2. To study the diversity of the mosquitoes in the study area.

3. To assess the mosquitoes with environmental parameters (temperature, rainfall, relative humidity).

CHAPTER TWO

LITERATURE REVIEW

IN THE CONTEXT OF NEPAL:

In Nepal some works has been carried out on mosquitoes regarding the distribution, diversity and importance. Some of which are as follows:

Theobald (1910) reported one more species of Non *Anopheline, Culex (Culiciomyia) Longifurcatus* from the Eastern Nepal.

Puri (1955) and Peter et al. (1958) were the first to report the species of *Anopheles* while Peter and Dewar (1956) were the first to record certain *culicine* occurring in Nepal.

Peter and Dewar (1956) published a preliminary record of the *Culicine* and *Toxorhynchities* mosquitoes of Nepal. Their collections were made in the vicinity of Hetaura and Bhimphedi in Makwanpur District of Narayani zone from which they recorded 29 species in 8 genera and 12sub genera.

Wattal et al. (1958) published his work about the mosquito fauna of India which have been very helpful in interpreting the findings in Nepal.

Stone, knight and Starcke (1959), Joshi et al. (1965) also reported 2species of *Culex* (*culex*) from the Kathmandu district, Bagmati zone.

Brydon et al. (1961) increase the number of *Anopheles* in 31 species and Joshi et al. (1964) added *Anopheles kochi*.

In the following year, Joshi et al. (1965) made a major contribution by reporting 59 species of *culicine* including 28 new country records.

Joshi, G., S.Pradhan and Darsie (1965) found out that the collection made by entomological staff during numerous *Anopheline* survey in connection with malaria and eradication campaigns since 1956 which resulted 3 more genera they are: *Culiseta, Malaya* and *Topomyia,* four more subgenera: three of the genus: *Aedes-Aedimorphus, Mucidus* and *Neomelaniconion*; and from the genus *Culiseta-Culiseta* and 28 more species distributed among 9 genera and 11 subgenera as new country record. The

collections have been made mostly in the southern plains (Terai), including some area in inner Terai to the elevation of 4,500ft.

Shrestha (1966) published an extensive review of the mosquito's fauna of Nepal. He recorded 97 species including 36 *Anophelines* and 61 *culicines*.

Over the years, various treatments of specific genera, subgenera or species groups, some of which mention Nepal, have been very useful in constructing the identification keys.

Thurman (1959), Delfinado (1966), Bram (1967), Reid (1968), Knight (1968), Tyson and Zavortink (1970). Mattingly (1971), Harrison and Scanlon (1975), Sirivanakarn (1976), Abercrombie (1977), Peyton (1977), Huang (1979), Harrison (1980),Reinert (1984), Harbach (1988) findings are very helpful in constructing the identification keys.

Rao et al. (1973), Bhat (1975), Das et al. (1987), Malhotra (1987) and Nagpal and Sharma(1987) did some more recent works in the findings of the mosquito fauna.

Rao (1981) includes the record of *Anopheles* of Nepal. No new additions to the fauna were recorded until 1989.

Certain species have been included in the Nepal mosquito fauna though the courtesy of N.Burgess, Department of Military Entomological, Royal Army Medical College, Milibank, London, Who collected mosquitoes in Nepal from1983to 1988.They are *Ae.khazani*, An. Indefinites, *An.nivipes*, *Ar. Annulityarsis*, *Ar. Aureolineatus*, *Ar. Dentatus.*

Pradhan (1988) reported 7 new country records which include: *Ae.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 recorded130 species and subspecies in 14 genera of mosquitoes known from Nepal.

Darsie & 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 & Brydon (1960) from Lamjung district in the North Central Nepal.

Darsie and pradhan (1994) reported that in Nepal, 42 species of *Anopheline* mosquitoes has been identified. Out of these seven has been identified incriminated as vector of primary importance. These are *A.minimus, A.fluviatilis, A.maculatus, A.dravidicus, A.pseudowillmori, A.willmori and A.anullaris.*

Darsie (1994) found out that since1987, 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.

Darsie (1994) reported 41new country records. There is now 171species known from the country. For the record of fauna of mosquitoes in the country, the author had spent one summer in each region.

IN THE GLOBAL CONTEXT:

James (1901-03) observed at Mian Mir (Lahore) that the population of larvae and adults of *Anopheles subpictus* diminished very rapidly in December and not a single adult was found until the beginning of July.

According to Senior White (1928), its optimum temperature was 32˚c.

Pruthi (1931) observed that at 32-34˚c its larvae could live, but could seldom metamorphose into adults, whereas at 28-30˚c, both pupation and emergence were facilitated.

Nuttall and Shipley (1902), Ross (1911) and Bacot (1915) pointed out on the other hand that the emergence of males and females were more or less equal.

Leicester (1908) reported *Aedes (Stegomyia) gardnerii var imitator* from north eastern India.

Leicester (1908) collected *Armigeres (Leicesteria) dolichocephalous* from Manipur.

Stephens and Christopher (1908), Gorden (1922), Young (1922) and Harold (1923) had noted that in summer the males of *Anopheles subpictus* emerged in excess.

Christopher (1911) draws attention to the fact that during winter, *Anopheline* larvae were scanty and occurred only in water rich vegetation.

Hodgson and King (1914) found that the optimum temperature of *Anopheles* lay between 20 and 26˚c.emergence were facilitated.

The findings of different workers with regard to the ratio of sexes among freshly emerged mosquitoes are variable. It was generally believed by various observers Lamborn (1922), Russell (1925), Bradley (1926), Boyd (1930), Mehta (1934), Sen. (1935) and Rozeboom (1936) that female mosquitoes invariably predominated.

Barraud (1922, 1928) reported *Aedes (finlaya) alboniveus* and *Aedes punctissimus* from north east India.

Borel (1928) also reported *Aedes (Stegomyia) pseudalbopictus* from north east India.

It is almost a truism that temperature is of outstanding importance in a study of insect ecology. According to Chapman (1925) extremes of temperature limit the activities of animal and incidentally determine their abundance during the annual cycle.

Senior White (1928) further showed that the optimum temperature for *Anopheles stephensi* was 24˚c.

Chowdhury (1931) concluded that the *Anopheles subpictus* was a truly hibernating species in the Punjab, probably only as adult, as its larvae could not be found during winter, the author detected its larvae and adults during winter in the Punjab.

Roy (1931) observed that at 24˚c temperature for *Anopheles subpictus* usually matured in 16-20 days. He also pointed out that its eggs hatched even after an exposure up to 5 days at 11˚c, but could not survive exposure beyond 6-8 days.

Barraud (1934) reported *Malaya (Maorigoeldia) jacobosoni Edwards* from Jalpaiguri and Darjeeling district of West Bengal

Herden, F.W and Poolson, B.J (1969) collected 45 species of mosquitoes both larvae and adult from Hancock country, Mississippi from May 1964 to December 1968.*Culex salinarius, Anopheles crucians, Aedes solicitans* and *Aedes vexans* seems to be the most numerous. CDC. miniature light trap containing CO2 provided the best means of collecting numerous species in a short time.

Richiardii. M.W. (1969) recorded mosquito population in southern England through sampling method, which was sampled from 1964 to 1966 with light trap and trap baited with animal. Both zoophilous and ornithophilous species were caught in cylindrical trap baited with rabbit, but very few individuals were caught when they were baited with chicken. Direct bait, catches, however showed that ornithophilous species were not attracted to rabbit outside trap. The most abundant catches were *Aedes detritus* and *Mansonia*.

Knight and Stone (1977) recorded only nine mosquito species of the genus *Armigeres*.

Ward (1984) reported *Culex infula* was encountered for the first time in North Eastern India at an altitude of 600-1200m of Manipur.

Scientific investigators are constantly looking for new mosquitoes, as well as reviewing previously identified specimens for new information or identifying characteristic. Recently such a review made a change in the name of many mosquitoes belonging to the genus *Aedes*.

Reinert. (1984) made a change in the sub genus of *Aedes chlorotatus* to the status of genus.

Nagpal and Sharma (1987) collected 1574 species of *Anopheles nivipes* from north eastern India. They also reported twenty seven species of *Aedes* from the north east.

Rajput and Singh (1988) recorded *Mimomyia (Etorieptiomyia) luzonensis* at an altitude of 600-1200m from Manipur.

Malhotra and Mahanta (1994) reported 130 sps of mosquitoes from north eastern India. These belong to 12 genera, Viz., *Anopheles* (37 sps), *Aedes* (27sps.), *Armigeres* (13sps.), *culex* (30sps.), *Coquillettidia* (2sps.), *Heizmannia* (2sps.), *Malaya* (2sps.), *Mansonia* (4sps.), *Mimoyia* (3sps.), *Toxorhynchites* (1sps.), *Tripteroides* (2sps.), *Uranotaenia* (7sps.).

Alten.B.et al. (1999) studied on the seasonal composition and population dynamic of mosquitoes in Belek region of turkey. In their study light trap was installed at four different site .In between May and December 4,542 specimen representing seven species of *Culex tritaeniorhynchus*, *Aedes caspius*, *Aedes cretius,* Aedes *vexans* and *Culiseta annulata.*

Jiri et al. (1999) study on difference of respond to the temperature and density of two strains of mosquito, *culex pipens* and *c.molestus* .they reared the strain for more than five years under the same laboratories condition to find to what extent the reaction of these strain to constant temperature.

J.Sutherland et al. (2000) found out many kinds of mosquito in State. They found sixty species in New Jersey each of which has different habitat, behavior and preferred source of blood. Among them about ten of these species are so numerous and such vicious biters of man and animals in the state.

Floore et al. (2002) stated that there are over 2500 different species of mosquitoes throughout the world about 200 species occur in the United States with 77 species occurring in Florida. A new species was reported from Florida.

Annonymous (2003) published an article on the diversity of mosquito, which was analyzed from data obtained from Jinan International air port in Shandong, China .The species richness, relative frequency vector capacity, inter-specific encounter and population intensity was analyzed. 2008 mosquitoes were collected, of which 1086 were from human habitation 362 from the surrounding of airport square and 570 from an airport hotel. *Culex pipiens palleus* was dominant especially in human habitations.

Caglar, S.S. (2004) found out that in southern Sudan, many wetlands have been constructed, which raised the problem of mosquitoes in human settlements. The diversity of mosquitoes in constructed wetlands was compared to natural wetlands. Mosquito abundance and species richness were higher in wetland.

Fischer and Schweigmann. (2004) conducted a study in Buenos Aires city of Argentina, 89 pools were sampled weekly for fort one year period .The aim was to investigate the seasonal dynamics of three *Culex sps*. Breeding in temporary rain pools and to analyze the relationships of the presence of these sps. to pool dimension, pool age, vegetation, and insolation degree. The three species showed differences in their seasonal patterns, *Culex dolosus* being present during the whole year, *Culex pipiens* mainly in the summer season and *Culex mami* almost exclusively during the fall.

The stastical analysis performed revealed significant positive relationships of all three mosquito species to increasing surface area, whereas no relationship to insolation degree was detected in the studied pools. *Culex pipiens* and *Culex dolosus* showed positive relationships to increasing vegetation cover, whereas the presence of *Culex dolosus* was also related to pool age.

Russell, C. Richard (2005) studied on the species diversity of mosquito on Florida The mosquitoes were collected with the help of CO2 – baited light trap on big pine, from 2000-2004. Twenty species of mosquitoes were collected during this study, the most commonly collected being *Anopheles atropos*, *Culex bahamensis Deinocerites cancer*, *Ochlerotatus taeniorhyncus*. Most of the mosquito species were collected during "high season" month (June to September) than in low season (January to march).

Cruz, Oswaldo (2006) published a new technique for the collection of *Aedes aegypti* mosquito. He found out the BG-Sentinel Trap (BGS-Trap), a new trap for capturing adult *Aedes aegypti*. The BGS-Trap has been recently developed by BioGents GmbH (Regensburg, Germany) and utilizes patent pending technology from the University of Regensburg. The trap consists of an easy to transport, collapsible white bucket with white gauze covering its opening. In the middle of the gauze cover, there is a black tube through which a down flow is created by 12V DC fan that causes any mosquito in the vicinity of the opening to be sucked into a catch bag. The catch bag is located before the suction fan, therefore avoiding damage to specimens passing through the fan. The BG-Sentinel Trap uses a blend of mosquito attractants consisting of lactic acid, ammonia, and caproic acid, substances which are also found on human skin. The blend is constantly emitted in a fixed ratio from a long lasting multi-component dispenser. The dispenser emits the attractants for up to five months. During the tests, the BGS-Traps were simply placed on the ground.

CHAPTER THREE

DESCRIPTION OF THE STUDY AREA

Bhaktapur District:

The study area is situated in the Bhaktapur district. According to the population status of Bhaktapur district, it is the smallest city of the Nepal. 64 % of people of the Bhaktapur depend upon agriculture. Bhaktapur district consists of16V.D.Cs The altitude of Bhaktapur is about 1,330 from the sea level. It is about 7.8 km eastward from the Kathmandu. (Joshi, 1999)

Nangkhel V.D.C.:

The selected study area is Nangkhel V.D.C. The Nangkhel V.D.C. of Bhaktapur is situated between 85˚ 25' 45s and 85˚ 26' 55s North latitude and between 27˚ 37' 45s and 27˚ 39' 58s East longitude. The V.D.C. is at the height of 1,325 to 2,036 from the sea level. (Joshi, 1999).The climate of this V.D.C. is characterized by typical monsoon climate with rainy summer and dry winter. Day temperature in summer frequently rises above 30˚c and falls below 20˚c at night and 18˚c to 0˚c or even less during winter season from December to February. Pre-monsoon season during March to May is mostly dry and warm. This period is characterized by hazy atmosphere with dirty winds. The local temperature condition depends greatly on the degree of exposure to the Sun. (Metrological department, Babarmahal).

About 930 houses of different constructions are present in the V.D.C. along with cow/buffalo/cattle sheds, mainly ponds, ditches, puddles; rice paddy fields, bamboo bushes are present around the human dwellings. The total population of this V.D.C. is about 5,213 among which 2,630 are male and 2,583 are female. (Subhani, et.al.2002)

CHAPTER FOUR

MATERIALS AND METHODS

4.1. **Materials:**

The relevant materials required in the study are listed as follows:
Table 1.

S.NO.	EQUIPMENTS	FUNCTIONS
1.	Hand aspirator, mosquito net	For the collection of mosquitoes.
2.	Killing bottles	For killing of mosquitoes.
3.	Torch lights	For searching of mosquitoes.
4.	Camel hair brush	For taking out of mosquitoes from the aspirator.
5.	Pointed forceps	For taking out of mosquitoes from the killing bottles.
6.	Vials/Papers	For the collection of killed mosquitoes.
7.	Labeling pen	For labeling the collection.
8.	Cotton	For soaking the ethyl acetate in killing bottles.
9.	Ethyl acetate	As killing agent in killing bottle.
10.	Naphthalene balls	As preservative.
11.	Binocular microscope	For the study of morphological characters/ for identification.
12.	Entomological pins, Ivory papers,colourless nail polish	For staging of mosquitoes

4.2. Method:

The following method was applied during the study period:

4.2.1. Sampling method:

Random sampling method was used for the collection of the sample. Twenty houses of V.D.C.was selected. The houses were sampled 1 to 20.Indoor collection was done in morning from 6 to8AM.15 to 30minutes collection was done in each house. Monthly four days of collection was done for the sample. The evening collection was done from 5.30 to7.30 PM.

4.2.2. Collection technique:

The collection method was done with the help of hand aspirator of size14 by 4cm. (Clark, GG. et al. 1994) inside self baited mosquito net of mesh size 0.05mm. The sample collection was done by spreading the mosquito net (185 by 90cm) and closing outlets as doors, windows. The appeared specimen was instantly sucked by aspirator. The collection was done in the first week of the consecutive month, The collected specimen was instantly transferred to the killing bottle.

4.2.3. Killing method:

Collected mosquitoes were killed by keeping them in the killing bottle where ethyl acetate as killing agent. After killing specimens were transferred into papers.

4.2.4. Staging method:

Triangular card pointed at one end was cut out of Ivory paper. A drop of colorless nail polish was placed at the apex of the triangular paper. Each collected species was fixed with the help of colorless nail polish. The tip of the card point was bent down at right angle so that the specimen is upright in position. The bent tip was attached the thorax or pleuron. (Service, MW. 1993)

The other end of Ivory paper was pricked with entomological pin and labeled with date and location, after that such specimen were fixed in thermo Cole kept in box. Care was taken in order to prevent them from damage of their legs, wing, abdomen, antennae maxillary palp and proboscis. (Service, MW. 1993)

4.2.5. Preservation:

In order to preserve the specimen from attack of ant and other insect, Naphthalene balls were kept inside the box by attaching with glue.

4.2.6. Identification:

Detailed morphological study was done in the laboratory of the Epidemiological department of Teku, Kathmandu. The collected specimen was identified by following the taxonomic keys published by Mattingly(1971), Barraud(1934), Huang(1977), Knight(1968), Reinert(1973), Tyson(1970), Thurman(1959),Bram(1967), Sirivanakarn (1976).

4.7. Statistical Analysis:

4.7.1. Species Diversity Index:

Species diversity was calculated by using the formula (Shannon and Wiener1949),

$(\because pi = ni/N)$

$H_{max} = \log K'$

$H' = - \Sigma pi(\log pi)$

$$E = \frac{H'}{H_{max}}$$

Where,

Pi = proportion of Individuals of i^{th} species to the no. of individuals of all the Species (ni/N)

H_{max} = Maximum possible diversity

H' = Shannon-Wiener diversity index

N = Total number of individuals of all species.

Ni= No. of individuals of species

K = No. of species.

e = Relative density/Evenness index.

4.7.2. Correlation Coefficient test:

Correlation coefficient (r) was used to determine the significance of mutual relationship between climatic factors(temperature, rainfall, relative humidity) and the number of mosquito species collected by using Karl Pearson product moment formula (Gupta 1990)

$$r = \frac{\Sigma xy - \frac{\Sigma x.\Sigma y}{n}}{\sqrt{\Sigma x^2 - \frac{\Sigma x^2}{(n)}.x.\Sigma y^2 - \frac{(\Sigma y)^2}{n}}}$$

Where,

X = dependent variable (no. of mosquitoes)

Y = independent variable (temp., r .h. etc.)

r = correlation coefficient

n = pair of observations

CHAPTER FIVE

KEY FOR INDENTIFICATION

5.1. KEY FOR IDENTIFICATION OF ADULT FEMALE MOSQUITOES OF NEPAL (Mattingly 1971):

1. Proboscis long strongly received posterior border of wing emarginated just beyond tip vein cu2 ……. ……..……...*Toxorhynchities splendens*

Proboscis not so long and only slightly curved, if at all posterior border of wing evenly rounded or only slightly emarginated …….2

2(1). Scutellum, evenly rounded with setae evenly distributed, maxillary palpi about as long as proboscis……...*Anopheles*

Scutellum trilobed, with setae in three distinct groups; maxillary palpi shorter than proboscis ……..... 3

3(2). Proboscis with flexible joint, tip swollen, with long setae …….....*Malaya genrostris*

Tips of proboscis only slightly swollen, if at all, with neither flexible joint nor long setae apically……...…….. 4

4(3). Scutum with longitudinal stripe of bread, flat scales usually white or silvery, prespiracular setae present…….....*Topomyia aureoventer*

Scutum with other pattern; prespiracular setae present or absent……....5

5(4). Cell R2 of wing always shorter than vein R2+3 anal vein ending apically before fork of veins cu, and cu2 ……..... *Uranotaenia*

Cell R2 at least as vein R2+3, Anal vein ending apically distal to the fork of veins Cu1 and Cu1 and CU2 ……...…… 6

6(5). Prespiracular area with setae sub costal vein with group of seta; basally on the ventral aspect……. *Culiseta niveitaeniata*

Prespriacualr area and ventral aspect of sub costal vein bare ……7

7(6). Mesopostnotum with setae Scutum covered with bright metallic decumbent scales……..... *Heizmania*

Mesopostnotum without setae Scutum with other types of scales……..…… 8

8(7) Fore and midtarsomere 1 distinctly longer than other 4 tarsomeres combined tarasomeres 4 or fore and mid legs, short about as long as wide

……... *Orthopodomyia anopheloides*

Fore and mid tarsomere 1 shorter than the other 4 tarsomeres combined, tarsomere 4 of fore and mid legs much longer than wide ……....….9

9(8).Postspiracular setae present…….....10

Postspiracular setae absent…….12

10(9).Dorsal surface of wing with scales broad; abdomen bluntly rounded apically

……...*Mansonia*

Dorsal surface of wing with scales narrow; abdomen more or less pointed apically

……11

11(10).Proboscis rather stout, laterally compressed and curved, occiput with broad decumbent scales……...*Armigeres*

Proboscis fairly slender, not compressed nor notably curved; occiput usually with at least some decumbent scales narrow……....*Aedes*

12(9) Alula bare or with flat decumbent scales ……...*Mimomyia*

Alula fringed with narrow scales…….....(13)

13(12) Pulvilli present; tarsal claws small…….....Culex

Pullvilli absent; tarsal claws prominent……...…*Coquillettidia crassipes*

5.2. KEY FOR IDENTIFICATION OF ADULT FEMALES OF GENUS AEDES (Barraud 1934, Huang 1977, Knight 1968, Reinert 1973 and Tyson1970):

1. Hind tarsi without pale-scaled bands…….....…2

At least some hind tarsi with basal and/or apical pale-scaled bands……...6

2(1).Proboscis almost entirely pale-scaled; hind tarsi with longitudinal stripes of pale scales…….……..pallidostriatus

Proboscis entirely dark-scaled or at most with pale scales ventrally; hind tarsi without pale stripe……...…..…3

3(2).Scutum with broad longitudinal bands of golden scales sub-laterally; lower mesanepimeral setae present ……...*lineatopennis*

Scutum with other pattern; lower mesanepimeral setae absent…….....4

4(3).Scutum with 2 pairs of distinct sub median spots of broad, white scales,1 pair on anterior promontory and other on scutal angle;mid and hind femora and tibiae speckled…….…...*punctifemoris*

Scutum without distinct white-scaled spots, with other pattern; mid and hind femora and tibiae not speckled, with other pattern of dark and pale scales……..…..5

5(4).Scutum with patch of silvery scales in anterior 0.66, sometimes divided by median black-scaled stripe; hind femur with apical 0.33 entirely dark-scaled ……..…….…....*albolateralis*

Scutum with dark scales mixed with golden scales dorsally, with patches of silvery broad, flat scales in front of wing root;hindfemur with apical ring of silvery scales……..……*dissimilis*

6(1) .Some hindtarsomeres with both basal and apical pale-scaled bands …….……7

Hindtarsomeres with basal pale-scaled bands only on at least some segments……...…9

7(6) .Abdominal terga without transverse basal pale-scaled bands ……*assamensis*

Abdominal terga II-VII with narrow to moderately broad, transverse basal pale bands……..…..8

8(7) .Scutum with golden scales varying from large anterior patch to longitudinal lines, background of dark brown scales; fore- and mid femora broadly pale in basal 0.5……...…*aurcostriatus var. greeni*

Scutum with white to creamy scales forming lyre shaped pattern; fore- and mid femora with narrow longitudinal lines of pale scales…. ……...…..*pseudotaeniatus*

9(6) .Proboscis with distinct pale-scaled band near middle……...……10

Proboscis entirely dark scaled or at most pale-scaled ventrally…..……..……15

10(9) .Scutum with distinct spots of pale scales on dark-scaled background; femora with preapical pale-scaled bands …….…..*vittatus*

Scutum with other scale pattern; femora without preapical pale bands …..…….……11

11(10) .Abdomen mostly covered with pale yellow scales; scutum with tufts of brown and white scales mixed… ……..……..…….*scatophagoides*

Abdomen dark-scaled with white to golden scales in various patterns; scutum without scale tufts ……...……...……12

12(11) .Scutum with narrow median and sub median longitudinal stripes of golden scales; hindtarsomeres 4, 5 all dark scaled ...……..……...*chrysolineatus*

Scutum ornamented with pattern of gray-white to silvery scales; at least hindtarsomere 4 with pale scales ……...……...…13

13(12) .Wings with spots of pale scales; all femora and fore- and midtibiae with many white scaled bands ……..…….…….....*poicilius*

Wings without pale-scaled spots; femora and fore- and midtibiae with at most sub-basal white-scaled band ….…….14

14(13) .Hind tibiae with pale-scaled band in basal 0.5 ……..……*thomsoni*

Hind tibiae mostly dark-scaled, without pale band …..……....*annulirostris*

15(9) .Hindtarsomeres with narrow basal pale-scaled bands on at least some segments; fore- and midlegs with claws toothed.……..…….……16

Hindtarsomeres with wide basal pale-scaled bands on at least some segments; fore- and midlegs with claws simple ……..…..17

16(15) .Abdominal terga usually with median pale scaled patches, not forming complete transverse bands; scutum with large pale-scaled patch anteriorly ……...……*gubernatoris*

Abdominal terga with complete pale-scaled transverse bands; scutum with pale scales at scutal angles ……...……*caecus*

17(15) .Dorsocentral setae present ….……...……...….18

Dorsocentral setae absent ……...…….……...……...19

18(17) .Scutum with patch of broad flat white scales over wing roots, without round silvery-scaled spots postero-laterally …..……...……..……*albopictus*

Scutum without patch of broad flat white scales over wing roots, with silvery scaled spots on scutal angle ……...……..*unilineatus*

19(17) .Scutum with anteromedian white-scaled patch wider than long, reaching to scutal fossae laterally; some white scales in antealar area broad, flat ……..…….gardnerii imitator

Scutum with anteromedian white-scaled patch longer than wide, not reaching scutal fossae; all white scales in antealar area narrow……..……..*w- albus*

5.3. KEY FOR IDENTIFICATION OF ADULT FEMALES OF GENUS ANOPHELES (Thurman 1959):

1. Wings with 3 or fewer dark spots on Costa, involving Costa and vein R or

Wings all dark scaled (subgenus – Anopheles)...….…….....2

Wings with 4 or more dark spots on Costa, involving Costa and vein R, wing never all dark-scaled (subgenus – Cellia) .……...…….....14

2(1) .Wings without definite pale-scaled markings …..…...…….*aitkenii bengalensis*

Wings with some pale-scaled markings ……3

3(2) .Palpi entirely dark-scaled ……....……..……...….4

Palpi with pale-scaled bands ……..……...……...…….7

4(3) .Hind femur with broad white-scaled band..……..……..……5

Hind femur without broad white band ……..…….……...6

5(4) .Hind femora with pale scales ventrally on basal 0.33; apical portion of at least 3 wing veins pale (veins R2 and anal and at least one another) ……*lindesayi lindesayi*

Hind femora not pale ventrally on basal 0.33, at most with narrow circular band at base; apical portion of only wing veins R2 and anal pale-scaled..... *Lindesayi nilgiricus*

6(4) .Abdominal sterna with scattered pale scales ……..….……..…*barbirostris*

Abdominal sterna without scattered pale scales ……...…….…*ahomi*

7(3) .Hind femora-tibial joint with prominent tuft of black and white scales ……....8

Hind femora-tibial joint without such a tuft... ……..……...9

8(7) .Sub costal pale spot absent on wing …..……...*annandalei*

Sub costal pale spot present …….…….....*interruptus*

9(7) .Basal 0.25 of Costa with pale spots interrupting black scales ……..…..……..…10

Basal 0.25 of Costa completely dark-scaled, or at most with scattered pale scales (hyrcanus Pallas group) …….…….....12

10(9) .Wing vein A with pale scales in apical 0.5; midfemur without large pale-scaled spot dorsally near apex ……...…….....*gigas gigas*

Wing vein A entirely dark-scaled; midfemur with large pale-scaled spot dorsally near apex ……...…….….11

11(10) .Pale spots in wing fringe opposite apices of R4+5,usually vein M1 and sometimes other veins, but variable, in addition to the usual large pale spot between veins Cu2 and A……..…….*gigas var. simlensis*

Wing fringe dark opposite vein R4+5 and with no other pale spots except the large one between veins Cu2 and A…….…….*gigas var. baileyi*

12(9) .Basal dark spot on wing vein Cu small, separated by its own length from the middle dark spot in anal vein; pale-scaled bands on hind tarsi narrow,tarsomere 4 without basal pale band ……..……...*sinensis*

Basal dark spot on vein Cu large, separated from middle dark spot on anal vein by less than its own length; pale-scaled bands on hind tarsi moderately to very broad, tarsomere 4 usually with basal pale band …..…….…13

13(12) .Pale band on apex of hindtarsomere 3 and base of 4 seldom longer than length of hindtarsomere 5; abdominal tergum VIII usually with narrow scales

…….……*nigerrimus*

Pale band on apex of hindtarsomere 3 and base of 4 longer than length of hindtarsomere 5; abdominal tergum VIII seldom with scales ...……*peditaeniatus*

14(1) .Femora and tibiae speckled with dark and pale scales ……..…….15

Femora and tibiae not speckled ……...24

15(14) .Some or all of hindtarsomeres 3-5 pale-scaled ……..…..16

Hindtarsomeres 3-5 entirely dark-scaled ……..…….…….22

16(15) .Hindtarsomere 5 with basal dark-scaled band; abdominal sterna with row of conspicuous black –scaled tufts; palpi with 4 distinct pale scaled bands, including apical band …….…...…..*kochi*

Hindtarsomere 5 completely pale-scaled; abdominal sterna without such tufts; palpi with 3 distinct pale bands, including apical band ……..……17

17(16) .Hindtarsomere 5 and part of 4 completely pale-scaled ……....……...18

Hindtarsomere 5, all of 4 and part of 3 completely pale-scaled ……..……...19

18(17) .Scales on dorsum of abdomen either quite few or, if more numerous, do not form marked patch of flat scales on segment II……*maculates maculatus*

Abundant scales on dorsum of abdominal segments III-VIII, and with a marked patch of flat scales on I……..……...*maculatus willmorei*

19(17) .Hindtarsomeres 5, all of 4 and part of 3 pale-scaled ….……*theobaldi*

Hindtarsomeres 3-5 completely pale-scaled ……...……20

20(19) .Palpi speckled, apical and sub apical pale-scaled bands equal in length ……*splendidus*

Palpi unspeckled, apical and sub apical pale-scaled bands unequal …...……...…….21

21(20) .Abdominal terga VII, VIII covered with golden scales; wing with basal 0.25 and apical 0.33 of Costa mostly pale-scaled ……...…….*jamesii*

Abdominal terga VII, VIII covered with dark scales only; wing with basal 0.25 and apical 0.33 chiefly dark-scaled ……..……*ramsayi*

22(15) .Palpi with 3 pale-scaled bands, usually speckled, the apical and sub apical pale bands equal ……..……..……*stephensi*

Palpi with 4 pale-scaled bands; apical and sub apical pale bands unequal …….....23

23(22) .Hind legs with tibiotarsal joint broadly and conspicuous, banded with white scales……..……*balabacensis*

Hind legs without such tibiotarsal band…….……*cesseliatus*

24(14) .Some or all of hindtarsomere 3-5 completely pale-scaled…….……...25

Hindtarsomeres 3-5 not pale-scaled…….29

25(24) .Only hindtarsomere 5 and part of 4 completely pale-scaled……..……..….26

Hindtarsomeres 3, 4, 5 completely pale-scaled ……..……..27

26(25) .Palpi with 3 pale-scaled bands……...…….*majidi*

Palpi with 4 pale-scaled bands……...……*karwari*

27(25) .Wing vein cu mainly dark-scaled, with dark spot at bifurcation of Cu1 and Cu2……..……..*annularis*

Vein Cu mainly white-scaled, with no dark spot at bifurcation of veins Cu1 and Cu2……28

28(27) .Apical part of hindtarsomere 1 dark-scaled; abdominal sterna with scattered broad white scales ; scales present on abdominal terga III-VIII ; scales also on mesokatepisternum ;wing scales paler, dark spot at apex of vein R4+5 with apical dark spot about as long as fringe scales……*pallidus*

Apical part of hindtarsomere 1 with some pale scales ; few or no pale scales on abdominal sterna, except on VI,VIII and occasionally V ; scales present on abdominal terga VI,VII and sometimes V; mesokatepisternum without scale patch ; wing scales darker, dark spot at apex of vein R4+5 about 2.0 length of fringe scales

……*philippinensis*

29(24) .Tarsomeres of forelegs with broad, pale-scaled bands ……..…….…….30

Tarsomeres of forelegs entirely dark-scaled or with very narrow pale bands ...……..31

30(29) .Palpi with preapical dark band sub equal to apical pale band ; presector dark spot on wing with part on vein Sc more than 0.5 length of that on Costa ……....*subpictus*

Palpi with preapical dark band not more than 0.5 length of apical pale band ; presector dark spot with part on vein Sc less than 0.5 length of that on Costa ……..…….*vagus*

31(29) .Palpomere 1 dark-scaled ……..……..…..*turkhudi*

Palpomere 1 pale-scaled ……...……32

32(31) .Wing vein R4+5 mainly dark-scaled …….*culicifacies*

Wing vein R4+5 mainly pale-scaled ……...……..33

33(32) .Palpi with apical pale-scaled band sub equal to sub apical pale band ……..…34

Palpi with apical pale-scaled band much longer than sub-apical pale band …….36

34(33) .Vein A of wing with 3 dark-scaled spots and pale fringe spot opposite apex; proboscis with apical 0.5 yellow-scaled ……....…….....*aconitus*

Vein A with only 2 dark-scaled spots and no pale fringe spot opposite apex; proboscis with at most ventral pale-scaled patch …..…….....35

35(34) .Wing with basal 0.25 of Costa without pale-scaled interruption ….....…*varuna*

Wing with basal 0.25 of Costa with at least presector pale spot on at least 1 wing……...……...*minimus*

36(33) .Wing with basal 0.25 of Costa entirely dark-scaled ….…..……..*fluviatilis*

Wing with basal 0.25 of Costa with presector and sometimes humeral pale spots ……..……..*jeyporiensis*

5.4. KEY FOR IDENTIFICATION OF THE ADULT FEMALES OF GENUS ARMIGERES (Thurman1959):

Sterna II-VI entirely pale-scaled……*kuchingensis*

Sterna III-VI dark-scaled, with sub apical bands of pale scales……..*subalbatus*

5.5. KEY FOR IDENTIFICATION OF THE ADULT FEMALES OF THE SUBGENERA OF THE GENUS CULEX (Bram, 1967):

1. Four or more strong lower mesanepimeral setae present; relatively large species……*lutzia*

Mesanepimeral setae absent, or if present, with only 1.2 weak setae ……....…..2

2(1) .Pleuron with distinct scale patches at least on the upper and lower meskatepisternum and on anterior mesanepimeron ……..……..…*culex*

Pleuron without distinct scale patches……3

3(2) .Acrostichal setae well developed…….....(in part) *Eumelanomyia*

Acrostichal setae not well developed except at extreme anterior promontory and rarely near prescutellar space …….…….…….4

4(3) .Lower mesanepimeral seta absent: decumbent scales on occiput narrow …….. (in part) *Eumelanomyia*

Lower mesanepimeral seta present; decumbent scales on occiput broad, if only on ocular line ……..…….……5

5(4) .Pleural area with broad dark integumental band extending from postpronotum to mesanepimeron ……..…….*Culiciomyia*

Pleural area concolorous, without broad dark integumental band……....*Lophoceraomyia*

5.5.1. KEY FOR IDENTIFICATION OF THE ADULT FEMALES OF GENUS CULEX, SUBGENUS CULEX (Sirivanakarn, 1976):

1. One or two lower mesanepimeral setae present; proboscis without distinct pale-scaled ring; tarsomeres without pale bands at joints ……2

Lower mesanepimeral setae absent; proboscis with distinct pale-scaled ring; tarsomeres with basal and apical pale bands……..……6

2(1) .Anterior surface of midfemur without median longitudinal pale-scaled stripe ……..3

Anterior surface of midfemur with median longitudinal pale-scaled stripe……5

3(2) .Abdominal terga without basal transverse, pale-scaled bands; pleuron with striking pattern of dark and pale integumental stripes ……..…….…...*fuscocephala*

Abdominal terga with basal transverse pale-scaled bands; pleuron without striking pattern of dark and pale integumental stripes …….……..……..4

4(3) .Pleural integument with distinct pattern of dark stripes across mesokatepisternum and mesanepimeron; scutal integument reddish brown ……..……...…….*hutchinsoni*

Pleural integument without dark stripes; scutal integument yellowish to pale brown ……...….*quinquefasciatus*

5(2) .Postspiracular area and base of prealar knob without scale patches ……*vegans*

Postspiracular area and base of prealar knob with distinct scale patches ……...….*theileri*

6(1) .Wing without pattern of pale-scaled spots or streaks.…….……..7

Wing with pattern of pale-scaled spots or streaks on at least 2 areas of Costa and 1 area on other veins ……...……...……..18

7(6) .Abdominal terga II-VII largely clothed with yellowish or golden scales ……..……..*epidesmus*

Abdominal terga with dark- and pale-scaled bands or entirely dark …..……...8

8(7) .Abdominal terga II-VI entirely dark-scaled, without pale bands and apicolateral pale patches …….....(in part) *whitei*

Abdominal terga II-VI with basal or basal and apical pale-scaled bands or with apicolateral pale patches ……...……..9

9(8) .Abdominal terga II-VI with apical and/or basal pale-scaled bands ……...……..10

Abdominal terga II-VI with basal pale-scaled bands only ……..……...…….11

10(9) .Wing with dark scales on all veins; abdominal terga II-VI with dark areas not sprinkled with pale scales …….*sinensis*

Wing with mixed pale and dark scales; abdominal terga II-VI with dark areas sprinkled with pale scales ……..…….....*bitaeniorhynchus*

11(9) .Erect scales in center of vertex of head whitish; anterior 0.7 of scutum densely covered with white scales ……..……..12

Erect scaled in center of vertex pale yellow, dingy white or all dark; anterior 0.7 of scutum covered with beige, yellow, golden or dark scales ……...……13

12(11) .Anterior surface of fore- and midfemora without speckling of pale scales ;white-scaled patch on scutum dense, extending to wing root, posterior to that all dark-scaled; wing veins R1, R4+5 and Cu with narrow scales ….……....*gelidus*

Anterior surface of fore- and midfemora extensively speckled with pale scales; pale-scaled patch on scutum thinner, grayish-white, extending posterior to wing root in 4 lines; wing veins R1, R4+5 and cu with broad scales ……....*whitmorei*

13(11) .Midfemur with longitudinal stripe of pale scales on anterior surface; postspiracular area with small patch of semi erect scales on lower anterior aspect ……....14

Midfemur entirely dark-scaled or speckling of pale scales not forming definite stripe; postspiracular area without scales on lower anterior aspect ……....15

14(13) .Longitudinal pale-scaled line on anterior surface of midfemur broken into small spots at middle; costal vein entirely dark-scaled ……*barraudi*

Longitudinal pale-scaled stripe on anterior surface of midfemur complete; pale-scales present on base of costal vein at least to humeral cross-vein ……...…..*edwardsi*

15(13) .Anterior surface of fore- and midfemora with speckling of several pale scales at least on apical dorsal surface……..…….....(in part) *whitei*

Anterior surface of fore- and midfemora entirely dark-scaled …...……...…….…..16

16(15) .Erect scales on vertex mostly dark; anterior surface of hind femur pale-scaled with narrow black-scaled ring apically; scutum covered with dark coppery gold scale……...…*tritaeniorhynchus*

Erect scales on vertex pale yellow in center, dark-scaled posterolaterally; hind femur marked otherwise; scutum with scales paler …...…….……17

17(16) .Speckling of pale scales usually present on femora and proboscis ; scutum with scales brown and pale mixed in varying degrees ; hind femur without dark-scaled apical band, usually with dark sub apical band extending basally to form stripe…….....*vishnui*

Femora and proboscis never speckled with pale scales; scutum with yellow to silvery scales; hind femur with dark band apically, contrasting with pale-scaled areas

……..…….*pseudovishnui*

18(6) .Second pale-scaled costal spot involves veins C, Sc, R, and sometimes Rs and Cu; basal pale bands of abdominal terga narrow, usually less than 0.25 length of segment……..……...……..*mimulus*

Second pale-scaled costal spot involves only veins C and Sc; basal pale bands of abdominal terga broad, at least 0.25 length of segment ……..…..19

19(18) .Scutal scales predominantly brownish; midtibia with longitudinal stripe of pale scales on anterior surface ……...……..*jacksoni*

Scutal scales predominantly pale; midtibia without longitudinal pale stripe on anterior surface ……..……*mimeticus*

5.5.2. KEY FOR IDENTIFICATION OF THE ADULT FEMALES OF GENUS CULEX, SUBGENUS CULICIOMYIA (Barraud, 1934 and Bram, 1967):

1. Integument of pleuron with prominent dark spot dorsally on mesanepimeron: light brown spot on integument dorsally on mesokatepisternum ...……..*nigropunctatus*

Integument of pleuron with brown stripe extending from postpronotum to mesanepimeron…….….22(1)

Narrow scales on vertex of head brown in color ; cell R2 of wing about 2.25 length of vein R2+3 ……..……..……*pallidothorax*

Narrow scales on vertex creamy in color; cell R2 about 3.0 length of vein R2+3 ……..…...…*viridiventer*

5.5.3. KEY FOR IDENTIFICATION OF THE GENUS CULEX, SUBGENUS EUMELANOMYIA (Sirivanakarn, 1972):

1. Acrostichal setae and lower mesanepimeral seta absent ……..……*brevipalpus*

Acrostichal setae and usually lower mesanepimeral seta present ……2

2(1) .Decumbent scales on anterior dorsal margin of vertex broad, white or gray, those in central part broad and dark in color ……...…….*malayi*

Decumbent scales on vertex narrow, fine, mostly pale yellow in color .....*foliatus*

5.5.4. KEY FOR IDENTIFICATION OF THE ADULT FEMALES OF GENUS CULEX, SUBGENUS LOPHOCERAOMYIA (Sirivanakarn, 1977):

Decumbent scales on dorsum of vertex mostly narrow, linear ; scales on veins R2 and R3 of wing narrow, linear……..……*minor*

Decumbent scales on dorsum of vertex mostly broad; scales on veins R2 and R3 usually broad, clavate …….….*infantulus*

5.5.5. KEY FOR IDENTIFICATION OF THE ADULT FEMALES OF GENUS CULEX, SUBGENUS LUTZIA (Bram, 1967):

Abdominal terga V-VIII entirely pale-scaled, or with very broad apical pale-scaled bands; terga II-IV entirely dark-scaled, or with very narrow apical pale bands; median pale band of proboscis broad, clearly encircling it …..……....*fuscanus*

Abdominal terga entirely dark-scaled, or with apical pale bands of about same width; median pale band of proboscis narrow, most prominent on ventral aspect …….....…..*halifaxii*

5.6. TAXONOMIC CHARACTERISTICS OF IDENTIFIED SPECIMENS:

5.6.1. Identifying Characteristics of family Culicidae:

1. Wings are characteristic by scales along the wing veins.
2. Long with piercing mouth part, proboscis extends far beyond clypeus.
3. The antennae of male are very plumose while those of the female have only a few short hairs.

5.6.2. Identifying characteristics of Genus *Armigeres*:

1. Dorsal surface of wing with scales narrow.
2. Proboscis is rather stout, laterally compressed and curved.
3. Occiuput with broad decumbent scales.
4. Abdomen more or less pointed apically.

Plate 1. *Armigeres sp.*

5.6.3. Identifying Characteristic of Genus *Culex:*

1. The thorax is white or silvery marking in *Culex*.
2. In *Culex* the tip of the female abdomen is generally blunt with thoracic retracted and thorax is usually dull colored.
3. The palpi less than one fifth as long as proboscis.
4. The wing scales are narrow.

Plate 2. *Culex sp.*

5.6.4. Identifying characteristic of Genus *Anopheles:*

1. Postnotum with out setae.
2. Scutellum not lobed.
3. Palps long in both the sex.
4. Wing usually spotted or molted.
5. Resting position usually not humped.
6. Proboscis nearly parallel with axis of body.

Plate 3. *Anopheles sp.*

5.6.5. Identifying characteristic of Genus *Aedes:*

1. Postnotum with out setae.
2. Scutellum usually trilobed.
3. Palpi short in female.
4. Wings rarely spotted.
5. Resting position hump backed.
6. Abdomen of female pointed with exerted cerci.

Plate 4. *Aedes sp.*

CHAPTER SIX

RESULT

6.1. Table of month wise collection of mosquitoes in the study area:

Table 2.

		Number of collection during different months					
S.no.	Name of genera	May	June	July	Aug	Sep	Total no. of collection
1.	*Anopheles sps.*	3	5	13	28	17	66
2.	*Culex sps.*	4	9	20	33	21	87
3.	*Armigeres sps.*	10	15	37	48	35	145
4.	*Aedes sps.*	0	2	7	9	1	19
-	-	-	-	-	-	-	317

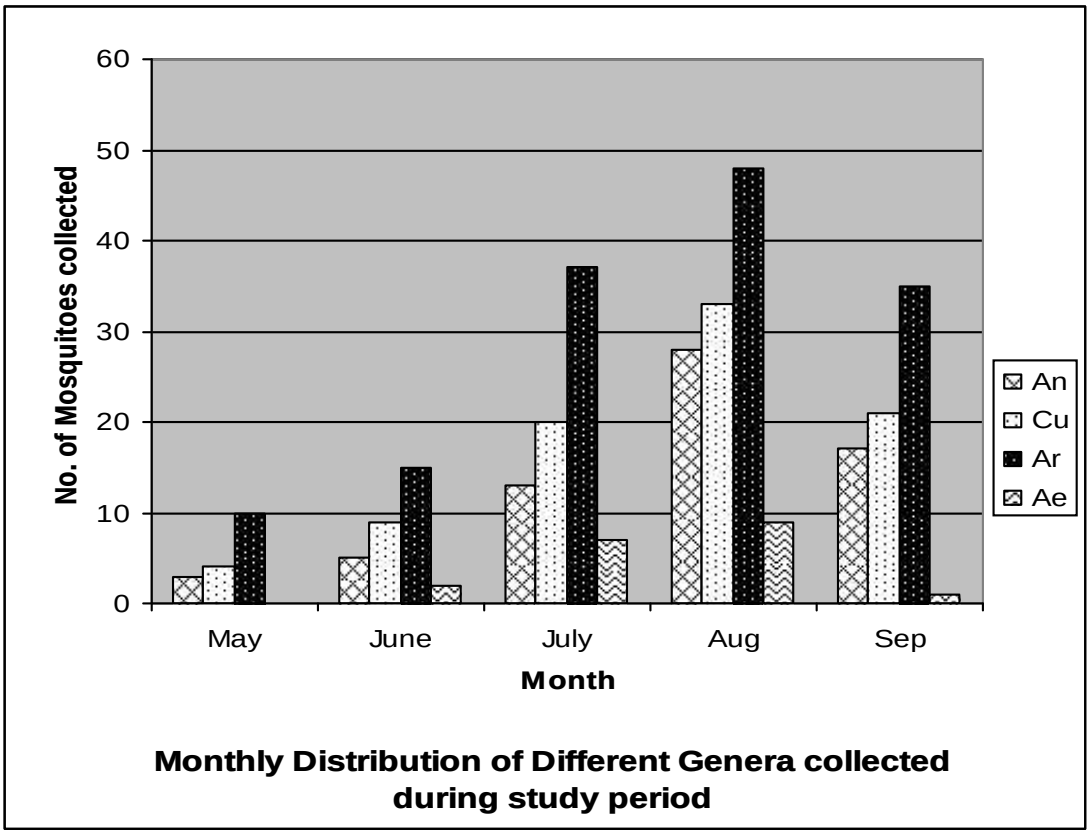


Fig.1.

(An-Anopheles, Cu-Culex, Ar-Armigeres, Ae-Aedes)

The month of July and August shows the highest collection of the mosquito of genera Armigeres due to presence of suitable breeding place with appropriate RH and Temperature. The population shows the sharp decrease in the month of September with decrease in temperature.

6.2. Abundance of mosquitoes in the study area:

The mosquitoes were collected from May to September in the study area. Five monthly observations were made during the study period; in which three hundred seventeen specimens were collected from each monthly sample of twenty. The collection was done with the help of aspirator and mosquito net. The mosquito net was spread and collection was done by closing the out lets. (Doors, windows) then by sucking through the aspirator.

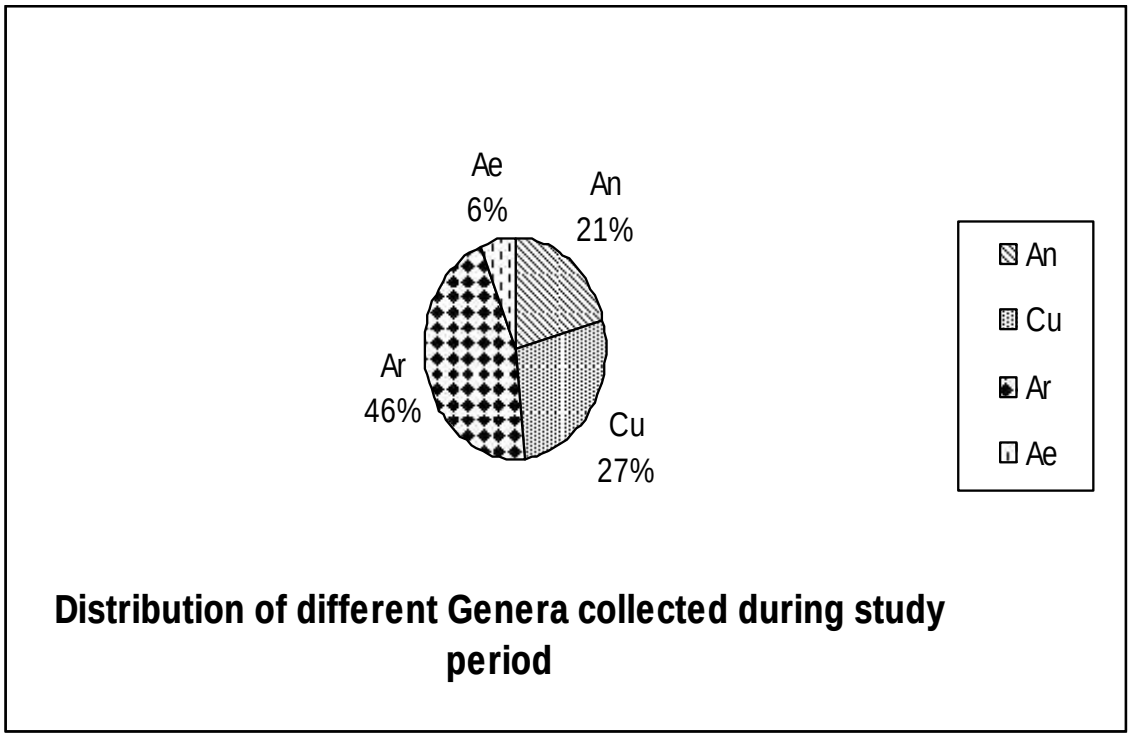
The collected specimens were found to be of four genera of mosquitoes namely: *Anopheles, Aedes, Armigeres* and *Culex*.

Among the four identified genera, the most abundant genera in the study area were found to be *Armigeres* with total number of one hundred forty five. Similarly the Genus *Culex* was found to be in the second position with total number of eighty seven. The total count of *Anopheles* during the study period was found to be sixty six. The genus *Aedes* was the least abundant among the other three genera with total count of only nineteen.

Total number and percentage of different genera collected during five month period in the study area:

Table 3.

S.No.	Genus	Number of collection	Percentage
1.	*Anopheles sps.*	66	21%
2.	*Culex sps.*	87	27%
3.	*Armigeres sps.*	145	46%
4.	*Aedes sps.*	19	6%



Distribution of different Genera collected during study period

(An-*Anopheles*, Cu-*Culex*, Ar-*Armigeres*, Ae-*Aedes*)

The distribution of the genera in the study period shows that *Armigeres* population found to be the most abundant in the study area with maximum percentage of 46%.

The least abundant genera is found to be *Aedes*; with minimum percentage of 6%.The other two genera were collected with percentage of 27% *culex* and 21% of *Anopheles*.

6.3. Diversity of mosquitoes:

To calculate the relative abundance of individual species, the diversity index method was applied. Diversity indices were based upon the relationship between the total no. of individual or species. A major of species diversity was useful when investigating the interaction of physical and biotic factor in an ecosystem. The commonly used diversity index is Shannon's index (H').

Table 4.

Number of collection during different months									
S.no.	Name of genera	May	June	July	Aug	Sep	Total no. of collection(ni)	pi = ni/N	H' =-∑pi log pi
1.	*Anopheles*	3	5	13	28	17	66	0.208	0.141
2.	*Culex*	4	9	20	33	21	87	0.274	0.154
3.	*Armigeres*	10	15	37	48	35	145	0.457	0.155
4.	*Aedes*	0	2	7	9	1	19	0.059	0.072
-	-	-	-	-	-	-	N = 317	-	-

From the calculation of diversity index it was found that the genus Armigeres was the most diverse genera among the collected genera, i.e. H' = 0.155. Similarly, Anopheles (H' = 0.141), Culex (H' = 0.154) and the most least diverse genera was Aedes (H' = 0.072).

6.4. Correlation between the mosquito and the climatic parameters (temperature, rainfall and relative humidity):

The total count of the mosquitoes collected in different month is correlated with different environmental parameters viz. temperature, Relative humidity and Rainfall. The relation was found significantly positive.

6.4.1. Table of Correlation Coefficient:

For Temperature (˚c):
Table 5.

X	Y	∑X″	(∑X)″	∑Y″	(∑Y)″	∑XY	∑X∑Y	r value
17	22.1							
31	25.8							
77	24.8							
118	24.8							
74	24.7							
∑X=317	∑Y=122.2	26579	100489	2994.22	14932.84	7839.3	38737.4	0.4123

The value of r for temperature is calculated as 0.412308.The population density of mosquito has positive relation with temperature. The value of correlation coefficient(r) is found to be positive; the positive value shows the significant result. The value also shows that the mosquito population reaches its peak with raise in temperature. The population density reaches its peak in the month of August.

For Rainfall (mm):
Table 6.

X	Y	∑X″	(∑X)″	∑Y″	(∑Y)″	∑XY	∑X∑Y	r value
17	195							
31	308.1							
77	572.2							
118	546.9							
74	235.9							
∑X=317	∑Y=1858.0	26579	100489	814917	3450677.8	138907.8	588859.2	0.7432

The value of r for rainfall = 0.7432, this positive value indicates the positive relation between the population density of the mosquito and average rainfall.

The correlation between rainfall and population density is positive, so it shows a significant relation.

For RH (%):
Table 7.

X	Y	∑X″	(∑X)″	∑Y″	(∑Y)″	∑XY	∑X∑Y	r value
17	94							
31	103.1							
77	106.3							
118	105.9							
74	102.8							
∑X=317	∑Y=512.1	26579	100489	52548	262246.41	33082.6	162335.7	0.7696

Similarly, relative humidity (%) with appropriate temperature. of 24.8˚c. is the most suitable condition for the breeding of adult in the study area. The value of r = 0.7696 for RH (%) is also significant.

CHAPTER SEVEN

DISCUSSION

During the five month study period in the Nangkhel V.D.C. of the Bhaktapur district 317 specimens of mosquitoes were collected. Four genera were determined among the 14 genera of the mosquitoes as recorded by Darsie and Pradhan (1990) in the context of Nepal. The four genera which were recorded during study period were: *Culex, Armigeres, Anopheles* and *Aedes.* Most of the specimens of the recorded genera were found to be abundant during the month of the August. This month supports the peak in the population density of the mosquito due to high availability of breeding places with suitable temperature and relative humidity.

The most abundant collected genera during the study period were found to be *Armigeres* with total number of one hundred forty five. This indicates that the study area has suitable habitat and climatic condition for the genus *Armigeres*.The habitat of this genus is mainly bamboo bushes, flowers, polluted waters, and artificial containers as stated by Barraud (1934). This genus is a persistence daytime biter. It is collected during dusk. The collection of this genus was high in those human dwellings which were near by bamboo bushes. Most of the human dwellings in the study area were in the vicinity of bamboo bushes and polluted waters. So, the density of this genus was found high due to presence of suitable habitat. Such human dwellings were in the risk of annoyance of this genus.

The second abundant collected genus was *Culex* with total number of eighty seven in the study area during study period because of presence of suitable habitat. The genus is a rural genus, commonly encountered in rice field, shallow marshes, pools, ponds, and ditches containing fresh or polluted water as stated by Sirivanakarn (1976). This genus was mostly collected in the dwellings which were in the vicinity of paddy fields. According to Sirivanakarn (1976) the adult of this genus mostly preferred cattle sheds and piggeries; the adults were abundantly collected in the month of August and September, when paddy plant reaches ½ m.

The human dwellings in the study area were in close vicinity of rice paddies. In the mixed dwellings also the density of the genus *Culex* was found high.

The genus *Aedes* was found to be the least abundant genera with total number of nineteen from the study area due to absence of suitable habitat. According to Darsie and Pradhan, this genus prefers dense forest and rock holes but the study area was not in the vicinity of dense forest. The adult female of this genus was capable of mass migration so due to this reason this genus was probably collected in the study area, as there was no suitable habitat for this genus. The collection of this genus was mainly from the human dwellings at dusk.

Similarly genus *Anopheles* was the second least abundant collected genera with total number of sixty six. This genus is a twilight feeder and mainly prefers hilly areas and places where trees have been recently cleared as stated by Shrestha (1966) but the collection of this genus was mainly from the dwellings in the vicinity of rice paddies. The collection of this genus is low probably due to unsuitable optimum temperature and the relative humidity for the completion of their lifespan. According to Shrestha (1966) this genus requires a maximum of 60˚ F for hatching its egg.

Among the four collected genera, the genus Armigeres was found in the whole study period starting from 1st week of May to 1st week of September, i.e. in five months of study period. The collected four genera : *Culex, Armigeres, Anopheles* and *Aedes* have positive results with environmental parameters like: temperature, rainfall and relative humidity which shows significant.

So, the most diverse fauna in the study area during the study period was found to be *Armigeres* due to presence of suitable habitat and climatic condition. Similarly, the genus *Culex* stood in the second position and the least diverse fauna were found to be *Anopheles* and *Aedes.*

The number of the mosquitoes increased continuously with the increase in the monthly temperature. The most suitable temperature for the mosquito was found to be in the month of the August, where the population of the mosquito reached in its peak. In this month the flooding due to rainfall stop in the breeding places, ditches, ponds, pools and paddy field were filled with stagnated water.

The mosquito population also found to be increased with the increase of the relative humidity (105.9%). In the first week of June, where the heavy rainfall appeared, resulted the lower number of the mosquito collection. This is due to because of the flooding of the breeding places.

CHAPTER EIGHT

CONCLUSION

8.Conclusion:

The study on the Diversity of the mosquito in the Nangkhel V.D.C of the Bhaktapur found out about three hundred seventeen specimens during the collection started from May to September. The most diverse fauna of the mosquito during the study period was the genus *Armigeres* (H = 0.155), the population of this genus was high due to suitable habitat in the study area, this genus prefers bamboo bushes. Similarly, the second diverse fauna was the genus *Culex* (H = 0.154), the habitat of this genus was paddy fields and the cattle sheds. The least diverse fauna was the genus *Anopheles* (H = 0.141) and *Aedes* (H = 0.072). The total population of the mosquitoes collected in different month was correlated with different environmental parameters viz. temperature, Relative humidity and Rainfall. The correlation value for temperature (r = 0.412380), for rainfall (r = 0.7432) and for relative humidity (r = 0.76963). This positive value showed the significant relation. Hence, the population density of the mosquito was positively correlated with temperature, rainfall and relative humidity. The population of the mosquito was high in the month of the August which was due to presence of high availability of breeding places. The population of mosquito was low in the month of May, this was due to because of absence of breeding places. This concludes that the month of the August has suitable temperature for the survival of the mosquitoes and the density also depends on their habitat.

CHAPTER NINE

RECOMMENDATIONS

9. Recommendations:

1. The study on the biology and the habitat distribution of the mosquito is highly recommended for the eradication of the several mosquito-borne diseases like: Malaria, Encephalitis, Filariasis, Dengue. etc.
2. Detail survey and taxonomic identification on the mosquito is recommended, as the mosquito being a vector of various human and animal diseases.
3. To understand the epidemiology of the mosquito-borne disease and their control, survey in the distribution of the mosquitoes in an area is recommended.
4. Awareness programme among the villagers about the mosquito-borne diseases should be launched.
5. Priority should be given by government for the research of vectors.

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Annex:

Number of mosquitoes collected during the study period:

Name of mosquito	*Anopheles sps.*	*Culex sps.*	*Armigeres sps.*	*Aedes sps.*
Date	-	-	-	-
2nd May	0	0	2	0
3rd May	1	0	3	0
4th May	2	3	4	0
5th May	0	1	1	0
2nd June	0	1	2	0
3rd June	2	2	5	0
4th June	3	3	7	2
5th June	0	3	1	0
2nd July	2	5	7	1
3rd July	5	3	11	3
4th July	3	7	10	2
5th July	3	5	9	1
2nd August	7	9	12	1
3rd August	6	7	9	3
4th August	9	11	17	5
5th August	6	6	10	0
2nd September	3	4	7	0
3rd September	5	7	5	0
4th September	8	8	15	1
5th September	1	2	8	0

Total number and percentage of different genera during five months study period:

S.No.	Name of mosquito	Number of collection	Percentage
1.	*Anopheles sps.*	66	21%
2.	*Culex sps.*	87	27%
3.	*Armigeres sps.*	145	46%
4.	*Aedes sps.*	19	6%

Rainfall in (mm) of the study area in different year:

Year Month	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
May	72.8	239.1	109.3	225.2	183.0	285.4	109.7	189.3	104.2	232.8
June	341.6	261.8	331.5	314.8	390.1	299.3	332.7	150.6	164.7	213.6
July	709.0	406.7	610.7	568.5	483.0	612.1	566.3	621.8	364.2	340.9
August	530.2	541.5	528.7	506.0	427.2	631.5	511.5	366.3	657.7	222.4
September	146.5	201.0	279.9	240.3	272.0	248.3	445.4	290.1	259.2	306.6

(Source: Metrological department, Babarmahal, Kathmandu, Nepal)

Relative humidity in (%) of the study area in different year:

Month Year	May	June	July	August	September
1997	83.8	91.8	98.1	96.6	93.8
1998	80.6	87.7	99.5	98.4	95
1999	87.1	95.9	96.8	94.8	92.7
2000	87.5	96	97.1	97	92.5
2001	88.5	94.6	95.1	96	93.4
2002	80.5	94	91	94	90.1
2003	75	93.5	93.9	93.5	92.1
2004	85.3	98.1	93.8	98.1	93.8
2005	82.3	94.5	95.2	94.5	88.3
2006	89.4	93.6	91.4	93.6	91.5

(Source: Metrological department, Babarmahal, Kathmandu, Nepal)

Temperature in (˚C) of study Area in different year:

Month Year	May	June	July	August	September
1997	23.2	24.0	22.1	21.0	22.3
1998	23.4	25.3	22.3	21.2	22.5
1999	23.1	23.1	23.2	22.0	23.6
2000	24.1	24.2	23.3	22.9	22.4
2001	23	23.4	23.1	23.2	22.4
2002	23	22.1	23.2	21.5	20.0
2003	23.3	23	22.7	22.2	23.1
2004	22.8	22.1	21.1	23.7	21.4
2005	22	22.6	22.1	21.9	22.6
2006	21.4	21.9	22.2	22.0	20.6

(Source: Metrological department, Babarmahal, Kathmandu, Nepal)

Average of three different parameter of the study Area:

Month Parameter	may	June	July	August	September
	22.3	25.8	24.5	24.8	24.5
	194.5	308.1	572.2	546.9	235.95
	94	103.1	106.3	105.9	102.8

Country records of Malaria in Nepal:

Year	Cases
1990	22,856
1991	29,135
1992	23,234
1993	16,380
1994	9,884
1995	9,718
1996	9,020
1997	8,957
1998	8,498
1999	8,959
2000	7,980
2001	6,420
2002	12,786
2003	9,508
2004	4,637
2005	7,068
2006	5,422

(Annual report 2002, 2005 and 2006 March, Epidemiology and disease control Division, His Majesty's Govnt. Ministry of health Services.)

Malaria cases are not found in the Bhaktapur and Kathmandu districts till today.

Country records of Japanese Encephalitis in Nepal:

Year	Cases
1990	227
1991	665
1992	650
1993	702
1994	1836
1995	1240
1996	1450
1997	2953
1998	1161
1999	2924
2000	1721
2001	1908
2002	842
2003	931
2004	1543
2005	2824
2006	1500

(Annual report 2002, 2005 and 2006 March, Epidemiology and disease control Division, His Majesty's Govnt. Ministry of health Services, Teku, Kathmandu, Nepal)

Record of Japanese Encephalitis in Bhaktapur district:

Year	Cases
1994	2
2000	2

(Record from Bhaktapur hospital, Bhaktapur)

549 and 542 cases were found in the country in 2005 and 2006. (Annual report 2005, 2006, Epidemiology and disease control division, Teku, Kathmandu, Nepal)