CHAPTER 5. DISCUSSION

Worldwide urbanization, industrialization and deforestation are growing phenomenon. It shows adverse affect on climate change (Duane J. Gubler (1998 a; Paul Reiter-2001, Paul Epistan 1998; Norman Gratz - 1999). Globally in the last three decades number of mosquito born diseases reemerged and resulted number of deaths (WHO, Paul Reiter- 2001). In this phenomenon malaria is leading mosquito born disease in tropical and subtropical countries. It is also a burning problem of developing countries, only malaria kills a child every 40 second globally. According to WHO 300-500 million people in Africa and 1.1 to 2.7 million people in South Sahara are killed either with malaria or in contribution with other disease (Vinod Joshi et. al. 2006). Especially in India at various place like Kolkata (Mahir Pramanik et. al 2006), North East region (Nagpal and Sharma 1987 and 1995), Bikaner, Rajasthan (D. K. Kochar et. al (2007); Orissa (S. S. Sahu et. al. 2008) but now it is widely spread in all the states of India. Simultaneously Chikungunya reported from different states since 2005, it is not reported death but loss the much more amount for recover as well as lifelong pains for patient. Researchers said resurgence of chikungunya is due to climate change (Paul Episton1998; Duane J. Gubler 1998; Paul Reiter – 2001; Shinde et. al. 2011). In 2008 and 2009 recorded 4500 cases with 935 and 754 deaths respectively (www.pdfmachine.com and www.nvbdcp.gov.in), same time Maharashtra contributes 164 and 106 deaths (www.nvbdcp.gov.in). All over India
female *Anopheles* is main malaria vector, *Aedes* for dengue, chikungunya and *Culex* for elephantiasis keeping these in view the present entomological survey is conducted during 2009 to 2012 at Jalna district (M. S.) regarding environmental factors. Marathwada region has poor health, transport and irrigation facilities. The study area is also part of Marathwada region. The scenario of Jalna shows that there is no proper development of city, low sanitation facilities, poor road constructions and sewage mismanagement; it provides abundant breeding sites for mosquito. Particularly in post monsoon season, there is more chance to survive abundantly of *Anopheles* and *Aedes* in Jalna because of abundant breeding sites and favorable climatic condition and in summer season more activeness of *Aedes* due to suitable climatic condition viz. temperature and optimum humidity. This survey and monitoring work was carried out in eight localities. These spots were chosen from the view of residential, educational and public places, there more chances of the transmission of the mosquito born diseases.

During this study period from June 2009 to May 2012, a total 5640 mosquitoes were collected from different localities of Jalna district. There are four genera and six species viz. *Anopheles subpictus*, *Culex quinquefasciatus*, *Culex tritaeniorhynchus*, *Aedes aegypti*, *Aedes albopictus* and *Armigeres subalbatus*.

The results obtain during study period at different localities of Jalna district such as, the most prevalent species in the genus *Armigeres*, *Armigeres subalbatus* shows n=1438 (25.50%), followed by *Aedes aegypti* n= 1425 (25.27%) and moderate density observed in *Culex quinquefasciatus* n=1163 (20.62%) and *Anopheles subpictus* n=1129 (20.08%) while minimum density observe in *Aedes albopictus* n=404 (7.16%) and *Culex tritaeniorhynchus* shows lowest density of n=81 (1.44%). The distribution and density of species varies from place to place and seasons to season. Overall results obtained due to correlation analysis at all the eight places indicated that temperature, rainfall and humidity play an important role in the population of the organisms. Increased in temperature decreases the population, while increased rainfall and humidity increases the population. However, the relationship varies from species to species and location wise.

The Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) has highlighted that by 2100 the global temperature would increase
by 1.8°C – 4°C. The Fourth Assessment Report of IPCC (2007) has clearly highlighted the possible increase in vector-borne diseases spatially and temporally. Global warming, in turn, is increasing the occurrence of extreme weather events; this combination of weather instability and warming is making an impact on infectious diseases, namely their vectors and hosts (Epstein, 2001). The factors responsible for the emergence/resurgence of vector-borne diseases are complex. They include insecticide and drug resistance, changes in public health policy, emphasis on emergency response, de-emphasis of prevention programs, demographic and societal changes, and genetic changes in pathogens Lederberg J, Shope RE, Oaks SC (1992).

Climate provides favorable condition for mosquito distribution and density. It is main cause of spreading and outbreak of infectious disease like malaria, Chikungunya and dengue caused by some female species of mosquitoes. Malaria is reemerging as the no. 1 infectious killer and it is the number one priority tropical disease (WHO-2002). The scenario of Maharashtra as well as India is not differing from the said; in 2008 – 09 India contribute 1282 death. Epidemiological scenario of malaria differs from state to state and region to region in India (D. K. Kochar et. al. 2007; Battacharya S.et. al. 2006; D. Ragunath and Durga Rao 2008; McMichael 2007; Mahir Pramanik et. al. 2006 and Anurag Bhargava et. al. 2007).

A steady rise in annual temperatures has been associated with expanding malaria transmission in the Usamabara Mountains in Tanzania (Matola et al. 1987), and highland malaria has been reported in Kenya (Some 1994). Same phenomenon has seen in the study area i.e. minimum atmospheric temperature of Jalna increases 3°C (RML services; KVK Jalna) in this decay and play an important role to transmitted mosquito borne disease as well as their outbreak. It is proved in 2005 with outbreak of Chikungunia and in 2008 with large outbreak of dengue in rural area with 10 deaths (L. V. Shinde 2011). In December 2009 and January 2010 the outbreak of Chikungunya are also reported from Savargaon; Partur talluka and Ghansangavi talluka of Jalna District, during the peak hour of illness near about 80 per cent population were suffered with Chikungunya.

The results obtain during study period at Jalna maximum surveillance observe in species *Aedes aegypti* and moderate density of *Armigeres subalbatus*, while other species such as *Anopheles subpictus* and *Culex quinquefasciatus* shows minimum
Discussion

density. Other two species *Culex tritaeniorhynchus* and *Aedes albopictus* do not observe at Jalna during study period. During June to October climatic condition is favorable for mosquito breeding and surveillance. The average minimum temperature is 11.6°C and maximum temperature is 42.6°C, average monthly rainfall is moderate 1.43 to 7.93 mm while the average humidity is optimum 65.63 to 73.20% during this months.

The maximum surveillance of mosquito species found in September and October month. During these two months the average monthly temperature is 22.3°C to 31.5°C, rainfall is 3.20mm to 6.20mm and average humidity is 68.93 to 82.30%.

During study period the results of statistical analysis done by one way ANOVA, shows that there is statistically highly significant variation in the population among species at 1% level; however the seasonal variation is also significant at 1% level. The correlation coefficient between climate variable and monthly population of mosquito shows that, the overall results indicate that there is a possibility of increasing the population of *Aedes aegypti* and other species with increase in rainfall and humidity while the population decreases with increase in temperature at Jalna collection site.

The result of Badnapur is quite different to all the talukas (places) of Jalna district. It shows that surveillance of only two species *Aedes aegypti* and *Armigeres subalbatus* during study period. During study period maximum surveillance of *Aedes aegypti* were observed. *Armigeres subalbatus* were as moderate while other species do not found at Badnapur.

Badnapur is also shows same duration for mosquito breeding and surveillance. The average minimum temperature is 13.3°C and maximum temperature is 40.2°C, average monthly rainfall is moderate 1.48mm to 10.24mm while the average humidity is optimum 46.60 to 81.20% during this months.

The maximum surveillance of mosquito species found in August and October month. During these two months average monthly minimum temperature is 22.2°C to maximum 32.1°C, rainfall is 2.10mm to 7.35mm and average humidity is 72.02 to 81.20%.

During study period from June 2009 - May 2012, the results of statistical analysis by one way ANOVA shows that there is statistically highly significant
variation in the population among species at 1% level; however the seasonal variation is non-significant at Badnapur. The correlation coefficient between climate variable and monthly population of mosquito shows that, all the variables shows positive correlation with rainfall and humidity while negative correlation with maximum temperature during three year collection period at Badnapur. *Aedes aegypti* shows significant positive correlation with rainfall and humidity in year 2010 to 2012 while it shows significant negative correlation with all parameters in year 2009-2010.

Ambad taluka shows maximum surveillance of *Anopheles subpictus* and *Aedes albopictus* while moderate density observes in *Culex quinquefasciatus* during study period. Minimum surveillance found in *Armigeres subalbatus* and lowest density shows *Aedes aegypti*. Species *Culex tritaeniorhynchus* do not reported at Ambad collection site.

Meteorological data shows that environmental factors are favorable for mosquito breeding and surveillance during June to November during three year at Ambad. The average minimum temperature is 11.8°C and maximum temperature is 42.6°C, average monthly rainfall is moderate 1.20mm to 8.80mm while the average humidity is optimum 11.20 to 84.33% during study period.

The highest density of mosquito species found in August to October month. During these three months the average monthly minimum temperature is 16.8°C to maximum 31.4°C, rainfall is 1.36mm to 8.16mm and average humidity is 56.33% to 80.70%.

Statistical analysis by ANOVA results concludes that the variation in the population of various species is highly significant but seasonal variation is statistically non-significant for three year study. The species *Culex quinquefasciatus*, *Aedes aegypti* and *Aedes albopictus* shows significant positive correlation with minimum temperature and optimum humidity while significant negative correlation with maximum temperature during all the three year study period. So Ambad collection site shows that population increases when increase optimum humidity and rainfall while the population decreases increasing maximum temperature.

Ghansawangi taluka (place) shows highest density of *Anopheles subpictus* and *Culex quinquefasciatus*, moderate density observe in *Aedes albopictus*. Minimum
density shows by *Armigeres subalbatus* while lowest density found in *Aedes aegypti*. *Culex tritaeniorhynchus* do not show surveillance in Ghansawangi during study period.

The climatic conditions of Ghansawangi show July to November is favorable for mosquito breeding and surveillance. During this month’s recorded minimum temperature is 16.70°C and maximum temperature is 36.40°C. Rainfall shows that range between 2.50 mm to 8.80 mm and average monthly humidity is 60.00% to 83.30%.

Maximum surveillance of mosquitoes found in September and October month. The climate record of these two months is shows average minimum temperature is 20.40°C and maximum temperature 34.50°C while optimum humidity 68.30% to 75.20%.

Statistical analysis of results by ANOVA, shows that there is highly significant variation in the population among species at 1% level; however seasonal variation is also significant at 5% level during study period at Ghansawangi. The correlation coefficient between climatic factors and monthly population of *Aedes aegypti* and *Aedes albopictus* as well as other mosquito species shows that similar trends to other locations such as increase the population when increase the rainfall and humidity while decrease the population when increase the maximum temperature during all three year study period. The climatic conditions of Ghansawangi are most favorable for mosquito breeding and surveillance as compare to other locations.

During study period Partur location shows that maximum density of *Culex quinquefasciatus* and *Aedes aegypti* while moderate density of *Armigeres subalbatus*. Minimum density observed in *Anopheles subpictus*. But *Culex tritaeniorhynchus* and *Aedes albopictus* did not recognize during three year at Partur.

During July to October climatic conditions is more favorable for mosquito breeding and surveillance at Partur. During this period the average minimum temperature is 18.6°C and maximum 33.1°C, rainfall 1.30 mm to 9.20 mm while humidity is 62.30% to 80.00%. The maximum surveillance of mosquito species observes in month of August and September during whole study period at Partur. During these two months temperature is in between 20.2°C to 33.1°C, rainfall 1.30 mm to 6.33 mm and humidity 62.30% to 73.60%.
Statistical analysis by ANOVA shows that, the variation in the population of different species highly significant during study period however seasonal variation is also significant except the year 2009-2010 at Partur. The correlation coefficient between climate change and monthly population of mosquito species indicate that *Culex quinquefasciatus, Aedes aegypti* and *Armigeres subalbatus* shows significant positive correlation with increase rainfall and humidity while negative correlation with increase the temperature. Unfortunately *Anopheles subpictus* did not show any correlation with climatic factors during study period at Partur. Overall a climatic condition of Partur locality is favorable for mosquito breeding and surveillance.

During study period Mantha shows maximum density of *Aedes aegypti* and moderate density recorded by *Culex quinquefasciatus* and *Armigeres subalbatus*. Lowest density found of *Anopheles subpictus*. Other two species *Culex tritaeniorhynchus* and *Aedes albopictus* did found out at Mantha locality.

Meteorological record of Mantha shows that July to November is more favorable for mosquito surveillance and distribution. During these period minimum temperature is 17.2°C and maximum temperature 34.5°C, rainfall 1.20 mm to 8.80 mm while humidity in between 55.10% to 83.30%. The maximum surveillance of mosquito observes in month of September and October during three year study period. Theses two months record about temperature, rainfall and humidity were shows 20.8°C to 34.5°C, 2.50 mm to 6.67 mm and 63.20% to 74.4% respectively.

ANOVA results shows that similar results, about variation in the population among species is statistically highly significant at 1% level however seasonal variation is significant at 5% level. The correlation coefficient results conclude that during 2009- 2010 did not show any correlation of climate and monthly population. But the year 2010-2012 shows significant positive correlation with rainfall and humidity while negative correlation with maximum temperature. Increase in population when increase the rainfall and humidity while decrease the population when increases the maximum temperature at Mantha collection site.

During survey Bhokardan locality shows that the maximum density of *Culex quinquefasciatus* and moderately found *Armigeres subalbatus*. Lowest density observes in *Aedes aegypti* while *Anopheles subpictus* found rarely. Surveillance of *Aedes albopictus* and *Culex tritaeniorhynchus* did not recognize during study period.
The study period shows that highest density record in month of August to November at Bhokardan. During this duration the average minimum temperature is 16.10\(^{\circ}\)C and maximum temperature 32.40\(^{\circ}\)C, average monthly rainfall in between 1.40 mm to 8.80 mm and humidity in between 56.20\% to 83.20\%. The highest surveillance of mosquito species found during the August and September of every year. This two month shows that average minimum temperature is 22.20\(^{\circ}\)C to 32.40\(^{\circ}\)C, rainfall 3.70 mm to 8.16 mm and optimum humidity 66.00\% to 83.20\%.

Statistical analysis ANOVA results shows that, there is highly significant variation in the population among species but seasonal variation is non-significant during three year duration. Correlation coefficient between climate variable and monthly population did not show proper correlation between us. It is possibility the continuous fluctuation of climate and seasonal density. Climatic factors affect the distribution and density of mosquito species at Bhokardan.

Jafrabad location shows maximum surveillance of *Armigers subalbatus*, moderate density of *Anopheles subpictus*. Lowest density observes *Aedes aegypti* while rarely found *Culex tritaeniorhynchus*. *Culex quinquefasciatus* and *Aedes albopictus* did not found during study period at Jafrabad.

Jafrabad shows August to November is favorable for mosquito breeding and surveillance. Meteorological record during this month’s shows the minimum temperature is 18.3\(^{\circ}\)C and maximum 33.5\(^{\circ}\)C, rainfall in between 1.20 mm to 9.30 mm and humidity 69.90\% to 84.30\%. The highest density of specimen found in September and October. These two months show average temperature is 20.2\(^{\circ}\)C to 33.5\(^{\circ}\)C, average rainfall 3.40 mm to 8.20 mm and humidity 65\% to 84.30\%.

During study period statistical analysis by ANOVA, shows that there is variation in population of various species is highly significant at 1\% level however the seasonal variation significant during study period. All species shows positive significant correlation with climate change and monthly population. Overall result of three year study concludes that increase the population of species with increase the rainfall and humidity while decrease the population with increase the maximum temperature.

Overall meteorological record of all eight localities of study area shows that the average monthly temperature in between 16\(^{\circ}\)C to 36\(^{\circ}\)C, rainfall 1.20 mm to 9.00
mm and optimum humidity greater than 60%. The mentioned factors are favorable for mosquito breeding and surveillance. During the study period June to November shows the highest surveillance of mosquitoes of all localities. This period provide abundant breeding beds as well as suitable climatic factors. So the climatic factor is responsible for mosquito species distribution and surveillance at Jalna district.

Correlation analysis was conducted relating to monthly population of mosquito vs. monthly climate measures (temperature, rainfall and relative humidity) following different periods of collection. Overall results were obtained correlation analysis at all the eight places indicated that temperature, rainfall and humidity play an important role in the population of the organisms. Increased temperature decreases the population, while increased rainfall and humidity increases the population. However, the relationship varies from species to species and location wise.

The molecular systematic study is useful for mosquito’s correct identification and diversity study. A universal DNA-based barcode system (Molecular taxonomy) that is applicable to all animal species will provide a simple, universal tool for the identification of mosquito species. The barcode system is based on sequence diversity in a single gene region (a section of the mitochondrial DNA cytochrome c oxidase I gene, COI). When the reference sequence library is in place, new specimens and products can be identified by comparing their DNA barcode sequences against this barcode reference library (Lakra et al, 2011, Persis et al, 2009).

A total 5640 mosquitoes were collected during study period. These are morphologically identify and preserve for molecular study. For molecular study samples selected by species and location wise, it covers the whole sampling sites. Near about 120 mosquitoes were used for DNA isolation. In which 50 samples failed in DNA isolation, remaining 70 samples were proceed for PCR amplification. In which only 32 samples shows good quality PCR products and then it used for DNA sequencing. These 32 samples show good quality sequences then it used for phylogenetic analysis.

Our study confined to 1 family, 2 subfamilies, 4 genera and 6 species of mosquito. These are Anopheles subpictus, Aedes aegypti, Aedes albopictus, Culex quinquefasciatus, Culex tritaeniorhynchus and Armigeres subalbatus.
A total 32 specimens belonging to six species, four genera and two subfamilies were studied for COI divergence from Jalna district of state Maharashtra, India. The sequences with read length of 658 nucleotide base pair with no insertions deletions or stop codons were submitted to Barcode of Life Data systems (BOLD) and Gene Bank with accession number KC970268 to KC970299.

DNA-based species identification systems depend on the ability to distinguish intraspecific from interspecific variation. In the present study, COI sequence differences among species were, the average conspecific K2P divergence for mosquito species (0.56%) in this study it is similar to those earlier reported in Candian mosquito (0.55%; A. Cywinski et al., 2006), slightly higher than the earlier reported in China (0.39%; Gang Wang, 2012) and also higher than in study of Indian mosquito species (0.18%; Pradeep Kumar, 2007).

The mean conspecific, congeneric and confamilial divergence using K2P distance was 0.56%, 11.51% and 15.38% respectively. The average intraspecific K2P divergence was 21 folds higher than the interspecific divergence. The K2P divergence increase with increase in the taxonomic level.

The overall average nucleotide composition (GC%) was 32.15%, while at first codon position it was higher 44.23% as compared to second 42.91% and third codon position 9.3% respectively. The nucleotide variation was analysed by computing maximum composite likelihood using transition and transversion bias.

The nucleotide substitution from A to G is 29.24%, while from T to C is 19.79% and their frequencies were 28.34% (A), 38.33% (T/U), 15.70% (C) and 17.62% (G) respectively. The transition/transversion rate ratios are k1=8.036 (purines) and k2= 4.023 (pyrimidines). The overall transition/transversion (ts/tv) bias is $R = 2.554$.

The average intraspecific K2P divergence was 21 folds higher than the interspecific divergence. The K2P divergence increases with in the taxonomic level. Maximum genetic divergence was observed in *Aedes aegypti* with 2.79% intraspecific divergence. The lowest mean intraspecific neighbouring distance is observe in *Culex quinquefasciatus* (0.12%) where as highest in *Aedes aegypti* (0.98%), it is higher than compare to earlier study of Gang Wang et al., 2012, A. Cywinski et al., 2006 and Pradeep Kumar, 2007.
The current study indicates the utility of using single-gene sequences (5’ region of mitochondrial Cytochrome Oxidase subunit one gene), toward identification of the mosquito species. The NJ tree computed was in general agreement with the taxonomy based on morphology as reported previously (Hebert et al. 2003a, b; Hajibabaei et al. 2006).

This molecular study provides the first COI barcodes for mosquitoes in Jalna district in Maharashtra, India and provides further evidence of the effectiveness of DNA barcoding in identifying recognized species in study area. This is the first step of future planning of mosquito molecular systematic in study area. An insufficient number of specimens were studied but it is important for this area in the future. COI databases also need to include specimens of the same species collected from different geographical locations in order to determine the extent of intraspecific variation. A complete evaluation of the effectiveness of DNA barcoding for the Culicidae can be achieved through multinational research.