ECOLOGICAL RELATIONSHIP AMONG
GROUND WATER BREEDING
MOSQUITOES AND POPULATION
DYNAMICS OF KARACHI AND THATTA DISTRICT

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A thesis submitted in partial fulfillment of the requirements of the degree of Doctor of Philosophy in the Faculty of Science
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The present research of larval breeding sites was performed. The larvae of 14-species of mosquito comprising 3-genera viz. *Anopheles*, *Culex* and *Aedes* were collected from different ground water habitats in Karachi and Thatta of Sindh province, Pakistan, during four years period from 2004 to 2007.

A total of 94800 mosquito larvae were collected, out of all these larvae 13.82% was belonged to genus *Anopheles*, 81.03% to genus *Culex* and only 4.77% belonged to genus *Aedes*.

Over all collection during 2004 to 2007, the *Anopheles* was found 16.00%, 14.11%, 11.93% and 13.38% respectively. While *Culex* larvae were found 79.90%, 82.41%, 80.84% and 82.31 respectively during 2004 to 2007, whereas *Aedes* larval population was recorded as 4.09%, 3.48%, 7.23% and 4.31% during four years.

Among *Anopheles* six species were found which are *An. annularis*, *An. culicifacies*, *An. pulcherrimus*, *An. nigerrimus*, *An. stephensi* and *An. subpictus* (12,974 larvae were studied and identified). Similarly six species of *Culex* genus (77,192 larvae were studied and indentified) as *Cx.*
bitaeniorhynchus, Cx. fuscocephalus, Cx. pipiens fatigans, Cx. pseudovishnui, Cx. tritaeniorhynchus and Cx. vagans. In the same way two species of Aedes, aegypti and Aedes albopictus were also found.

Culex genus was the most common and showed highest percentage among Culex species, the Cx. tritaeniorhynchus and Cx. pipiens fatigans exhibited the greatest range of habitat.

Among Anopheles, An. culicufacies and An. stephensi breeds in almost any available type of water, but An. stephensi was inversely related to temperature as for as Aedes mosquito concerned, Ae. aegypti percentage was always very high as compared to Aedes albopictus. Highest number mostly was found in the month of October, every year.

It is to be noted that larvae fauna depends not only the type of habitat but also on the physical and chemical composition. Some species have positive association and some restricted only in clean water.

In the present thesis different experiments were conducted for the resistance on mosquito larvae of District Karachi and Thatta, Mosquito larvae were exposed to neem formulation, the Biosal (10 EC) available locally in the market, a synthetic pyrethroid, deltamethrin (10 EC) and
Acorus calamus extract were used. The LC_{50} values were calculated simultaneously the two enzyme (GOT, GPT) were estimated in mosquito larvae. Residue analysis and rate of biogradation was also noted by HPLC.

The LC_{50} values of Biosal deltamethrin and Acorus calamus extract against mosquito larvae were computed as 1605.05 ppm, 0.6119 ppm and 70.64 ppm respectively.

The enzyme activity pattern and inhibition by Biosal, deltamethrin and Acorus calamus extract were estimated after treatment with LC_{50} values of the under test insecticides. The inhibition of GOT produced by biosal, deltamethrin and Acorus calamus extract was calculated as 5.78%, 26.66% and 5.83% respectively. Whereas for GPT the inhibition was 3.95%, 19.65% and 13.77% respectively.

The residue analysis by HPLC shows that 97.79%, 87.87% and 78.18% residue of biosal were detected in 24, 48, and 72 hours exposed samples as compared to standard biosal sample. While 98.66 %, 98.49% and 70.45% residues of deltamethrin were detected in 24 hours, 48 hours and 72 hours exposed samples. Whereas 94.63%, 71.65% and 66.25 % residue of Acorus calamus extract were found in 24 hours, 48 hours and 72 hours.
exposed larvae. The residue analysis by HPLC indicated that the biodegradation is faster in biosal as compared to Deltamethrin and *Acorus calamus* samples, which indicate that Biosal degrade quicker and this may be concluded that toxicity of pesticide if compared to each other, is in the following sequence

Biosal > *Acorus calamus* > Deltamethrin.

Enzyme inhibition values and HPLC residue analysis, possibly proves the hypothesis that the pesticides obtained from neem and other plant and trees are not only less harmful but safer for our environment, but less prone to the development of resistance.
Abstract Urdu
CERTIFICATE

Certified that the research work reported in this thesis entitled “Ecological relationship among ground water breeding mosquitoes and population dynamics of Karachi and Thatta district” has been carried out by Tanveer Fatima under my supervision and guidance in the Department of Zoology, Federal Urdu University of Arts, Science and Technology, Karachi. The work reported here is original and suitable to submit for the award of Ph.D. degree.

Signature:      Signature:

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Supervisor,                                Co-Supervisor,
Department of Zoology,                  Dept Pharmacology,,
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Science and Technology,                Karachi,
Dedicated
To My Beloved
Parents
INTRODUCTION

Insects were present and established on our planet well before the advent of man. Man explored well his environment and is still trying to reach the core of every existing things, places or life in his surroundings. He realized that a group of insects may cause great nuisance to mankind. Mosquitoes have always established their inclusion among the group of insects known as the worst enemy of man.

Medical Entomologists have discovered that mosquitoes do transmit various fatal diseases to man and animals. Mosquitoes also provide a nursery for certain stages of parasite without which parasites life history could not be completed. Mosquito born diseases have an economic impact, including loss at commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-born diseases (Fradin and Day, 2002). Mosquitoes are the major vector for the transmission of different diseases like malaria, dengue fever, yellow fever, hemorrhagic fever, filariasis, schistosomiasis and Japanese encephalitis (JE) etc. (James, 1992; Gubler, 1998). Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema (Peng et al., 1999).
Mosquitoes are two-winged flies belonging to the order “Diptera” suborder Nematocera and family Culicidae. Anophelinae and Culicinae are the sub families of Culicidae. Variety of species of this group is well known as the vector of several diseases. Mosquitos species like *Anopheles stephensi* and *Anopheles culicifacies* are the vector of malaria disease. *Aedes aegypti* and *Aedes albopictus* are the vector of yellow fever, hemorrhagic fever, dengue and many virus vectors. *Culex tritaeniorhynchus* is the vector of Japanese β-encephalitis, west nile and dengue etc and of course the common house mosquito like *Culex pipiens fatigans* is the vector of elephantiasis, avian malaria and influenza.

Except Christopher, 1933 and Barraud, 1934 no comprehensive studies on mosquito have been made in Pakistan. Most of the workers viz., Ansari and Shah (1950); Mohan (1950); Qutbuddin (1960); Ikeshaji (1968); Khan and Salman (1968); Reisen (1978); Reisen and Siddiqui (1979); Suleman (1985); Reisen and Milby (1986); Naqvi *et al.*, (1988) and Suleman and Khan (1993) reported population density or survey of mosquito populations in different villages, valleys, forests and cities of Pakistan.

In the Indo-Pak sub-continent studies have characterized, *Anopheles* breeding habitats by Senior-white (1928); Chirstopher (1933); Russell and
Rao (1942); Pal (1945); Ansari and Shah (1950); Ansari and Nasir (1955); Qureshi (1965); Aslamkhan and Salman (1969); Sinha (1970) and Culex spp., Mohan (1950); Ikeshaji (1968); Sirivananakaran (1973) and Reisen and Siddiqui (1979).

Fauna of Pakistan was first published by Aslamkhan (1971). The first mosquito species recorded from Karachi city was *Aedes aegypti* by Barraud (1934). Later *Anopheles stephensi* by Talibi and Hussain (1958) and Afridi *et al.*, (1958), *Culex tritaeniorhynchus* Baker and Aslamkhan (1968) and *Anopheles subpictus* Aslamkhan and Baker (1969) were detected in Karachi city.


Up till now little systematic effort has been made to study the Culicidae of Pakistan. The fauna of British India dealing with *Anopheline*
(Christophers, 1933) and *Culicine* (Barraud, 1934) mosquitoes still remain the standard reference work. From 1934 till 1971, only one species and one variety of *Anopheline* and 3 species of *Culicine* mosquitoes have been added to the mosquito fauna of Pakistan.

An excellent survey of India and Pakistan has been given by Qutbuddin (1960). The very little work has been done on the taxonomy (Khokhar and Tariq, 1966; Tariq, 1967 and Aslamkhan, 1971).

The Culicidae of Pakistan remains poorly known except for the *Anopheline* mosquitoes, which are better known because of their involvement in the transmission of malaria. The distribution of *Anopheles* is given by Covel (1927, 1931); Barraud (1933) and Puri (1936, 1948).

The *Anopheline* mosquitoes of Pakistan have been described by Talibi and Qureshi (1956) and that of Punjab by Ansari and Shah (1950). Supplementary information about the *Anopheles* comes from the work on malaria, Ecology and biology (Nasiruddin, 1952b; Naqvi and Qutbuddun, 1954; Ansari and Nasir, 1955; Talibi and Hussain, 1958). Information about the Culicine mosquitoes of Pakistan (specially Karachi) is still very meager. However, studies on bionomics, filariasis and cytogenoyics have added some information concerning the distributional record of mosquitoes has been reported by different workers (Nasiruddin, 1952a; Qutbuddin,
Man’s worst natural enemy is still the mosquito. Annually on this planet, over one and a one-half million people die from malaria caused by mosquito. In Pakistan, Baker et al., (1973). Malaria has increased enormously in 1970-1971. In 1961, when the malaria eradication program was initiated, there were an estimated 7 million cases of malaria by 1967. The number of cases has been reduced to 9,500. Moreover, there are large number of other important diseases other than malaria, which are transmitted by mosquitoes, like yellow fever, encephalitis, filariasis, Dengue, west Nile, influenza and haemorrhagic fever etc. These mosquito born diseases have caused immense misery and suffering as well as tremendous economic loss in the peoples and countries where mosquitoes are found.

The war against mosquito began over 100 years ago when it was discovered that it is a carrier of malaria. Long before insecticides, there were some successful campaigns against the mosquito. Since the immature stages of the mosquito are aquatic, the removal of all standing water or, the spreading of oil on the surface was effective in mosquito control. For the
last 60 or 70 years use of insecticides have been thought as the ultimate weapon. We now know that chemical control has many serious drawbacks. In addition it is the problem of environmental pollution.

Karachi is a cosmopolitan, largest and heavily populated city of Pakistan. A large number of people excessively use insecticides for mosquito control. Due to misuse, over use or unnecessary use of insecticide resistance has developed in the mosquito of Karachi and other parts of Pakistan. However despite all the harmful effects, no systematic work for determination of resistance has been done in this region. However, few workers (Rathore et al., 1986; Naqvi, 1987; Azmi et al., 1991 and Azmi, 1992) have reported resistance in insects in Pakistan Karachi. Insecticide resistance is increasingly becoming a problem for malaria vector control programs and other mosquito born diseases. Resistance may develop due to changes in the mosquito’s enzyme pattern systems.

Insecticides that can be used in malaria control are increasingly becoming limited. *Culex tritaeniorhynchus*, was found susceptible to permethrin and resistant to DDT, dieldrin, fenitrothion and propoxur (Bansal and Singh, 1995) and resistance to organophorous insecticides.
The frequent use of systematic insecticides to manage insect pests leads to a destabilization of ecosystem and enhanced resistance to insecticides in pests (Kranthi et al., 2001; Mohan and Gujar, 2003), suggesting a clear need for alternative, so biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans and other advantages (Liu et al., 2000). In addition, increasing documentation of negative environmental and health impact of synthetic insecticides and increasingly stringent environmental regulation of pesticides (Isman, 2000) have resulted in renewed interest in the development and use of botanical insect management products for controlling mosquitoes and pest.

At present Karachi is the largest and heavily populated city of Pakistan. Its problems are multifold as compared to other cities, with further increase in its population, so the people of Karachi are facing a number of mismanagement. Such as water shortage, poor sanitation, unhygienic conditions, often the epidemic and spread of different vector born diseases from time to time which occur in Karachi.

Unfortunately little attention has been paid to work out the biodiversity and population of mosquitoes in Karachi. Present research work was conducted to establish baseline faunistic of mosquito species,
population densities and to locate and assess potential breeding sites in the Karachi and Thatta region, along with the identification of species found in there Districts, seasonal fluctuation and habitats will be also assess.

The other important objective of this study was to determine the toxicity of neem based phytopesticide (Biosal) and *Acorus calamus* extract (A.C.) and deltamethrin (synthetic) pesticide against mosquito larvae.

The results of this research work will be used to assist in monitoring mosquito population densities and related to toxic effects for future management strategies.

These studies are concerned with obtaining basic information about the biology and ecology of the different species of mosquitoes, in order to develop one or more methods of biology control.

**The objectives of the Research work are as follows:**

To find the data on population dynamics provide essential background information for the evaluation of both insecticide and biological control and to find the population status of mosquito larvae in various parts of the city during four years (2004–2007) research work.

To develop database, illustration of breeding habitats for different mosquito species found from all districts of Karachi. To find tolerance
level in mosquitoes against, Biosal, (azadirachtin) Deltamethrin (SP) and
Acorus calamus (β-azarone) extract, which were used for the control by
various researches. This work will also provide proper information to
prevent or control the current “Dengue Epidemic disaster” in Karachi.

Achievements:

Initiation of these studies has been undertaken in response to the
above objectives, which were possibly achieved by the determination of
toxicity level by using WHO method. Evaluation of Enzyme levels by
spectrophotometry for evaluating the possible resistance status, further
verification was done by using HPLC techniques by determining residue
level. This data gives complete analytical proof of phenomenon of possible
resistance.

The other achievement are identification of different mosquito
species, population densities and seasonal fluctuation of the larval
population were determined and have been reported.
2. REVIEW OF LITERATURES

2.1. MOSQUITO POPULATION

Ansari and Shah (1950) reported the different types of mosquitoes found in Punjab region, in which they reported different genera of mosquitoes abundantly found in that region. Mohan, in the same year also surveyed on certain uncommon habits of *Cx. tritaeniorhynchus* and described unique nature and habit of that species.

Hussain and Talibi (1956) working in Karachi, notified the incrimination of vectors of malaria, and indicated that *An. stephensi* plays an important role as a vector of malaria.

Qutubuddin (1960a) gave detailed account of fauna of mosquitoes from Hangu valley, Kohat, West Pakistan. Previous study of the area restricted to cantonment of Kohat (Naqvi and Qutubuddin, 1954) whereas some mosquitoes species were reported but this study comprises not only exploration of the fauna of mosquitoes but also their variable breeding places throughout the year, factors impact of them, zoogeography of that overlapped to Palaearctic extension to Afghanistan. It was the first time report of 4 genera, 9 subgenera and 22 species. These species were not only concerned with malaria but also with microbacterian filariasis. Author
also showed topography of Hangu valley in detailed whereas all possible breeding pools were pointed out during the year by many climatic factors.

Qutubuddin (1961) reported the fauna of mosquitoes and population density in Kohat, Hangu Valley, West Pakistan. He specially noted that *Aedes aegypti* was abundantly found in that area. May (1961) described ecology of malaria disease. According to him *P. falciparum* was able to invade red blood cells of any age, which describe its considerable infectivity.

Rehman and Muttalib (1968) presented the different species of malarial parasites, their transmission in Karachi city and incrimination of *An. stephensi* as a malaria vector. Baker *et al.*, (1968) worked on salivary glands chromosome map for *An. pulcherimus*. The different banding pattern of this species showed similarities with *An. gambiae* and *An. stephensi* especially at the free ends of the arms. Rehman *et al.*, (1968) conducted survey on malaria situation in Karachi, during the months of September and October. They also reported *A. stephensi* as an urban vector from Karachi. Ikeshaji (1968) presented population density and surveyed *Culex* mosquitoes from different areas of Pakistan.
Wolffe and Aslamkhan (1969) investigate and find filariasis in Karachi. In the previous study of Korke (1929, 1933), Krishnaswami et al., (1963) disclosed no evidence of filariasis in Indian subcontinent. For the study of this infection, survey of Karachi was undertaken to find those persons which came from endemic areas. About 209 peoples were investigated for the detection of *Waucheria bancroftii*, only 4 peoples were infected who came from three cities of India and one from East Pakistan. These immigrants were found infected from *Culex pipens fatigans* from their homeland but in their blood, no larvae were seen, so in Karachi city, no evidence has been seen for transmission of this infection.

Khan and Suleman (1969) studied bionomics of the mosquitoes of the Changa Manga, National Forest in Punjab. They surveyed and reported Anopheline groups because of their involvement in malaria transmission.

Aslamkhan (1971) has collected one year data of *Aedes (Aedimorphus) punctifemoris* (Ludlow) from specific villages, ground water since 1968 to 1969 and collected over 20,000 specimens of this species. It is important to find this species as a first time record from Indian subcontinent and first time redescribed from Pakistan. The main features of this species not only abundancy but also their taxonomic study with illustration and bionomics was discussed.
Aslamkhan et al., (1972) presented a preliminary report of *Anopheles stephensi* from Karachi with their morphological and genetical variations. They found about 50 variation from natural population. Several workers have worked on the races of *An. stephensi* (Knowles and Basu, 1934; Mulligan and Baily, 1936; Ramsay and McDonald, 1936; Sweet et al., 1938) for finding of results in natural population of *An. stephensi*. They observed seasonal emergence of adults in December 1970, and after three months, they found a great numbers of different variations of *An. stephensi* (mutants).

Baker et al., (1973) studied genetically controlled of mosquitoes in Pakistan. To evaluate the number of strains of mosquitoes, a considerable amount of basic genetic data was carried out through previous investigation. In the laboratory they experimented a lot of mosquito strains by their chromosomal aberrations, also temperature sensitive conditional lethal mutation. This advance work with association of non transmission disease forms in mosquitoes, where they kept on maximum range of temperature 32°C. Another method was used as sterile male technique in which sperms of particular species irradiated then released out in nature, when sterile male mate the wild female, the eggs do not hatch, so the mosquito population suppressed and extinct gradually. Same method was
used for other insect disease carrier. The isolation of many mutants revealed information of this type.

Mahmudul-Ameen and Moizuddin (1973) described life history and developmental stages in local mosquito control schemes, especially larval stages. Attempts were made to find out the longitivity of adult mosquitoes. They found that longitivity of ♀ appears to decrease if isolated from the males and fed continuously on glucose after a blood meal.

Zahar (1974) conducted survey and reported that A. subpictus was abundantly found in Lahore and Karachi, Pakistan the role of this species as a vector was not confirmed. In the same year Das et al., (1979) reported the Anopheles intensity as malarial vectors in different parts of Mediterranean region in which they noted refractoriness to Plasmodial maturation.

Reisen and Aslamkhan (1976) observed swarming and mating behavior of Anopheles culicifacies Giles in the cattle shed of Sattoki, Lahore, Punjab, Pakistan. They noted time and intensity of light and observed swarming before sunset, pairing and mating. During mating, they collected a number of copulated pairs by net then dissected out and observed their blood feeding correlated quantity.
Reisen et al., (1976) has investigated the population dynamics and several arboviruses vector, *Culex tritaeniorhynchus* in Asia, especially different villages of Punjab, Pakistan. They observed seasonal abundance variation with climatic factors, humidity and temperature. Due to floods in Asian countries, the population of this species increased in the paddy and rice field. In Pakistan, this species growth was related with fluctuating temperature. For instance, summer and winter season is flourish period of this species, while in autumn, they become inactive.

He also investigated diet activity pattern of 18 mosquitoes species with indoor and outdoor collection. They worked out Lahore, District of Punjab (Pakistan). They observed abundancy of mosquito species about their biting in buffalo shed. Except two species, *Culex fuscocephalus* and *Cx. epidesmus*, all species exhibited their fed crespuscular time and two hours later of it. *Culex tritaeniorhynchus* exhibited their feeding early morning. They studied Culicine and Anopheline mosquitoes population dynamics. During daylight, densities of endophilic anophelines increase but rather decrease in nights. They spent three nights in mudshed of buffalo, where they counted in regard of different parts of minutes.

Maddox et al., (1977) analyzed first time in Pakistan, susceptibility of mosquitoes with a pathogen protozoan *Nosema algerae*. In laboratory,
the pathogen can be susceptible, so out of 6 species of mosquitoes, 4 were potential heavy with pathogens while remaining two were light. This pathogen as biological agent was analyzed by these 6 species of mosquitoes for medical importance in Pakistan.

Resein et al., (1978) presented striking features of the adult resting mosquitoes collected from human habitations. They noted low relative abundance of *Anopheles* in house hold whereas *Culex* species were highly abundant. WHO (1978) reported malaria situation and also suggested that effort is to be concentrated in areas of great economic importance.

Aslamkhan et al., (1979) described pupa of *Aedes* (*Diceromya*) *micropterus* (Gillies, 1961) for the first time in literature. Adult (Gillies, 1961) and larva (Barraud, 1934) described with illustration already. The present report was not only description of pupa but also with illustration in detailed.

Resein and Boreham (1979) reported *A. stephensi* and *A. subpictus* population mostly predominant in Buffalo houses because they preferred bovid hosts.

Reisen and Khan (1979) studied and analyzed population behavior with different reactions such as mating, survivorship, size and gonotrophic
rhythm in *Anopheles stephensi*. This species of mosquitoes is quietly vector of malaria. For the study of its behavior, three different strategies of mark-recaptured were used in distantly areas of mudshed of cattles. They observed swarming period, a numbers of pairing species in mating, size of population with their aggregation, rate of mortality with survivorship and annual abundance.

Reisen and Siddiqui (1979) did experiment in the field and laboratory for estimation of immature survivorship of *Culex tritaeniorhynchus*. They used mark-release-recaptured method for numbers and their developmental stages. Two types of estimation methods were applied, horizontal and vertical estimation. Both methods were used in the pond as nature habit or frequently approach; increasing of densities of these larvae delayed development and decrease survivorship. In Lahore Punjab, different water breeding sites were observed and different attributes were summarized. In the field, different instars of their species were marked from the same pond, same time of the year and designed survivorship curves with calculated rate of mortality and their life tables.

Niaz and Reisen (1981) experimented the larval development of *Culex tritaeniorhynchus* by induced a substance quiescence in cool season. They gave different values of temperature rather median to lowest degree
and observed that in cool temperature, larval development of this species is reduced and did not emerge but in the presence of quiescence, glycerides, they become large and heavier. The rate of potential of energy, mating, blood feeding and synthesis of triglycerides from sucrose result in increase in low degree of temperature.

Reisen et al., (1981) analyzed very exhaustive study of mosquitoes population with relationship to their abundance, larval development and tolerance against physico-chemical factors. During this study a large scale area have been visited in Lahore, Punjab, Pakistan. They observed susceptibility in different habits of different species of mosquitoes. Alongwith different alkalines, turbidity and chemical variables have been studied in mosquito populations. They observed that larval forms of 15 species of mosquitoes showed great response against these physico-chemical factors. It is important things that author also participated in this survey herself and she with staff visited all types of mosquitoes habitats such as ditch, seepage, canal, drain etc. All biodata of these species were statistically analyzed by technical and relevant expertise.

David et al., (1985) described the malaria situation in Karachi specially periurban areas where immigrating groups including Afghan refugees and Biharis were settled. They described four types of
chromosomal inversions found in Karachi. They showed that *A. stephensi* status as a vector was related to chromosomal polymorphism in behavior. Nalin *et al.*, (1985) presented role of *Anopheles stephensi* as an urban malarial vector from Karachi, Pakistan. They also studied on genetic changes of *An. stephensi* and their systems and chromosomes were examined.

Kamimura *et al.*, (1986) conducted a survey to find the species of mosquitoes which transmit the diseases in Karachi, with special reference of Japanese encephalitis virus, transmitted by *Culex tritaeniorhynchus*. They also identified four other species.

WHO/SEARO (1987) reported mosquito vector genus *Anopheles* including 300 species each with a wide range of ecological niches and behavioural pattern.

Naqvi *et al.*, (1988) reported the mosquito population in three different pool communities within Karachi University area. They collected 450 samples and analyzed that *Anopheles stephensi, An. culicifacies, An. subpictus, Ae. aegypti, Cx. tritaeniorhynchus* and *Cx. fuscanus* were found rarely but the abundant species was *Cx. p. fatigans*. 
WHO (1989) reported malarial prevention and eradication program throughout Pakistan and also described world-wide threat of malaria and malarial situation in Pakistan.

Zaidi and Kazmi (1990) reported many vector species from Pakistan, and according to them four species appeared to be of significant value including *An. culicifacies*, *An. stephensi*, *An. superpictus* and *An. fluviatilis* as suspected malarial vectors.

Service (1993) studied on mosquitoes and reported and compiled data of anopheline vector species of malaria in the epidemiological zone. The work notified the North American, Central American, South American, North Eurasian, Mediterranean, Afro Arabian, Afrotropical, Indo Iranian, Indo Chinese hill, Malaysians, Chinese and Australasian malarial vectors.

Suleman *et al.*, (1993) reported 31 species of mosquitoes belonging to 6 genera. Genus *Anopheles* was represented by 10 species, *Aedes* by 8, *Culex* by 9, *Culiseta* by 2 and *Armigeres* and *Mansonia* each by a single species. Their data described the species composition of mosquitoes in Peshawar (NWFP) area is considerably different from the Punjab. The one year study on breeding ecology in that year anophelines showed that 53%
of the larvae from all anopheline belonging to the *An. stephensi*, 16.1% *An. fluviatilis*, 15.9% *An. maculates*, 6.5% *An. culicifacies*, 3.4% *An. pulcherimus*, 1.8% *An. annularis*, 1.5% *An. splendidus*, 1.9% *An. subpictus*, 0.5% *An. Pallidus* and 0.4% *An. nigerrimus*. Among anophelines, the two known malaria vectors, *An. culicifacies* and *An. stephensi*, and a suspected vector, *An. fluviatilis*, three species *Culex. quinquefasciatus*, *Aedes. albopictus* and *Armigeres subalbatus*, are most troublesome for humans.

Sulemen *et al.*, (1993) reported apart from the *An. stephensi* and *An. culicifacies*, the two species *An. pulcherrimus* and *An. fluviatilis* as suspected vectors especially in the mountain and foot hill areas of northern districts of NWFP, Peshawar and Punjab.

Tassanakajon *et al.*, (1993) detected *P. falciparum* in mosquitoes by polymerase chain reaction using a primer set derived from a repetitive deoxyribonucleic acid sequence specific to *P. falciparum* for detecting parasite DNA in mosquitoes. Snounou *et al.*, (1993) identified 4 species of malarial parasites in field samples by using polymerase chain reaction and designed nested PCR which was more sensitive and used for the genus – specific and species – specific primers.
Reisen (1995) studied two geographical isolated population of mosquitoes. *Culex tarsalis* in Coachella and San Joaquin valleys of California. He analyzed seasonal fluctuation and impact on development of larvae and their adult size. He observed that this species widely distributed in California and active in summer and winter latitude of North and South respectively. He did experiment on population dynamics of *Cx. tarsalis* on the basis of different temperatures of their development. He studied these experiments from 1991 to 1993 and found that winter season was similar to *Cx. tarsalis* which is found in Coachella and San Joaquin valleys but after spring, one group influenced by gradual decrease or increased temperatures while other group showed this tendency in different season. He also showed this fluctuation of parameters with their survivorship.

Kazmi (1995) reported a total of 26 *Anopheles* species, for the first time from Pakistan. According to him only 14 species were malarial carriers. He also concluded that *A. stephensi* and *A. culicifacies* were seriously implicated in the transmission of this disease. In the same year Higgins and Azad (1995) revealed that the loss of sensitivity and specificity during the PCR amplification due to the presence of inhibitors present in the mosquito tissues.
Sharma (1995) worked on the host feeding pattern of malaria vectors *An. culicifacies*, *An. subpictus*, *An. stephensi* and *An. fluviatilis* in Haryana and Himachal Pradesh during 1990-1992. The anthropophilic index was low as compared to zoophilic index. The anthropophilic index was reported 12.5 for *An. culicifacies* in cattle sheds. The zoophilic index for *An. fluviatilis* and *An. stephensi* was 62.50 and 100% in human dwellings.

Roberts (1996) studied the ovipositional preferences and larval survival in brackish water during breeding of the mosquitoes. Four species of mosquitoes were abundant in concrete reservoir tanks containing brackish water that ranged from 16 to 39% seawater. The ability of the larvae to survive in various salinities was compared for each species with the ovipositional preferences of the adult females to determine whether the 2 traits were correlated. Southern house mosquito, *Cx. quinquefasciatus* Say, normally was not present in the tanks but survived well in salinities up to 25% seawater. However, gravid females almost always oviposited in freshwater. *Cx. sitiens* Weidemann larvae survived best in saline water (66% sea water), but oviposition was greatest in 28% sea water; both larval survival and the frequency of oviposition were low in freshwater. *Cx. sinaicticus* Kirkpatrick survived salinities up to 50% seawater, but the females refused to blood-feed; therefore, their ovipositional preferences were not
tested. Larvae of *An. stephensi* Liston and *An. culicifacies* Giles survived best in freshwater, but some *An. stephensi* were able to tolerate up to 50% seawater. The females had a similar ovipositional preference for freshwater. The preferred salinity for oviposition did not correspond with larval survival for *Cx. quinquefasciatus* and *Cx. sitiens*, but did compare well in *An. stephensi* and *An. culicifacies*.

Bhatt and Kohli (1996) reported the biting rhythms of few anophelines in Central Gujarat. In which a total of 41,552 anophelines comprising 16 species were collected during 70 all-night bovine-bait collection carried out in six villages of Kheda district, Gujarat. *An. subpictus*, *An. varuna*, *An. culicifacies*, and *An. stephensi* unimodal biting rhythms. Most feeding occurred during the early night with occasional increase during pre-dawn/dawn hours. The *An. pallidus* inhibited bimodal biting rhythm with two well-defined peaks. *An. turkhudi*, *An. tessellatus*, *An. fluviatilis*, *An. aconitus*, *An. annularis*, *An. barbirostris* and *An. nigerrimus* had multimodal biting rhythms or were arrhythmic. *An. culicifacies*, *An. varuna*, *An. aconitus* and *An. tessellatus* exhibited a marked seasonal shift in feeding activities with most biting occurring at dusk in colder months and late at night during warmer months.
Kar et al., (1996) described the domestic breeding sources of *An. stephensi* in India. In this connection longitudinal study was taken up for one year in 10 different types of breeding habitats in Dindigul town, Tamil Nadu, revealed that out of 51,785 habitats 225 (0.43%) were found positive for *An. stephensi* immatures. The overall positivity varied between 0.03 to 1.31% with peak density during July. The observed habitat-wise positivity was overhead tanks 0-7.07%; wells 0-1.69%; underground tanks 0-2.26%; tappits 0-2.36%; outside tanks (permanent) 2.42%; outside tanks (temporary 0-0.39%; inside tanks (permanent) 0-20%; inside tanks (temporary) 0-3.6%; barrels 0-1.32% and others 0-25.0%. In 16.0% habitats *An. stephensi* was found breeding with *An. subpictus, Ae. aegypti, Ae. vittatus* and *Cx. quinquefasciatus* in different combinations. Overhead tanks were found to contribute maximum *An. stephensi* breeding in this area.

Naqvi et al., (1997) observed mosquitoes population from 12 different localities within Karachi region. They noted larval abundance estimated by counting the number of larvae found in the dips. They also described that temperature and humidity produced significant effect on mosquitoes population i.e. high temperature and low humidity level declined the population. Singh and Bansal (1996) especially described its
control and treatment measures. Cox-Singh et al., (1997) revealed that malaria remained a disease of under-developed and remote regions of the world.

Prakash et al., (1998) reported anopheline fauna of the north-eastern states of India with notes on vectors of malaria. In this connection, they recorded state-wise checklist of anopheline mosquitoes, so far, from the north-eastern region of India is prepared. It includes 42 mosquito species of genus *Anopheles*, the maximum (40) from Assam and the minimum (18) from Nagaland. Notes on various species groups of genus *Anopheles* and malaria vectors in the north-eastern states have been provided.

Beebe and Cooper (2000) reported that appearance of groups and complexes of cryptic or sibling species in many of the anopheline taxa and also studied morphological characters to identify the vector species involved.

Suleman (2000) conducted study on the biodiversity of mosquitoes and epidemiology of malarial transmission in children. He noted fourteen species of mosquitoes from the areas of Pakistan. Among these only seven species of *Anopheles* were found important.
Hozhabri *et al.*, (2000) estimated the prevalence of malaria amongst the children with fever or history of fever during the investigation, they examined thick and thin blood films with Giemsa stain.

Nayar *et al.*, (2001) isolated arboviruses from female mosquitoes under observation of light traps, collected 46-150 female mosquitoes, out of them 18 species of mosquitoes were observed and isolated different viruses. For isolation, serological tests were examined the PCR analyzed for nucleotide sequencing, in which 1 virus of KEY and 6 virus of TEN isolated and were identical nucleotide sequences.

Philips (2000) described the status of malaria and its control at the start of the millennium. He revealed that malaria still present as the greatest threat in all the parts of the world. He also reported the fact that its eradication has not been achieved even in the 21\textsuperscript{st} century. He suggested that the spectrometers development in molecular biology and allied biological sciences raise hope that new anti-malarial vaccine will be developed.

Amerasinghe *et al.*, (2002) published an article provides taxonomic keys for the identification of the fourth-instar larvae and females of 24 species of anopheline mosquitoes (seven species in subgenus *Anopheles* and 17 species in subgenus *Cellia*) recorded from Pakistan. The keys are
based on literature sources as well as the examination of field and museum collections.

Foss and Dearborn (2002) did preliminary faunistic survey of mosquito species (Diptera: Culicidae) with a focus on population densities and potential breeding sites in greater Portland, Marine a total of 27 species were collected during study (Technical report No. 42, CDC).

Lester and Pike (2003) analyzed the population dynamics of *Culex previglans* with inverse proportional of surface areas of anthropogenic and water container, also observed interaction between larvae of *C. previglans* and their predators. They observed density of *C. previglans* population throughout the year and obtained results that the native species of *C. previglans* was very abundant in small and particular area than open and vast surface; larvae feed by backswimmer, Notonecta, Damsel fly larvae and diving beetles. They reported that due to backswimmer, oviposition of *C. previglans* become reduced. They also analyzed aggregation of *C. previglans* larvae with correlation of depth of container.

Klinkenberg *et al.*, (2004) studied the population behaviour of malarial born vectors which depends upon changing environment, because Punjab province less epidemic for malaria rather than other area of Punjab and adjacent India. To find cause of influence the changes of environment
on malarial vectors, entomological data were carried out since 1970 to 1999 from District of Bhawalnagar, South Punjab and Indus Basin Irrigation system. After studied this data, it has been suggested that Anopheles stephensi is more prevalent throughout, due to large scale changes of water logging of soil with salinity which created the tolerance in A. stephensi, but some biotypes may be suspected this species, for this way the dominant species could not efficient for transmission of malaria in Punjab.

Deressa, et al., (2006) they examine the relationship between population and malaria transmission in Ethiopia, because Malaria is the number one public health problem in Ethiopia. Study of all malarious areas in Ethiopian lowland to highlands revealed that since 1965 to 2005, 35 millions peoples affected and death during that year. In this region, majority of areas are highly ecological degradation has led to increased vector born diseases, like malariais, trypanosomiasis etc. To calculate all interventions projects of eradication of malaria has been launched in Ethiopian areas till 2025, to suppress the population dynamics and transmission of malariasis.
2.2. TOXICITY AND ENZYMES

Amalraj et al., (1989) reported IGR effects of a substituted urea compound XRD-473 (OMS 3031) against the target species viz. Culex quinquefasciatus, Aedes aegypti, Anopheles stephensi and non-target species Toxorhynchites splendens. This compound inhibited the emergence of all these mosquito species with $E_{50}$ values of $9 \times 10^{-5}$, $1.09 \times 10^{-4}$, $2.22 \times 10^{-4}$ and $2.14 \times 10^{-4}$ mg (ai)/l, respectively. Emergence inhibiting activity of XRD was found to be more than Fenoxy carb and S 21149 against one or other species. In stagnant polluted water, the activity of the compound against C. quinquefasciatus was for shorter duration of 5 and 10 days at 0.02 and 0.2 kg (ai)/ha, respectively, whereas in clear water against An. stephensi the activity was for longer duration of 11 and 17 days at the same dosage. The control of Aedes aegypti was obtained more than two weeks in cement tank at 0.2 kg (ai)/ha dose, but at the lower dose of 0.02 kg (ai)/ha this compound was effective for less than one week.

Sergieva et al., (1989) characterized 7 mosquito strains and species from the collection of the E.I. Martisinovsky Institute with regard to the levels and mechanisms of their resistance to malathion and DDT. Resistance mechanisms of the strains An. stephensi, An. sacharovi, An.
atroparvus, Ae. aegypti and C. papiens were determined with the help of synergists. Malathion resistance in one of the An. stephensi strains is due to high carboxyl activity. Other strains featured various levels of DDT-resistance due to various mechanisms in Ae. aegypti. It was due to mixed function oxidases activity; in An. atroparvus, An. sacharovi and An. stephensi second strain to glutathione-dependent transferase, in C. pipiens, An. stephensi third strain and partially, An. sacharovi, to the probable presence of Kdr factor.

Thiery and De Barjac (1989) reported the larvicidal power of more than 180 bacteria, Bacillus sphaericus, strain belonging to six H. serotypes, against Cx. pipiens, An. stephensi and Ae. aegypti under standard standardized conditions. The most potent strains were distributed into serotype H5a5b, generally toxic to the three mosquito species, and serotype H6 and H25 were found toxic to C. pipiens and An. stephensi strains of serotype 26a, 26b and H2a2b were much less toxic and most often only on C. pipiens. The relative potency of each strain can be expressed by specific titers on the different mosquito species and by activity ratios derived from such titers.

Chockalingam et al., (1990) reported the toxicity of eight different plant products of different plants, which were used to determine their
larvicidal effect against third and fourth instar larvae of *Cx. quinquefasciatus*. Plumbagin isolated from *plumbago indica* was found to be more effective than other plant products against mosquito larvae with the LC$_{50}$ values of 10 and 17.5 ppm to 3$^{rd}$ and 4$^{th}$ instar larvae, respectively.

Chunina *et al.*, (1990) reported that the mosquitoes *Ae. aegypti* and *An. stephensi* contact with sublethal doses of deltamethrin and cypermethrin pyrethroids at larval stage and in grown stage, when diet includes sugar with pyrethroids, had no influence on the sensitivity of survived females to malaria agents, *P. gallinaceum* and *P. berghei*. Mosquitoes under experiment showed no obvious inhibition in comparison with the control ones.

Itoh *et al.*, (1990) reported the efficacy of pyrethroid-treated wide mesh netting against mosquitoes. They impregnated wide-mesh netting of 0.5 cm mesh size with each of five insecticides (permethrin, cypermethrin, d-phenothrin, esfenvalerate and fenpropatrin) at various concentrations ranging from 0.003125 to 0.025% and the effect of netting on caged *Cx. pipiens pallens* Coquillett was assessed. The mosquitoes were killed on contact with the treated net as they pass through. The mortality of mosquitoes was parallel to the concentration of an insecticide treated. The
biting of *An. gambiae* Giles on the rabbit caged in a net box treated with each of permethrin (0.6%) and fenpropathrin (0.3 and 0.6%) was also assessed. Most of the mosquitoes died before entering the net box, and the few that entered died before biting. Practical use of pyrethroid-treated, wide mesh bed net for preventing mosquito bite was evaluated from these results.

Al-Sharook *et al.*, (1991) reported the larvicidal effect of acetone extracts from *Melia volkensii* and *M. azedarach*, seeds were compared with the pure natural growth inhibitor azadirachtin A in their morphogenetic effects against *Cx. pipiens molestus*. There are significant differences between the insecticidal activity of both the crude extracts. *M. volkensii* acetone extract results in equal toxicity for larvae and pupae with LD$_{50}$ of 30 µg/ml. *M. azedarach* acetone extract is exclusively larvicidal with LD$_{50}$ of 40 µg/ml and has no inhibitory effect on the pupal stage. Like the crude *M. volkensii* extract, pure azadirachtin A is equally toxic to larvae and pupae with LD$_{50}$ of 1-5 mg/ml. the bioactive compounds from *M. volkensii* are thermostable and partially soluble in water.

Amalraj *et al.*, (1991) described the activity of a new synthetic pyrethroid, tralomethrin (OMS-3048) against vector mosquitoes. Tralomethrin was tested for its insecticidal properties in the laboratory
against normal strains of *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. sitiens*, *An. stephensi*, *An. culicifacies*, *Ae. aegypti* and *Armigeres subalbatus* and against strains of *Cx. quinquefasciatus* resistant to fenthion and malathion. Tralomethrin showed good larvicidal activity against all species tested. LC$_{50}$ ranged between 7.00x10$^{-6}$ and 9.10x10$^{-3}$ mg (ai)/1. Resistant strains of *Cx. quinquefasciatus* showed higher tolerance than the normal strain. Tralomethrin was more effective against adults of *An. culicifacies* (LD$_{50}$ 0.18 µg/cm$^2$) than the other species. Residual activity of this compound lasted for 15 weeks on thatch surface at a dose of 50 mg (ai)/m$^2$ against all the mosquito species tested. In the field, this compound was effective for a period of 1-2 days in polluted water viz., cesspits and drains and 10-24 days in less polluted water as in cement tanks, when applied at the rate of 0.002-2.0 mg (ai)/l against immatures of *Cx. quinquefasciatus*.

Kamal and Mangla (1991) reported the efficacy of pyrethrins extracted from the floral heads of *Dysodia tenuifolius* and *Ageratum conyzoides* against the larvae of *An. stephensi*, a carrier of malaria. *D. tenuifolius* showed slightly higher levels of pyrethrin content than *A. conyzoides*. The LC$_{50}$ of test material as calculated by probit analysis was
138 ppm as compared to that of standard pyrethrins which measured 0.0458 ppm.

Kamal and Mehra (1991) reported the activity and compatibility tests of pyrethrins extracted from the floral heads of *Tagetes minuta*. These isolates were also subjected to compatibility tests with rotenoids extracted from *Indigofera tintoria* and synergistic action of pipernyl butoxide against third instar larvae of *An. stephensi*. LC$_{50}$ mortality data showed a good compatibility between the two natural insecticides, and significantly superior kill was achieved when synergized with piperonyl butoxide at the ratio of 1:8 over the unsynergized extract.

Amalraj *et al.*, (1991) worked on the activity of a new synthetic pyrethroid, tralomethrin (OMS-3048) against vector mosquitoes. Tralomethrin was tested for its insecticidal properties in the laboratory against normal strains of *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. sitiens*, *An. stephensi*, *An. culicifacies*, *Ae. aegypti* and *Armigeres subalbatus* and against strains of *Cx. quinquefasciatus* resistant to fenthion and malathion. Tralomethrin showed good larvicidal activity against all species tested. LC$_{50}$ ranged between 7.00x10$^{-6}$ and 9.10x10$^{-3}$ mg (ai)/1. Resistant strains of *Cx. quinquefasciatus* showed higher tolerance than the normal strain. Tralomethrin was more effective against adults of *An.*
culicifacies (LD$_{50}$ 0.18 µg/cm$^2$) than the other species. Residual activity of this compound lasted for 15 weeks on thatch surface at a dose of 50 mg (ai)/m$^2$ against all the mosquito species tested. In the field, this compound was effective for a period of 1-2 days in polluted water viz., cesspits and drains and 10-24 days in less polluted water as in cement tanks, when applied at the rate of 0.002-2.0 mg (ai)/l against immatures of Cx. quinquefasciatus.

Ansari et al., (1991) evaluated the potentiality of juvenile hormone compound JHM/S-31183 against immatures of mosquitoes in natural habitats. Of two formulations tested 1% emulsifiable formulation was marginally superior than the granule formulation. Adult emergence of An. stephensi was completely inhibited upto 12 weeks when 0.5% granule formulation was applied @ 0.04 ppm in wells against 50% inhibition upto 8 weeks in pools. However, in Cx. quinquefasciatus the percent inhibition of adult emergence varied from 52 to 90%. 100% inhibition in pools upto one week at 0.04 ppm in An. stephensi was also obtained with 1% emulsifiable formulation but the effect was diluted in successive weeks. The impact of this formulation was not much pronounced against Cx. quinquefasciatus.
Sorokin et al., (1991) described the specific sensitivity in blood sucking mosquitoes including *An. stephensi*. Several populations of malaria mosquitoes which were previously discovered to have different irritability to two insecticides of the organophosphorous group, such as fenitrothion and malathion. Individual comparison of fenitrothion and malathion irritability levels in laboratory colonies of *An. stephensi, An. atroparvus, Cx. pipiens, Ae. aegypti* and *Ae. togoi* and in the natural population of *An. martinius* has shown that irritability to the two chemicals is specific in all the six species. This is probably due to recognizing the insecticide molecules by means of a so-called “living group” which has different structure in fenitrothion and malathion.

Baktharatchagan and Vasantharaj (1991) reported the evaluation of Trebon for insecticidal efficacy against mosquito larvae. Trebon (Ethofenprox) which is a new compound was evaluated against three species of mosquito larvae in the laboratory. Larval LC$_{50}$ values revealed that *Cx. quinquefasciatus* was more susceptible than *Ae. aegypti* and *An. stephensi*. The residual toxicity of Trebon was studied against culicine larvae in the rice agroecosystem at rates ranging from 0.05 to 0.2 kg ai/ha. Effective control was obtained for even beyond five weeks at the larvicidal rate of 0.2 kg ai/ha. Trebon is not safe to non-target aquatic organisms.
Kumar et al., (1991) reported the role of mono-oxygenases as a mechanism of resistance to the synthetic pyrethroid, deltamethrin in the larvae of *Cx. quinquefasciatus* Sy., *Ae. aegypti* L. and *An. stephensi* Liston developed by laboratory selections with deltamethrin, DDT or deltamethrin and the synergist, piperonyl butoxide (PBO) in the ratio of 1:5, was investigated. There was a significant correlation with mono-oxygenase activity and larval LC$_{50}$ to deltamethrin in various strains of all the three species. The present data, therefore, clearly suggest that deltamethrin resistance of the larvae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* is mainly due to the detoxification of deltamethrin by microsomal mono-oxygenases. High activity of G6PD observed in DDT-selected strains seems to be related to its role as a rate-limiting enzyme in GSH-dependent dehydrochlorination of DDT.

Efird et al., (1992) described the efficacy of various ground applied pyrethroids against adult mosquito of *An. spp.*, in the rice growing region of Arkansas. The ground-applied ULV, cold aerosol, insecticide sprays were evaluated against caged adult female *An. quadrimaculatus*. Treatments included 2 rates each of resmethrin, permethrin and a water-based permethrin formulation. Mortality at 24$^{th}$ post-treatment was not significantly (P$\geq 0.05$) different between the resmethrin and water-based
permethrin treatments. Both rates of permethrin were significantly (P<0.05) less effective than the other treatments.

Saxena et al., (1992) observed the effects of methanolic extract of *Ageratum conyzoides* which was used to observe the developmental defects on preimaginal stages of *An. stephensi*, a major vector for malaria in urban population. The crude extract was found to suppress the population of the vector at higher dosages, while lower dosages were found to induce several developmental defects and ultimately decreasing the growth index to a considerable extent.

Naqvi et al., (1994) reported the toxicity and abnormalities caused by neem fractions: RBU-9, RB-b and Margosan-O™ against 4th instar larvae of *Ae. aegypti* (PCSIR strain). RBU-9 is the UV-exposed air-dried ethanolic extract of ripe berries of neem whereas RB-b is the ethanolic extract of the outer coating of the neem ripe berries (excluding kernel). The third compound, Margosan-O™ was obtained by the courtesy of Mr. R.O. Larson and is a product of Vikwood Ltd., Sheboygan, U.S.A. It contains 0.3% azadirachtin. LC$_{50}$ (24h) was found to be 380 ppm, 490 ppm and 340 ppm, respectively. Most of the treated larvae were having narrow abdomen and the survived adults showed.
Tariq et al., (1994) reported the effects of fenpropathrin (pyrethroid) and two neem compounds (NfA and NfB) which were tested against the 4th instar larvae of Ae. aegypti L. (PCSIR strain), by using WHO method. The LC50 of fenpropathrin was found to be $2.25 \times 10^{-4}$ ppm.

Nagpal et al., (1995) reported about wood scrapings which were given the shape of a ball and soaked in 5, 10 and 20% neem (*Azadirachta indica*) oil diluted in acetone. Control of *An. stephensi* and *Ae. aegypti* breeding in water storage overhead tanks (OHTs) with the application of these balls was achieved for 45 days. Two balls soaked in 5% neem oil produced the best results among other concentrations tested.

Naqvi et al., (1995) reported the comparative effects of three neem products, Margosan-OTM, RBU-9 and RB-b against *Cx. p. fatigans* larvae. LC$_{50}$ was determined against the 4th instar larvae of *Cx. p. fatigans* and it was found to be 154.5 ppm, 502.5 ppm and 545.0 ppm for Margosan-OTM, RBU-9 and RB-b, respectively. Effect of neem fraction on acid and alkaline phosphatase was observed by LC$_{50}$ treatment of the respective three compounds. Inhibition of acid and alkaline phosphatase was observed in treated sample as compared to the untreated sample (control). Protein pattern by TLC method revealed inhibition and decreased mobility
of protein metabolites in LC$_{50}$ treated sample as compared to the control and check samples.

Bansal and Singh (1996) studied on the insecticide susceptibility tests on the adults of four anopheline species namely, *An. annularis*, *An. culicifacies*, *An. stephensi* and *An. subpictus* against the diagnostic doses of six insecticides, viz. DDT (4.0%), dieldrin (0.4%), malathion (5.0%), fenitrothion (1.0%), propoxur (0.1%) and permethrin (0.25%) in District Bikaner (Rajasthan). A time dependent effect has been observed with each insecticide. All the four species were found resistant to DDT and dieldrin and susceptible to fenitrothion and permethrin. *An. culicifacies* and *An. subpictus* showed susceptibility to malathion, while further verification for the other two species was required. However, with propoxur *An. annularis* showed resistance, whereas for other three species further studies are required. DDT and dieldrin, the two organochlorines, were found least effective as compared to organophosphates and carbamates.

Murugan et al., (1996) observed the effect of neem oil and neem seed kernel extract against mosquito larvae of *An. stephensi*. Antipupational effect of neem oil and neem seed kernel extract (NSKE) was evaluated against *An. stephensi*. As a result of treatment of water used for rearing, neem oil (5%) was more effective than NSKE, the larval
mortality being 99.3% as compared to 65.9% with NSKE (5%). But 10% NSKE gave 89.8% larval mortality. The percentage of pupation and adult emergence appeared to be dose-dependent, being respectively 19.3 and 11.2% with 3% neem oil as compared to 93.7 and 89.6% obtained in control. No pupation occurred with 5% neem oil treatment. With 5% NSKE, the adult emergence was 20.7%, whereas in 3% neem oil it was reduced to 11.2%. With 2.5% NSKE, the adult emergence was 54.8%, but drastically reduced to 3.1% when 10% NSKE was used vis-à-vis control (89.6%). Evidently, the percentage of pupation and adult emergence were markedly reduced by neem oil and NSKE treatments, suggesting the growth regulatory effects and post-ingestive toxicity of neem extractives in mosquito control.

Muse et al., (1996) reported the effect of boiled extract of the neem leaf against the larvae of An. gambiae. They suggested that the boiled extract of the neem leaf of Azadirachta indica inhibited the growth of larvae and caused a delay in pupation of An. gambiae. The effect was dose-dependent resulting in the death of larvae and formation of larval-pupal intermediates in different concentrations of extracts. There was no significant difference in the number of emerging adults and pupae
indicating that the extract has no effect on the development of the pupae and subsequent emergence of adults.

Naqvi (1996) published an article “Prospects and development of a neem based pesticide in Pakistan”. In which, the review of the work done on neem based pesticides, internationally and locally has been done. Various fractions and formulations of neem extract developed by H.E.J. Research Institute of Chemistry, University of Karachi, have been discussed.

Ansari et al., (1998) described the efficacy of synthetic pyrethroid-impregnated fabrics against An. stephensi, Ae. aegypti and Cx. quinquefasciatus, under laboratory conditions. Results revealed that deltamethrin was significantly superior in comparison to λ-cyhalothrin and cyfluthrin. Results of bioassay tests revealed that deltamethrin was 1.5 and 1.0 times more effective than λ-cyhalothrin and cyfluthrin, respectively, against An. stephensi exposed to cotton fabric treated at 100 g/m². Deltamethrin was 3.9 and 4.6 times more effective against Ae. aegypti and 3.53 and 4.0 times more effective against Cx. quinquefasciatus. Of cotton, nylon, polyethylene, and jute fabrics, the cotton was the best on the basis of median lethal dose (LD₅₀) and 95% lethal dose (LD₉₀) values and persistence of insecticide.
Naqvi (1998) published a review article of the research on medicinal and pharmacological aspects of neem, leaving aside the pesticidal aspect on which thousands of articles have been published by neem research workers throughout the world. Several books have been edited by Prof. H. Schmutterer of Giessen (Germany) and the work on pesticides in Pakistan has been reviewed by Naqvi (1998). Brief account of the chemistry, which was started by Prof. B.S. Siddiqui and later by Prof. W. Kraus and his team in Germany is given. The up-to-date research on pharmacological aspect has been given in this article.

Wang (1999) reported resistance to two pyrethroids in *Anopheles sinensis* from Zhejiang, China. Probabilities of pyrethroid resistant genotypes in natural populations of *Anopheles sinensis* Wiedemann were measured with deltamethrin and permethrin. The median lethal concentrations (LC$_{50}$s) of deltamethrin and permethrin in the susceptible larval population were 0.0209 and 0.1747 ppm, respectively. Under dosages that produced 99% mortality in susceptible laboratory strains of larvae, the lethal percentage of Cangnan larval field populations after 20 minutes of exposure was only 61.23% for deltamethrin and 64.92% for permethrin. This was much lower than those of other natural populations. Also, the probability of pyrethroid-resistant genotypes in Cangnan adult
Field populations was at the highest, reaching 0.5867. The results are discussed in relation to future mosquito control programs.

Amadioha (2000) reported that oil extracts exhibited the best control of the pathogen and subsequent disease followed by ethanol extract, cold water and then hot water extracts. The oil, ethanol and cold water extracts of neem compared favourably with carbendazim at 0.1% a.i in controlling the pathogen in vivo. Neem appears to have the potential to be used for managing rice blast in the field.

Caraballo (2000) described mosquito repellent action of Neemos®. 2-3 ml of Neemos®, when applied to the exposed body parts of human volunteers, provided protection for 8h from the bites of all anopheline species in two villages of the Sucre municipality, Bolivar State, Venezuela. Neemos is safe and can be used for protection from malaria in endemic countries.

Khan et al., (2000) observed the toxicity of neem extract (RB-a) against An. stephensi (mosquito) and Aphanius dispar (Killifish). The applied concentration of RB-a against Aphanius dispar were 20, 30, 40, 50 and 60 ppm, while the mortality percent was 6.67, 36.67, 53.33, 80 and 90%, respectively. The LC$_{50}$ was found to be 36.37 ppm and LC$_{90}$ was found to be 61.04 ppm. The applied concentrations of RB-a were 30, 60,
90, 120 and 150 ppm against *An. stephensi*, the mortality percent were observed as 15, 40, 60, 80 and 85%, respectively. The LC$_{50}$ against this pest was found to be 69.4 ppm and LC$_{90}$ was found to be 182.77 ppm.

Kolaczinski and Curtis (2000) reported comparison of two $\alpha$-cyano pyrethroids when impregnated into bednets against a pyrethroid resistant and susceptible strain of *An. stephensi* (Diptera: Culicidae) and their F$_1$ progeny. The two $\alpha$-cyano pyrethroid insecticides $\lambda$-cyhalothrin and $\alpha$-cypermethrin were tested as bednet treatments at a target dose of 20 mg m$^{-2}$. To establish their efficacy, female pyrethroid resistant and susceptible *An. stephensi* Liston, and the F$_1$-hybrids were allowed to fly freely in a room with a human subject under an impregnated net. Both treatments provided good personal protection by significantly reducing the number of blood fed mosquitoes compared to an untreated control net. Mortality after 24h was significantly higher for the $\alpha$-cypermethrin treated net when compared to $\lambda$-cyhalothrin. For each insecticide there were no significant differences in the proportion of susceptible homozygotes and F$_1$-hybrids found dead after a 24h holding period, which suggests that there would be no selection for pyrethroid resistant heterozygotes by either of the insecticides.
Latha and Ammini (2000) studied the leaves and tuber of *Curcuma raktakanda* as a mosquito larvicid against the early fourth instar larvae of four mosquito species, viz., *Cx. quinquefasciatus*, *Cx. sitiens*, *Ae. aegypti* and *An. stephensi*. The petroleum ether extract of the leaves and tuber exhibited toxicity towards all the test species. The LC$_{90}$ values of leaf extract for *Cx. quinquefasciatus*, *Cx. sitiens*, *Ae. aegypti* and *An. stephensi* were 46.77, 27.45, 58.75 and results suggested that a positive correlation of GST and DDTase activity might be species dependent.

Pathak *et al.*, (2000) reported the larvicidal action of essential oils from plants against the vector mosquitoes *An. stephensi* (Liston), *Cx. quinquefasciatus* (Say) and *Ae. aegypti* (L.). The larvicidal effects of essential oils extracted from the leaves of four plants, i.e. *Tagetes erecta*, *Ocimum sanctum*, *Mentha piperita* and *Murraya koenigii* have been evaluated against three major vector mosquito species namely, *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. *T. erecta* leaf oil was found to be the most effective at lower concentrations followed by *O. sanctum*, *M. piperita* and *M. koenigii*. The order in which the three mosquito species was affected by the four oils was different for each oil.

Siddiqui *et al.*, (2000) isolated two new triterpenoids, 6α-O-acetyl-7-deacetylnimocinol and meliacinol from the methanolic extract of the
fresh leaves of *Azadirachta indica* (neem). Their structures have been elucidated through spectral studies, including 2D-NMR (COSY-45, NOESY, HMQC and HMBC). The bioactivity of these as well as of nimocinol, reported earlier from the same source, is reported. The first compound and nimocinol showed toxicity in fourth instar larvae of mosquitoes (*Ae. aegypti*) with LC$_{50}$ values of 21 and 83 ppm, respectively. The second compound had no effect up to 100 ppm.

Tariq and Zafar (2000) reported about the population of Dengue vector mosquitoes increasing day-by-day in Karachi and other areas of Sindh, Pakistan, although continuous spray at city level, but population level did not change. It increases in every year passing.

Thomas *et al.* (2000) observed the insecticidal properties of essential oil of *Cannabis sativa* Linn. against mosquito larvae. Laboratory studies carried out with the essential oil of an indigenous plant, *Cannabis sativa* to evaluate its mosquito larvicidal properties revealed that the oil could induce 100.0 percent mortality at concentrations of 0.06, 0.1, 0.12 and 0.2 ml/litre of water in the larvae of *Cx. tritaeniorhynchus*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* respectively. The LC$_{50}$ and LC$_{90}$ values estimated for *Cx. tritaeniorhynchus*, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were 0.0101 and 0.0295, 0.026 and 0.0749,
0.0273 and 0.0919 and 0.0453 and 0.1803 respectively. The essential oil of *C. sativa* plant was found to be more toxic to *Cx. tritaeniorhynchus* followed by *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus*. The aqueous oil extract was found to be more toxic than the ethanolic extract.

Hopwood *et al.*, (2001) reported that many insects are able to adjust their egg production according to physiological conditions such as nutrient supply and mating success. One way in which this is achieved is by resorption of some, or all, of the ovarian follicles at some stage during oogenesis. We have shown that the mosquito *An. stephensi* responds in this manner when ookinetes of the malaria parasite *Plasmodium yoelii nigeriensis* first begin to invade the midgut. Ultrastructural studies show that patches of follicular epithelial cells translational reading frame. A novel insertion derived from intron sequence was predicted to introduce in-frame stop codons following exon 11. Two truncated novel exon 1 variants were identified that are homologous to a previously published 5 sequence for this exon. The large number of AsNOS transcripts and diversity in AsNOS splicing and exon 1 sequences indicate that transcriptional complexity is a hallmark of both invertebrate and vertebrate NOS genes.

Tariq *et al.*, (2001) worked on the comparison of extract from neem fruit seed extract (RB-a) and neem fruit coat extract (RB-b) for the toxicity
and IGR effects against the fourth instar larvae of *Aedes aegypti* L. (Orangi Town wild strain) according to WHO method. The RB-b was found to be more effective and better IGR than RB-a. The LC$_{50}$ of RB-a was found to be 446 ppm while that of RB-b was found to be 319 ppm. The RB-a contains azadirachtin whereas RB-b has other compounds. e.g. meliacinin and azadirionic acid.

David *et al.*, (2002) reported the larvicidal properties of the dietary leaf litter originating from the vegetation surrounding the subalpine mosquito breeding sites were investigated by using 10-month decomposed alder leaf litter against different field collections of culicine taxa of various ecological origin (*Ae. cantans, Ae. caspius, Ae. cataphylla, Ae. detritus, Ae. punctor, Ae. pullatus, Ae. rusticus, An. claviger, Cx. hortensis, Cx. pipiens, Culiseta morsitans*). Larvae originating from sites with polyphenol-poor vegetation appeared more sensitive to ingested leaf litter than those originating from sites with polyphenol-rich vegetation. Within a given taxon (e.g., *A. rusticus, A. cataphylla, C. hortensis*), the overall levels of cytochrome P450 monooxygenase and esterase activities appeared higher in larvae able to feed on leaf litter than in pupae and adults unable to feed on leaf litter. This suggests the involvement of these enzymes in the detoxification mechanisms responsible for larval tolerance.
to polyphenols of the dietary leaf litter. Such a tolerance of the larval stage thus appears as fundamental in the ecotoxicological adaptation of mosquito taxa to the polyphenolic profiles of the riparian vegetation.

Madhyastha and Shetty (2002) reported that *An. stephensi*, an important vector of malaria continues to be distributed widely in the Indian subcontinent. This vector species has developed resistance for various insecticides. Therefore, it is desirable to develop alternate strategy, which does not involve resistance. In order to develop such strategy, it is mandatory that genetic studies of concerned vector species should be established. This paper describes the isolation and genetic studies of an eye colour mutant, ruby-eye (ru), and linkage studies involving another autosomal recessive mutant grayish brown larva (grb ru) in *An. stephensi*.

Tariq *et al.*, (2002) described the activity of extracts from neem fresh fruit seed extract (RB-a) and fresh fruit coat extract (RB-b) (also called FFS and FFC) to compare the toxicity and IGR effects of these against 4th instar larvae of malaria vector mosquito, *Anopheles stephensi* Liston (Orangi Town wild strain) according to WHO method. The fruit coats extract (RB-b) was found to be more effective and better IGR than fruit seed extract (RB-a). The LC$_{50}$ of RB-a was found to be 784 ppm while that of RB-b was found to be 290 ppm. RB-a contains azadirachtin
whereas RB-b is devoid of azadirachtin and has other compounds, including azadiradione, epoxyazadiradione, meliacinin, azadironic acid etc.

Tariq et al., (2004) studied toxicity of sixteen pure compounds from the fruit-coat of Neem Tree (Azadirachta indica A. Juss) against An. stephensi Liston. They used sixteen compounds against 4th instar larvae of malaria vector mosquito An. stephensi. The LC50 value of (1) Azadiradione (2) nimbocinol (3) 17-β-hydroxy nimbocinol (4) azadirone (5) deoxygedunin (6) gedunin (7) α-nimolactone (8) β-nimolactone (9) 14.15-Epoxyazadiradione (10) desfuranoazadiradione (11) meliacinine (12) azadironic acid (12a) methyle easter (13) limocin-A (14) limocin-B (15) desfuranoazadiradione (16) 23, 23-dihydronimocinol (1-16) in sequence was found to be: 15, 30, 15, 10, 150, 120, 60, 45, 18, 37, 13, 4.5, 2.8 (12a), 19, 19, 43 and 60 ppm respectively.

Tariq et al., (2006) described the toxicity of various fraction and subfractions from fruit coats of neem (Azadirachta indica A. Juss) against An. stephensi Liston. These fractions were tested against 4th instar larvae of An. stephensi Liston. These results were compared with permethrin (25 EC), showed mean mortalities at different doses with SD value. The extract of neem fruit coats alone (RB-b) was fractionated into various fraction i.e., EtoAc, RB-b ‘A’, RB-b ‘N’, PE ‘1’, The LC50 of RB-b and its
fractions in sequence was 290, 165, 142, 43, 159, 154, 106 and 15 ppm respectively.

Aldemir and Boscelmez (2006) described population dynamics of adult and immature stages of mosquitoes in Golbasi District, Ankara. They reported 9 mosquito species in 15 breeding habitats, most abundant species was *Cx. pipiens*. They observed that population dynamics of the all mosquito species in the July-September period in Golbasi District, Ankara.

Deressa *et al.*, (2006) they examine the relationship between population and malaria transmission in Ethiopia, because malaria is the number one public health problem in Ethiopia. Study of all malarian areas in Ethiopian lowland to highlands revealed that since 1965 to 2005.

Yasmin *et al.*, (2008) observed effect of a neem sample on protein patterns of *Bactrocera cucurbitae*. Adults were reared with different solution, females were provide to lay eggs. 23rd Instar expose to treated filter paper and determined LC$_{50}$, were calculated as 5.6%.
3. MATERIALS AND METHODS

3.1. POPULATION DYNAMICS:

The present investigation covered a period from 2004-2007. During this 4 years study, samples of mosquito larvae and adults were collected from Dhabejee, District Thatta and six different points belonging to five district or eighteen towns of Karachi. The amount and accuracy of information obtained about the mosquitoes population depends on the sampling and processing of the material.

In sampling the following aspects were taken into consideration: place, time, method, and the environmental conditions prevailing at the time of collection. Method used for processing of samples depends on the objective of the study and on the intended use of the data obtained. When the only information required is the population of immature and adult mosquitoes. Relative method was applied (Bishop et al., 1994).

3.1.1. Description of study area:

Mosquito samples were collected arbitrarily along most of the major areas of all five districts, such as district South, East, West, Central and Malir of Karachi city, and Gharo farm house and Dhabejee from district Thatta.
All promising sites were first inspected for the presence of mosquito larvae. After a series of sampling different habitats like pool, ponds, ditches, borrow pits, seepage canal and different types of artificial containers (Tyre tub, Buckets, overhead tanks, etc.) within different districts were selected. Localities of different districts are as under:

3.1.2. Districts of Karachi City

i. District East (D.E):

Gulshan Chowrangi, Nipa chowrangi, New Dhorajee (Block-4), Aziz Bhatti Park, and Urdu College Railway Station (13-D Area) were included for collection.

ii. District West (D.W):

Qasba, and Orangi town included in District East. Orangi town is a well known area of Karachi city. The whole area is heavily populated. Rahmat Chowk and around Qatar Hospital area was selected as sample point because different habitats were available for mosquitoes larvae breeding (collection).

iii. District South (D.S):

Bihar Colony, Civil Hospital, Baba-e-Urdu Road, Ranchore Line, Kharadar and Liyari trans park area. Mosquito breeding mostly occurs in pools and overflow water near park area, some seepage water also.
iv. District Central (D.C):

Paposh Nagar, Nazimabad, North Nazimabad, New Karachi, Surjani Town, Golimar, Liaquatabad, Firdous Colony and Pak Colony (Old Golimar) are well known areas. There is a Nala at the junction of Firdous Colony and Liaquatabad. That was a permanent collection point, beside this small pockets of water were available throughout the year.

v. District Malir (D.M):

Model Colony, Malir, Landhi and Gulzar-e-Hijri scheme-33 area, behind Karachi University are areas along Super Highway and Liyari River. Most of the populations consist of Katche abadi grown without any planning. Liyari Nadi is main mosquito breeding place. Due to growth of plants, the flow of water is slow, some ditches and water pockets are also present.

vi. District Thatta:

Dhabejee District Thatta is a populated area and the main market area near dispensary was selected as sample point. It is a mixed population area, with small houses behind the small colony, there was a big pond and within community some small pools, tanks, borrow pits due to construction was available for mosquitoes collection.
District Thatta was selected as collection point. There are many farm houses in Thatta, but Thakleeq Farm House is the best area in Thatta, for research point of view. Small pools, ditches, Borrow pits and seepage of rice field were present as mosquitoes breeding habitats.

3.1.3. Identification characters of different mosquito species (Larvae)

1- *Anopheles annularis*

1. Without a pronounced siphon on the last abdominal segment.

2. Inner clypeal hairs (ICH) widely spaced, distance greater than both ICH bases combined.

3. Outer clypeal hair branched.

4. Sutural hair simple or bifid at tip.

2- *Anopheles culicifacies*

1. Without a pronounced siphon on the last abdominal segment.

2. Inner clypeal hairs (ICH) widely spaced, distance greater than both ICH bases combined.

3. Outer clypeal hair simple.

5. Both mesothoracic hairs simple, palmate hairs of abdominal segment-1 plate-like (may be reduced to size).

6. Thoracic palmate hairs well developed.

3- *Anopheles nigerrimus*

1. Without a pronounced siphon on the last abdominal segment.
2. Inner clypeal hair ICH closely spaced, distance between bases no greater than the width of the hair base.
3. Outer clypeal hair branched.

4- *Anopheles pulcherrimus*

1. Without a pronounced siphon on the last abdominal segment.
2. Inner clypeal hairs (ICH) widely spaced, distance greater than both ICH basis combined.
3. Outer clypeal hair branched.
4. Sutural hair branched near base with more than two branches.

5- *Anopheles stephensi*

1. Without a pronounced siphon on the last abdominal segment.
2. Inner clypeal hairs (ICH) widely spaced, distance greater than both ICH basis combined.
3. Outer clypeal hairs simple.
4. One mesothoracic hair simple, other pectinate, inner clypeus slightly pectinate, thoracic palmate hair inapparent.
5. Palmate hair of abdominal segment Hair-like, reduced in size.

6- *Anopheles subpictus*

1. Without a pronounced siphon on the last abdominal segment.
2. Inner clypeal hairs (ICH) widely spaced, distance greater than both ICH bases combined.
3. Outer clypeal hair simple.
4. Both mesothoracic hairs simple, palmate hairs of abdominal segment-1 plate-like (may be reduced to size).
5. Thoracic plamate hairs non-differentiated.

1- *Culex bitaeniorhynchus*

1. With a pronounced siphon on the last abdominal segment.
2. Siphon longer, not saw-like.
3. Siphon with two or more sub-ventral tuft, base with distinct acus.
4. Siphon usually longer, pectin teeth restricted to basal ½ siphons.
5. Clypeal hair short and thick, ends blunt, darkly pigmented.
6. Pectin teeth obscure greatly reduced restricted to base 1/5 of siphon.
7. Comb scales 4-8, larvae usually associated with filamentous green algae.

2- *Culex tritaeniorhynchus*

1. With a pronounced siphon on the last abdominal segment.
2. Siphon longer, not saw-like.
3. Siphon with two or more sub-ventral tuft, base with distinct acus.
4. Siphon usually longer, pectin teeth restricted to basal ½ siphons.
5. Clypeal hair short and thick, ends blunt, darkly pigmented.
6. Pectin teeth extending beyond basal 1/5 and very noticeable.
3- *Culex psudoviashnui*

1. With a pronounced siphon on the last abdominal segment.

2. Siphon longer, not saw-like.

3. Siphon with two or more sub-ventral tuft, base with distinct acus.

4. Siphon usually longer, pectin teeth restricted to basal ½ siphons.

5. Clypeal hair short and thick, ends blunt, darkly pigmented.

6. Pectin teeth extending beyond basal 1/5 and very noticeable.

7. Comb scales variable in number, median spicule greatly enlarged, long and pointed.

8. Siphononal tufts two or more simple hair.

9. Comb scales 5-13 in numbers.

4- *Culex fuscocephalus*

1. With a pronounced siphon on the last abdominal segment.

2. Siphon longer, not saw-like.

3. Siphon with two or more sub-ventral tuft, base with distinct acus.

4. Siphon usually longer, pectin teeth restricted to basal ½ siphons.

5. Clypeal hairs long and thin, tapering to a fine point, hairs usually curved and unpigmented.

6. Upper and lower head hairs with 2 or 3 branches.

5- *Culex p. fatigans*

1. With a pronounced siphon on the last abdominal segment.

2. Siphon longer, not saw-like.
3. Siphon with two or more sub-ventral tuft, base with distinct acus.
4. Siphon usually longer, pectin teeth restricted to basal ½ siphons.
5. Clypeal hairs long and thin, tapering to a fine point, hairs usually curved and unpigmented.
6. Upper and lower head hairs with 5 or 6 branches.
7. Annal saddle hair single, siphonal index 3:1.

6- *Culex vagans*

1. With a pronounced siphon on the last abdominal segment.
2. Siphon longer, not saw-like.
3. Siphon with two or more sub-ventral tuft, base with distinct acus.
4. Siphon usually longer, pectin teeth restricted to basal ½ siphons.
5. Clypeal hairs long and thin, tapering to a fine point, hairs usually curved and unpigmented.
6. Upper and lower head hairs with 5 or 6 branches.
7. Annal saddle hair bifid or trifid, siphonal index 4:1 or greater.

*Aedes.*

1. With a pronounced siphon on the last abdominal segment.
2. Siphon longer, not saw-like.
3. Siphon without acus, only one siphonal tuft of setae present.
4. Antennal shorter than head, head hairs shorter than head length.
3.1.4. Larval Abundance:

Larval abundance was estimated by counting the number of larvae (*Culex, Anopheles* and *Aedes*) species found in ten to twenty (10-20) dips with a standard mosquito larvae dipper with extendable handle (2-3 feet). The numbers of dips varies with the size and depth of habitat, the density and dispersion of the larvae. Less dips were taken in small homogenous habitats such as ground pools, water-tub (for tyre repairing shops) having uniform mosquito abundance (approximately equal numbers of larvae per dips), while more dips were taken in heterogenous environment such as large ponds (Dhabejee and Gharo) drains etc., where mosquito larval abundance varied considerably from dip to dip.

All collections were taken back to the laboratory where 5-10 slides of different looking larvae were made and identification has done up to species level using binocular mosquitoes and standard keys (Du Bose and Curtin, 1905; Aslamkhan 1981; Harbach, 1985, 1988; Snow, 1990). The specimen and pertinent information were recorded in a database. Larvae from these dips collection were extracted, using strainer and droppers, than placed into a plastic bottle/jar (size: 5"x8") with wide mouth. Covered with net, having a hole in the centre, plugging with cotton.
Few larvae were preserved in a 50/50 mixture of water and 70% isopropyl alcohol. The remainder of the collection was reared to adults identified and counted. The percentage position of the total specimen identified, including both larval slides and emerging adult was used to estimate the mean numbers of larvae per dip for each species. Date, time, year and water temperature, number of individuals and current weather were recorded.

3.1.5. Physico-chemical conditions:

In order to study the physico-chemical conditions of water, different parameters were recorded. For this purpose concomitant with larval collections, water samples were also taken by dipping and filling the sample bottles (250 ml Brown bottles for O₂) at the edge of the every habitats of mosquitoes collection points. The samples could not be taken successfully in shallow, temporary ground pools. In large bodies of water, samples were taken with sampler.

Initially, all chemical analysis were performed in the field using a portable Hach Direct reading engineer's laboratory (Model OR EL/2 Hach chemical company, Ames, Lowa USA) and the procedures for CO₂, Alkalinity and dissolved oxygen outlined in Taras, et al., (1971).
To save time in the field, finally samples were brought to laboratory in an ice-box, refrigerated overnight, and analyzed. The following morning results were obtained in the laboratory. The next day data did not significantly differ from results obtained from concomitant samples analyzed in the field.

3.1.6. Physical parameters:

Physical parameters include water temperature, depth of the habitat, pH and conductivity. While chemical parameters include dissolved oxygen, total alkalinity, carbon dioxide, phosphates, nitrates, and sulphates.

a) Water temperature:

Water temperature was taken with the help of mercury thermometer. Thermometer put at the edge of habitat, where larval and water samples were collected. Reading here were most likely higher than those in deeper water, however they did represent the conditions under which the mosquitoes larvae were breed.

b) Depth:

Depth were measured by simple scale. Only that depths were measured from where samples were collected.
c) **Power of Hydrogen Ions (pH) and conductivity:**

The pH and conductivity of different samples were determined with pH meter (JENCO 607) and conductivity meter (MC-1 Mark 5) followed by calibration with standard solution.

**3.1.7. Chemical parameters:**

Chemical parameters include dissolved oxygen in mg/l, pH, Carbon dioxide, Nitrate, Sulphate, and Phosphate.

a) **Dissolved oxygen:**

Dissolved oxygen in mg/liter was estimated using Azid modification of the wrinkler method (Taras, *et al.*, 1971) with samples fixed in the field within 30 min. of collection.

b) **pH:**

pH was measured initially using pH-papers and wide range calorimetric method (Hach, 1973) and later using a corning Model 610 pH meter.

c) **NO₃, SO₄ and PO₄:**

Initially nitrate, sulphate and phosphate were measured by volumetric analytical method, but later on Hach Aqua check Water Quality
Test Stripe for phosphate (PO₄) sulphate (SO₄) and nitrate (NO₃), were used.

3.2. TOXICITY:

Potential or capacity of a test material (pesticides) to cause adverse effect on living organisms, generally a poison or mixture of poison. Toxicity is a result of dose or exposure concentration and exposure time, modified by variables such as temperature, chemical form, and availability. Toxicity tests are desirable in Water Pollution Evaluation because chemical and physical tests alone are not sufficient to assess potential effects on aquatic biota. Different species of aquatic organisms are not equally susceptible to the same toxic substances nor are organisms equally susceptible throughout the life cycle. In addition, organisms of the same species can respond differently to the same level of a toxicant from time to time.

Toxicity tests are useful for a variety of purposes that include determining:

a) Suitability of environmental conditions.

b) Favorable and unfavorable environmental factors.

c) Toxicity of wastes to a test species.
d) Relative sensitivity of aquatic organisms to an toxicant.

e) Pesticides are being added to ecosystem in an agricultural country specially to water, soil and plants.

f) It also depends on the species variations, susceptibility and tolerance of various chemicals due to sub-lethal or continuous exposure to pesticides.

3.2.1. Pesticides Used:

**Biosal 10 EC (azadirachtin a.i.):**

For the last two decades, a search has been going on for pesticide composition which would deter insects or other pest but would have done none or minimum harmful effect on the environment. In this context, a wealth of literature has been accumulated on the effectiveness of plants for pest control. Heading the list, from the standpoints of number of pest species affected, high quality, availability, safety and resistance to predators, is the subtropical "Neem" Tree, *Azadirachta indica* A. Juss, a member of the plant family Meliaceae.

Through empirical testing throughout the world azadirachtin rich extracts have been developed and marked for use in controlling the pest of edible and none edible crops. The HEJ Research Institute of Chemistry,
University of Karachi has developed a storage stable composition with the name of BIOSAL after year's long research. The formulated composition comprises about 3000ppm (0.32%) azadirachtin and about 7.5% neem oil. Neem oil is a torpenoid constituent of neem seed kernel contains azadirachtin as active ingredient. It consists of azadirachtin salannin, nimbin, deacetylnimbin, azadirone, azadiradione, epoxyazadiradione and azadironic acid along with several other minor triterpenoids.

Azadirachtin has antifeedant and growth regulation potency against several pests. Salannin, nimbin and deacetylnimbin, on the other hand have repellent action.

The physical properties of the formulated product Biosal is brown coloured syrup with slight fruity (vinegar like) smell. Its pH is in between 3-4 and contains about 0.5-1.0% of a sunscreen to prevent degradation. An emulsifying agent has been added to Bioaman, so that it may be dissolved in water easily to spray on water pounds various crops, vegetables, fruits and ornamental plants.

It is a storage–stable composition (retaining more than about 80% of its potency when in the form of an emulsion for 8-10 weeks and 65% of its potency even after two years. The standardization of Biosal is based on the main active ingredient, azadirachtin which is a white microcrystalline
solids. Melting point 155-8°C patented in Pakistan and registered by APTA committee for commercial use.

The efficacy of Biosal has been tested and found effective against various species of mosquitoes.

2. *Acorus calamus* extract (β–azarone a.i.):

*Acorus calamus* is a local plant found in Pakistan. The common name of this plant is sweat flag, also called as Butch in Urdu. The extract of the plant was extracted from its dried rhizome. The extraction work was carried in International Centre for Chemical and Biological Sciences, University of Karachi, under Indigenous Plant Based Pesticide project, funded by Govt. of Sindh to H.E.J. Res. Inst. of Chemistry and Department of Zoology.

The extract of contained 2% twin–80 as emulsifier. The main active ingredient, responsible for insecticide activity in β–azarone.

3. Deltamethrin (Pyrethroid):

Deltamethrin (10 EC) is a synthetic pyrethroid, commonly used as insecticide. It is highly toxic to insects therefore low concentration is effective. Due to high toxic effect 0.1% stock solution was made. After preliminary tests the following concentration were made from 1% stock
solution. The final concentrations were 0.1000, 0.0500, 0.0250, 0.0125 and 0.0065.

3.2.2. Preparation of chemicals

A. Biosal:

The 10% stock solution was prepared in distilled water. After preliminary test, five different doses were set, which are 2.5ml, 3.0, 3.5, 4.0 and 4.5ml and poured in 200ml water. For this purpose beaker of 250ml were used, containing 20 mosquito larvae/per beaker. The experiment was set in duplicate (W.H.O. 1970). The result for percent mortality was noted after 24 hours and the data was tabulated and analyzed according to Abbot Formula (Abbot, 1925).

B. Acorus calamus extract:

The oil containing 2% emulsifier of A.C. was considered as 100%. The 1% stock solution was made by diluting 1ml in 99ml of distilled water. The preliminary test were carried out and then five doses 2.0, 2.5, 3.0, 3.5 and 4.0ml in 200ml water were applied in 250ml beaker, having 20 mosquito larvae/beaker. The method adopted is known as World Health Organization method (W.H.O. 1970). The result were noted after 24 hours and the data was tabulated and analyzed by Abbot Formula (Abbot, 1925).
C. Deltamethrin:

0.1ml of deltamethrin was diluted in 100ml distilled water, making 0.1% solution. Then 25ml of 0.1% solution was diluted four times, in distilled water making 0.025% stock solution. The 0.1ml of stock solution (0.025%) was diluted in 100ml of water and then it was halved by mixing 50ml water+50ml solution making 0.0500%. In this way 0.0500%, 0.0250%, 0.0125%, 0.00625%, and 0.00312%, were made and applied in 200ml of water.

Further dilution were prepared in the same way. The following formula may also be used for the same purpose.

\[ C_1 V_1 = C_2 V_2 \]

3.2.2. Toxicity determination

Method of Treatment:

A group of 20, fourth instar mosquitoes larvae having uniform age and size was released in 250 ml beakers (WHO, 1970) using WHO method in 200ml of water. The mosquito larvae were treated with respective neem formulation (Biosal), Standard Deltamethrin (Pyrethroid) and Acorus extract having different concentrations. A set of six beakers was set up for each neem product in duplicate, five for five different concentrations, and
one for control. For standard Deltamethrin also (Pyrethroid), six beakers were set up, five for five different concentrations and one for control, but the 7 beaker for check was not set up because Deltamethrin was diluted in distilled water. Mortality counts were made after 24 hours. Each experiment was repeated 5 times. If in any experiment mortality rate increased more than 10% in the case of control the experiment was discarded. Moribund larvae were counted as dead. The observations were analyzed according to Abbot's Formula (1925) and recorded in the form of Tables.

**Lethal Concentration (LC$_{50}$) Values:**

Average values were calculated and mortality curve was drawn by HP Laser Jet 30 Software to find out LC$_{50}$ by taking dose on x-axis whereas the percent mortalities on y-axis for each compound or fraction. Statistical analysis was also done.

**Calculations:**

Formulae used for statistical calculations are as under:

\[
\text{Abbot's formula} = \frac{\% \text{ Mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 100
\]
S.D. = \sqrt{\frac{\sum x^2 - n \bar{x}^2}{n - 1}}

S.D.
S.E. = \frac{\text{Range}}{\sqrt{n}}

Range = X + 2.58 \times S.E.
C_1 \times V_1 + C_2 \times V_2

where,

\sum x^2 = \text{The notation for variance of variable x.}
n = \text{The total number of observations.}

X = \text{The average of variable x.}

S.E. = \text{Standard error.}
S.D. = \text{Standard deviation.}
C_1 = \text{Initial concentration of stock solution or original pesticide.}
C_2 = \text{Final concentration of the solution to be made.}
V_1 = \text{Volume of the stock solution or original pesticide to mixed or diluted.}
V_2 = \text{Volume of the solution to be made.}

3.3. BIOCHEMICAL ESTIMATION OF ENZYMES

a) Preparation of Homogenates:

For the biochemical analysis 200 Culex mosquito larvae were crushed in 4 ml bi-distilled water with mortar and pestle. They were
homogenized in OSK-9258 tissue grinder for 5 minutes at 1000 rpm. The homogenates were centrifuged at 5000 rpm for 15 minutes in LABOFUGE 2000, Heracus placed in cold chamber. Supernatants were taken in separate tubes and were used for biochemical experiments. During experiments the homogenate and reaction mixtures were kept in ice at 5°C approximately. This homogenate was used for the estimation of GOT and GPT. Enzymes were selected on the basis of previous literature. Instead of Sigma kits (generally used in USA) Croma test (linear chemicals S.L) kits were used as they are cheaper and easily available. These kits are being used in Medical Research at Medical and General Universities in developing countries. Procedure given with the kits was followed.

b) **Estimation of glutamate oxaloacetate transaminase (GOT); L-Asparate, 2-Oxoglutarate aminotransferase (E.C. 2.6.1.1) activity:**

Activity of glutamate oxaloacetate transminase (GOT) was determined by the colorimetric kit method, of Cromatest Lot No. 086936-39G, which is based upon the method of Reitman and Frankel (1957), for determination of serum aspartate aminotransferase.
**Principle:**

\[
\text{GOT} \\
\alpha - \text{oxoglutarate} + L - \text{aspartate} \rightarrow L - \text{glutamate} + \text{Oxaloacetate}
\]

Glutamic-oxaloacetic transaminase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl hydrazine.

c) **Estimation of glutamate pyruvate transaminase (GPT); L-alanine, 2-oxoglutarate aminotransferase (E.C. 2.6.1.2) activity:**

The activity of glutamate pyruvate transaminase (GPT) was determined by colorimetric kit method of Cromatest Lot No. 086580-391 M5 and REF. No. AL 146.

The estimation of GPT activity was essentially based on same principle as for GOT which is based upon Reitman and Frankel (1957) and the entire procedure adopted was almost the same as in the case of GOT, except for the buffer substrate solution which contains L-alanine and glutarate instead of L-asparate.

**Principle:**

\[
\text{GPT} \\
\alpha - \text{oxoglutarate} + L - \text{alanine} \rightarrow L - \text{glutamate} + \text{pyruvate}
\]

Glutamic-pyruvic transaminase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl hydrazine.
Assay Procedure for GOT and GPT:

Two sets of test tubes for control, check and treated in case of Biosal 10 EC (Neem formulation, while control and treated in the case of cypermethrin 10 EC (Pyrethroid) were taken for the measurement of activity. One set was labeled as S.B. (sample blank) and other set was labelled as R.B. (reagent blank). Half (0.5 ml) buffer substrate solution (R₁) in reagent blank and sample blank was taken and all the test tubes were kept in water bath for 5 minutes at 37°C. Test tubes were taken out and 0.1 ml homogenate in reagent blank mix was added, and incubated at 37°C for 30 minutes. Then 0.5 ml colour reagent was added in both sets and 0.2 ml homogenate in sample blank. It was mixed well and allowed to stand at room temperature for 20 minutes. Now 5 ml 0.4N NaOH solution was added in all the test tubes. Then after five minutes optical density (O.D.) of sample blank against the reagent blank at 546 nm by Schimadzu spectrophotometer UV-120, was noted and these values were calculated according to the table given in brochure.
The activity of GOT in the serum from the table.

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<th>U/l</th>
<th>Absorbance</th>
<th>U/l</th>
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The activity of GPT in the serum from the table.

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<th>U/l</th>
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3.4. Quantitative Analysis of Pesticide Residues By High Performance Liquid Chromatography:

During the recent years chromatographic technique have undergone a rapid development and high performance liquid chromatography (HPLC) is one of the achievement. The detection by this technique is simple, rapid and sensitive. HPLC have been used for the separation of pesticides, by using a packed column, (Zorbax TM NH₂) a polar bounded phase with particle size of about 7 µm in diameter. The columns were packed to uniform bed density by using a high pressure slurry loading technique. This column was used with fractionated n-hexane as mobile phase with a flow rate of 2.5 ml/min. A UV detector was used with a wavelength of 205 nm, pressure 200 kg/cm² and absorbance 0.08 with chart speed 10 mm/min., for the detection of pesticide.

Standard sample of Biosal, Acorus (AC) and Deltamethrin (D.M.) as well as the samples from treated Culex larvae were taken for HPLC analysis (all the three strains of mosquitoes treated with Biosal, deltamethrin and Acorus calamus extract were exposed for 24 hours, 48 hours and 72 hours to study residual effects.

Before the application of sample to HPLC, pesticide accumulated with fats should be extracted from the animal tissues (mosquito larvae),
and initial separation of interfering organic residue such as fats, pigments and other organics must be carried out. For each step involved in the method experiments were carried out in the following manner.

**Extraction of Fat from under test Insect tissues:**

The pesticides are lipophilic in properties and accumulate with lipids, therefore for the extraction of Biosal, Deltamethrin and *Acorus calamus* extract, fat must be extracted. For this purpose following procedures were adopted.

**Soxhalation method:**

For the extraction of pesticide residues from samples of mosquitoes larvae Holden and Marsden (1969) method was used. A known quantity of samples (200 mosquitoes larvae treated with Biosal, Deltamethrina and or Acorus extract separately, weighing about 100 mg) was macerated with anhydrous sodium sulphate (Na$_2$ SO$_4$) and was transferred into a thimble made of filter paper.

The thimble was then placed in the extractor which was fitted to the bolt head flask containing 60 ml. of n-hexane, which was then fitted with condenser connected to the tap water for cooling. The flask was then placed on a heating mantle. The process of extraction was carried out for two hours during which all the pesticide residues must have been extracted.
with solvent. Better recoveries were noticed by this method. The fat extracted solvent was then reduced to about 1 ml by evaporation. For complete recovery of pesticides the column chromatography (Sorption) was employed and the material was passed three to four times through the columns given below.

**Sorption:**

The process of sorption was carried out in chromatographic columns of alumina (Holden and Marsden 1969) and Silica (Kadoum 1967, 1968).

**Alumina and Silica columns:**

The alumina column was used as an alternative method for the separation of fat from pesticides which was used by Holden and Marsden (1969). The column was made by glass column having length 40-42 cm with internal diameter 6 mm. The column was filled with 2 grams of alumina without calcium of 0.3 micron size already, activated at 800°C for 4 hours in a furnace and then partly deactivated by shaking with 5% by weight of water. The concentrated extract was re-dissolved in 1 ml of n-hexane (fractionated) and transferred on the surface of the alumina column. Now the pesticides absorbed on the column were eluted with 12 ml of n-hexane and volume of eluted sample was then reduced to 1 ml which was passed through a new column. The new column of the same size was
packed with 2 gram of silica gel for column chromatography No. 60, 0.060 millimeter size, activated at 120°C for 2 hours, cooled and deactivated with 3.5% distilled water. For the removal of traces of moisture a layer of activated Na₂SO₄ was set on top of the silica-gel. The elution of pesticides was done by 5 ml of n-hexane and then with 12 ml of 10% diethylether in hexane. All the fractions were concentrated to 1ml separated and identified by HPLC. The same procedure was adopted using the different quantities of standard Biosal, Deltamethrin and Acorus extract per ml of n-hexane which are 2µg, 4µg and 6µg in case of Biosal, Deltamethrin and *Acorus calamus* extract. It was used to obtain standard peaks for comparison with the samples.

The chromatograph obtained by standard Biosal, Deltamethrin and *Acorus calamus* extract were used for comparison with the chromatograms obtained from treated samples of pesticides.
4. RESULTS

4.1. POPULATION DENSITY:

4.1.1. Results for the year 2004:

A total of 19414 mosquito larvae were collected and examined during January 2004 to December 2004, collectively from all, six Districts. Out of 19414 mosquito larvae, 3107 belonged to genus *Anopheles*, 15512 larvae were from *Culex* Genus and 795 larvae belonged to *Aedes* genus. (Table I).

Table also shows that population of all genera mostly occurs in those areas, which covers District Central but the population of *Culex* genus was most prevalent in all Districts among all three genera.

An in depth investigation of the larvae abundance in all six Districts of Karachi and Thatta, was initiated during January 2004 to December 2004. It shows that 19414 mosquito larvae were examined, out of 19414 larvae 16.0% were *Anopheles*, 79.9% were *Culex* and 4.0% belong to genus *Aedes*. (Table II). It also show that population of all three genera were high in District Central, but *Anopheles* showed no significant difference in all Districts, except District South *Culex* population was low in Thatta and Malir i-e. 10.49 % and 7.51% respectively. *Aedes* mosquito population was high in District Central and Malir but over all *Culex* population was much higher than *Anopheles* and *Aedes*. 
### TABLE I
District Wise Mosquitoes Larvae Collection Examined
(Jan 2004 - Dec 2004)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Collection Site</th>
<th>Total Mosquito Larvae Collected</th>
<th>Total <em>Anopheles</em> Mosquitoes</th>
<th>Total <em>Culex</em> Mosquitoes</th>
<th>Total <em>Aedes</em> Mosquitoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>District East</td>
<td>3825</td>
<td>544</td>
<td>3176</td>
<td>105</td>
</tr>
<tr>
<td>2</td>
<td>District West</td>
<td>3590</td>
<td>385</td>
<td>3130</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>District South</td>
<td>3236</td>
<td>188</td>
<td>2858</td>
<td>190</td>
</tr>
<tr>
<td>4</td>
<td>District Central</td>
<td>4495</td>
<td>739</td>
<td>3556</td>
<td>200</td>
</tr>
<tr>
<td>5</td>
<td>District Malir</td>
<td>2468</td>
<td>651</td>
<td>1627</td>
<td>190</td>
</tr>
<tr>
<td>6</td>
<td>District Thatta</td>
<td>1800</td>
<td>600</td>
<td>1165</td>
<td>35</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>19414</strong></td>
<td><strong>3107</strong></td>
<td><strong>15512</strong></td>
<td><strong>795</strong></td>
<td></td>
</tr>
</tbody>
</table>
TABLE II
Percentage Wise Mosquitoes Larvae Collection Examined
(Jan 2004 - Dec 2004)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Collection Site</th>
<th>Total Mosquito Larvae Collected</th>
<th>Total <em>Anopheles</em> Mosquitoes</th>
<th>%</th>
<th>Total <em>Culex</em> Mosquitoes</th>
<th>%</th>
<th>Total <em>Aedes</em> Mosquitoes</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>District East</td>
<td>3825</td>
<td>544</td>
<td>17.51</td>
<td>3176</td>
<td>20.47</td>
<td>105</td>
<td>13.21</td>
</tr>
<tr>
<td>2</td>
<td>District West</td>
<td>3590</td>
<td>385</td>
<td>12.39</td>
<td>3130</td>
<td>20.18</td>
<td>75</td>
<td>9.43</td>
</tr>
<tr>
<td>3</td>
<td>District South</td>
<td>3236</td>
<td>188</td>
<td>6.05</td>
<td>2858</td>
<td>18.42</td>
<td>190</td>
<td>23.90</td>
</tr>
<tr>
<td>4</td>
<td>District Central</td>
<td>4495</td>
<td>739</td>
<td>23.79</td>
<td>3556</td>
<td>22.92</td>
<td>200</td>
<td>25.16</td>
</tr>
<tr>
<td>5</td>
<td>District Malir</td>
<td>2468</td>
<td>651</td>
<td>20.95</td>
<td>1627</td>
<td>10.49</td>
<td>190</td>
<td>23.90</td>
</tr>
<tr>
<td>6</td>
<td>District Thatta</td>
<td>1800</td>
<td>600</td>
<td>19.31</td>
<td>1165</td>
<td>7.51</td>
<td>35</td>
<td>4.40</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>19414</td>
<td>3107</td>
<td>16.00</td>
<td>15512</td>
<td>79.90</td>
<td>795</td>
<td>4.09</td>
</tr>
</tbody>
</table>
Anopheles Population:

Table III show that a total of 3107 Anopheles mosquito larvae were collected comprising six (6) different species during January to December 2004. Out of all six species Anopheles culicifacies, (37.75%) was most common mosquito in Karachi and Thatta District. The second most common was Anopheles stephensi (21.82%).

Bar and pie (Fig. 1a and 1b) shows the relative abundance of different Anopheles species. It also shows that all the six species were recorded during whole year of 2004. Of the total record Anopheles culicifactus showed highest percentage i-e 37.75 % and Anopheles annilaris showed the lowest percentage which was 6.69% Anopheles puleherimus and Anopheles subpictus were very close to each other i-e 12.36% and 13.13 % respectively.

Graph and data (Fig. 2) shows seasonal variation of different Anopheles species, during 12 months period i.e. Jan 2004 to Dec 2004. It shows that Anopheles culicifacies were essentially bimodal with increased abundance depending on season. The 1st highest population was during middle of March and April, and the second peak was during September and October 2004. Graph also show that Anopheles stephensi was inversely related with temperature i.e. high population in winter (October and
November) and low in Summer i-e June and July, but *Anopheles subpictus* was directly related to temperature, man population increased with increase in temperature.
Table III
Different Percentage of *Anopheles* Species
In All Districts
Jan 2004 - Dec 2004

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Total</th>
<th>Relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Anopheles annularis</em></td>
<td>208</td>
<td>6.69</td>
</tr>
<tr>
<td>2</td>
<td><em>Anopheles culicifacies</em></td>
<td>1173</td>
<td>37.75</td>
</tr>
<tr>
<td>3</td>
<td><em>Anopheles nigerrimus</em></td>
<td>256</td>
<td>8.24</td>
</tr>
<tr>
<td>4</td>
<td><em>Anopheles pulcherrimus</em></td>
<td>384</td>
<td>12.36</td>
</tr>
<tr>
<td>5</td>
<td><em>Anopheles stephensi</em></td>
<td>678</td>
<td>21.82</td>
</tr>
<tr>
<td>6</td>
<td><em>Anopheles subpictus</em></td>
<td>408</td>
<td>13.13</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>3107</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
**Culex Population:**

A total of 15512 *Culex* mosquito larvae were collected during 12 month Jan 2004 to Dec 2004, from all Districts, comprising different species. In the genus *Culex*, the *Cx. p. fatigans* was the most common, and showed highest percentage i.e. (47.43%). The second highest was *Cx. tritaeniorhynchus* (34.20%). Percent population of *Cx. fuscocephalus* was 6.35% and *Cx. pseudovishnai* was 6.07%. Other two species *Cx. bitaenioorhyncus* and *Cx. vegans* were found in minimum percentage with 3.27 % and 2.68% respectively (Table IV).

Bar and pie (Fig. 3a and 3b) shows relative abundance of different *Culex* species. *Cx. p. fatigans* showed highest percentage i.e. 47.43% and the lowest was *Cx. vagans* i.e. 2.68% (Table IV).

Graph and data (Fig. 4) shows seasonal fluctuation of different *Culex* species during January 2004 to December 2004.

It shows that *Cx. p. fatigans* was inversely related to temperature, while *Cx. triaeniorhynchus, Cx. pseudovishnai* and *Cx. fuscocephalus* directly related to temperature.

According to table and graph, population of *Cx. p. fatigans* increased during November 2004 and January 2004. As temperature decreases population frequently exceeded.
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Total</th>
<th>Relative Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Culex bitaeniorhynchus</em></td>
<td>508</td>
<td>3.27</td>
</tr>
<tr>
<td>2</td>
<td><em>Culex pipiens fatigans</em></td>
<td>7358</td>
<td>47.43</td>
</tr>
<tr>
<td>3</td>
<td><em>Culex pseudovishnui</em></td>
<td>941</td>
<td>6.07</td>
</tr>
<tr>
<td>4</td>
<td><em>Culex tritaeniorhynchus</em></td>
<td>5305</td>
<td>34.20</td>
</tr>
<tr>
<td>5</td>
<td><em>Culex fuscocephalus</em></td>
<td>985</td>
<td>6.35</td>
</tr>
<tr>
<td>6</td>
<td><em>Cx. vagans</em></td>
<td>415</td>
<td>2.68</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>15512</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Aedes Population:

Table (V) shows that a total of 795 Aedes larvae were collected during year 2004. Out of 795 larvae belonged to Ae. aegyptio and 127 belonged to Ae. albopictus. Aedes genus was collected throughout the year 2004.

Bar and pie (Fig. 5a and 5b) shows relative abundance (percentage) of different two Aedes species. Ae. albopictus which was 15.97% and Ae. aegypti was 84.03% (Table V).

Graph and data (Fig. 6) shows seasonal fluctuation (variations) of two different species of Aedes genus population of Ae. albopictus were very low throughout the year but Ae. aegypti population was maximum in July and October.
Table V
Different Percentage of *Aedes* Species
In All Districts During
Jan 2004 - Dec 2004

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Total</th>
<th>Relative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Aedes aegypti</em></td>
<td>668</td>
<td>84.03</td>
</tr>
<tr>
<td>2</td>
<td><em>Aedes albopictus</em></td>
<td>127</td>
<td>15.97</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>795</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
BREEDING HABITATS:

With the analysis of mosquitoes larvae of fourteen (14) different species belonging to three genera their breeding habitats during twelve months (12) periods of 2004. (Table VI and Fig. 7) it was found that *Culex pipiens fatigans* collected from all seven different types of habitats and *Culex tritaeniorhynchus* found from six (6) out of seven (7) habitats, none from catch basin. Table also shows that *Culex tritaeniorhynchus* found from 4 out of seven habitats, none from borrow pits, catch basin and artificial containers, habitats.

In the same way (Table VI) shows that twelve (12) species out of fourteen (14) were found from pools as a breeding habitats eleven (11) species from borrowpils.

Among *Anopheles* mosquitoes, only *An. stephensi* larvae were found from all seven habitats out of seven (7). *An. culicifacies* and *An. pulcherrimus* both were collected from five (5) habitats out of seven (7). *An. culicifacies* mostly found from ditches and as *An. stephensi* from catch basin (Fig. 7) but as *An. pulcherrimus* showed no significant differences (Table VI and Fig. 7) also shows that 6 species out of 14 were found form artificial containers but only *Culex tritaeniorhynchus* and *Aedes* species were significantly higher in densities (Fig-7). Table VI and Fig. 7 also
demonstrated that the most commonly collected larval species were *Culex pipens fitigans*, *Culex tritaeniorhynchus* and *Anopheles stehensi*.

Similarly pool was the common habitats for *Anopheles* and *Culex* both, but for *Aedes* artificial container was highly significant habitat.
## TABLE VI
Breeding habitats of mosquito larvae
Jan 2004 - Dec 2004

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Mosquito Species</th>
<th>Habitats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pond</td>
</tr>
<tr>
<td>1</td>
<td><em>Anopheles annularis</em></td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td><em>Anopheles culicifacies</em></td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td><em>Anopheles nigerrimus</em></td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td><em>Anopheles pulcherrimus</em></td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td><em>Anopheles stephensi</em></td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td><em>Anopheles subpictus</em></td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td><em>Culex bitaeniorhynchus</em></td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td><em>Culex fuscocephalus</em></td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td><em>Culex pipiens fatigans</em></td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td><em>Culex pseudovishnui</em></td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td><em>Culex tritaeniorhynchus</em></td>
<td>6</td>
</tr>
<tr>
<td>12</td>
<td><em>Culex vagans</em></td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td><em>Aedes aegypti</em></td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td><em>Aedes albopictus</em></td>
<td>0</td>
</tr>
</tbody>
</table>
4.1.2. Results for the year 2005:

A total of 23395 mosquito larvae were collected and examined during January 2005 to December 2005, collectively from all six Districts. Out of 23395 mosquito larvae 3300 larvae were from genus *Anopheles*. 19280 mosquito larvae belong to genus *Culex* and 815 larvae were from *Aedes* genus (Table VII). Table also shows that population of mosquitoes belonging to all three genera mostly occurs in those areas which covers District Central and District East. Population of *Culex* genus is most prevalent among all the three genera.

*Anopheles* population:

An in depth investigation of the mosquito larvae abundance in all five District of Karachi and Thatta District, was conducted during January 2005 to December 2005 (Table VIII). It shows that total 23395 larvae were examined, out of 23395 larvae 14.1 percent (14.1%) were *Anopheles*, 82.41 percent (82.41%) were *Culex* and only 3.48% were genus *Aedes*. Table also shows the percentage of population of all three genera was highest in District Central and East, but *Anopheles* showed no significant differences in all Districts except South. *Culex* population was low in District Malir i.e. 10.49% and District Thatta 7.41%. *Aedes* population was high in District Central and Malir but over all *Culex* population was much higher than other two genera like *Anopheles* and *Aedes*. 
TABLE VII
District Wise Mosquitoes Larvae Collection Examined
(Jan 2005 - Dec 2005)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Collection Site</th>
<th>Total Mosquito Larvae Collected</th>
<th>Total Anopheles Mosquitoes</th>
<th>Total Culex Mosquitoes</th>
<th>Total Aedes Mosquitoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>District East</td>
<td>4613</td>
<td>748</td>
<td>3650</td>
<td>215</td>
</tr>
<tr>
<td>2</td>
<td>District West</td>
<td>3560</td>
<td>415</td>
<td>3050</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>District South</td>
<td>2971</td>
<td>246</td>
<td>2620</td>
<td>105</td>
</tr>
<tr>
<td>4</td>
<td>District Central</td>
<td>6161</td>
<td>716</td>
<td>5225</td>
<td>220</td>
</tr>
<tr>
<td>5</td>
<td>District Malir</td>
<td>3385</td>
<td>545</td>
<td>2720</td>
<td>120</td>
</tr>
<tr>
<td>6</td>
<td>District Thatta</td>
<td>2705</td>
<td>630</td>
<td>2015</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>23395</strong></td>
<td><strong>3300</strong></td>
<td><strong>19280</strong></td>
<td><strong>815</strong></td>
</tr>
</tbody>
</table>
### TABLE VIII

**Percentage Wise Mosquitoes Larvae Collection Examined**

*(Jan 2005 - Dec 2005)*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Collection Site</th>
<th>Total Mosquito Larvae Collected</th>
<th>Total <em>Anopheles</em> Mosquitoes</th>
<th>%</th>
<th>Total <em>Culex</em> Mosquitoes</th>
<th>%</th>
<th>Total <em>Aedes</em> Mosquitoes</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>District East</td>
<td>4613</td>
<td>748</td>
<td>22.67</td>
<td>3650</td>
<td>18.93</td>
<td>215</td>
<td>26.38</td>
</tr>
<tr>
<td>2</td>
<td>District West</td>
<td>3560</td>
<td>415</td>
<td>12.58</td>
<td>3050</td>
<td>15.82</td>
<td>95</td>
<td>11.66</td>
</tr>
<tr>
<td>3</td>
<td>District South</td>
<td>2971</td>
<td>246</td>
<td>7.45</td>
<td>2620</td>
<td>13.59</td>
<td>105</td>
<td>12.88</td>
</tr>
<tr>
<td>4</td>
<td>District Central</td>
<td>6161</td>
<td>716</td>
<td>21.70</td>
<td>5225</td>
<td>27.10</td>
<td>220</td>
<td>26.99</td>
</tr>
<tr>
<td>5</td>
<td>District Malir</td>
<td>3385</td>
<td>545</td>
<td>16.52</td>
<td>2720</td>
<td>14.11</td>
<td>120</td>
<td>14.72</td>
</tr>
<tr>
<td>6</td>
<td>District Thatta</td>
<td>2705</td>
<td>630</td>
<td>19.09</td>
<td>2015</td>
<td>10.45</td>
<td>60</td>
<td>7.36</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>23395</strong></td>
<td><strong>3300</strong></td>
<td><strong>14.11</strong></td>
<td><strong>19280</strong></td>
<td><strong>82.41</strong></td>
<td><strong>815</strong></td>
<td><strong>3.48</strong></td>
<td></td>
</tr>
</tbody>
</table>
Table IX shows that during 2005 year a total of 3300 *Anopheles* larvae were collected, comprising six (6) different species. Out of all six species *Anopheles culicifacies* was the most common mosquito species in Karachi and Thatta i.e. 1210 out of 3300, it means 36.67%. The second most common was *Anopheles stephensi* i.e. 24.55 percent (810 out of 3300). Bar and pie Figure shows the relative abundance of different *Anopheles* species. (Fig. 8a and 8b) reflect percentage of different species collected during Jan. 2005 to Dec. 2005. *An. annularis* showed lowest percentage (6.97%) and *An. pulcharimus* and *An. subpictus* were very close to and other i.e. 11.82% and 12.42 percent respectively.

Seasonal variation of different species belonging to genus *Anopheles* showed graph and data (Fig. 9) that during 12 months period i.e. from January 2005 to December 2005 *An. culicifacies* species was essentially bimodal with increased abundance depending on season. The 1st highest population was during March and April month, and the second peak position was during September and October, 2005. Graph and data (Fig. 9), also shows that *An. stephensi* population was high in winter season (November, December and January) and low population in summer (May, July and August) but *An. subpictus* mosquito was directly related to temperature i.e. low winter and high in summer.
### Table IX
**Different Percentage of *Anopheles* Species**
**In All Districts**
**Jan 2005 - Dec 2005**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Total</th>
<th>Relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Anopheles annularis</em></td>
<td>230</td>
<td>6.97</td>
</tr>
<tr>
<td>2</td>
<td><em>Anopheles culicifacies</em></td>
<td>1210</td>
<td>36.67</td>
</tr>
<tr>
<td>3</td>
<td><em>Anopheles nigerrimus</em></td>
<td>250</td>
<td>7.58</td>
</tr>
<tr>
<td>4</td>
<td><em>Anopheles pulcherrimus</em></td>
<td>390</td>
<td>11.82</td>
</tr>
<tr>
<td>5</td>
<td><em>Anopheles stephensi</em></td>
<td>810</td>
<td>24.55</td>
</tr>
<tr>
<td>6</td>
<td><em>Anopheles subpictus</em></td>
<td>410</td>
<td>12.42</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>3300</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
**Culex mosquito:**

A total of 19,280 *Culex* mosquito larvae were collected during Jan. 2005 to Dec. 2005, from all Districts, comprising six (6) different species in the genus. In the genus *Culex*, the *Cx. p. fatigans* was the most common species, and showed highest number (9,472 out of 19,280) and percentage (49.13%). The second highest number (6,825 out of 19,280) and percentage (35.40%) was of *Cx. tritaeniorhynchus* species. Percent population of *C. fuscocephalus* was 7.47%, *Cx. pseudovishunui* was 3.83%. Other two species like *Cx. bitaeniorhynchus* and *Cx. vagans* were found in minimum percentage i.e. 2.44% and 1.74% respectively (Table X).

Bar and pie (Fig. 10a and 10b) shows the relative abundance in percentage of different *Culex* mosquito species. It shows, all the six species were recorded during 2005. Of the total record *Cx. p. fatigans* and *Cx. tritaeniorhynchus*. Both the species showed high percentage i.e. 49.13% and 35.40% respectively and *Cx. bitaeniorhynchus*, and *Cx. vagans* showed the lowest i.e. 2.4% and 1.7% respectively.

Graph and data (Fig. 11) represents seasonal variation of different species of *Culex* mosquito, during twelve months period from January 2005 to December 2005. Graph shows that *Cx. p. fatigans*, number of larvae/10 dips became high in winter i.e. in the month of January,
November and December, while *Cx. tritaeniorhynchus* showed very high number of larvae/10 dips in the month of June, means *Cx. tritaeniorhynchus* population was directly related to the temperature. *Cx. fuscocephalus* was also directly related to temperature but number of mosquitoes larvae/10 dips were very low.

The rest of the mosquito like *Cx. bitaeniorhynchus*, and *Cx. vagans* showed very minimum number of larvae/10 dips throughout the year.
TABLE X
Different Percentage of *Culex* Species
In All Districts
Jan 2005 - Dec 2005

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Total</th>
<th>Relative Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Culex bitaeniorhynchus</em></td>
<td>470</td>
<td>2.44</td>
</tr>
<tr>
<td>2</td>
<td><em>Culex pipiens fatigans</em></td>
<td>9472</td>
<td>49.13</td>
</tr>
<tr>
<td>3</td>
<td><em>Culex pseudovishnui</em></td>
<td>738</td>
<td>3.83</td>
</tr>
<tr>
<td>4</td>
<td><em>Culex tritaeniorhynchus</em></td>
<td>6825</td>
<td>35.40</td>
</tr>
<tr>
<td>5</td>
<td><em>Culex fuscocephalus</em></td>
<td>1440</td>
<td>7.47</td>
</tr>
<tr>
<td>6</td>
<td><em>Culex vagans</em></td>
<td>335</td>
<td>1.74</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>19280</td>
<td>100</td>
</tr>
</tbody>
</table>
**Aedes mosquito:**

Table (XI) showed that a total of 815 *Aedes* mosquito larvae were collected during Jan. 2005 – Dec. 2005. Out of total 815 larvae 735 larvae belong to *Ae. aegypti* and only 80 larvae were of *Ae. albopictus*.

Bar and pie (Fig. 12a and 12b) shows relative abundance (percentage) of two species of *Aedes* mosquito, i.e. 90.18% of *Ae. aegypti* and 9.82% of *Ae. albopictus* (Table XI).

Graph and data (Fig. 13) shows seasonal fluctuation of both species during whole year of 2005 i.e. from January to December. Graph demonstrated that *Ae. aegypti* population (Number of larvae/10 dips) was high than *Ae. albopictus* population (Number of larvae/10 dips). It also indicate that population of *Ae. aegypti* became high three time, during whole year i.e. early March, July-August, and than October-November period.
Table XI
Different Percentage of *Aedes* Species In All Districts During Jan 2005 - Dec 2005

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Total</th>
<th>Relative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Aedes aegypti</em></td>
<td>735</td>
<td>90.18</td>
</tr>
<tr>
<td>2</td>
<td><em>Aedes albopictus</em></td>
<td>80</td>
<td>9.82</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>815</td>
<td>100</td>
</tr>
</tbody>
</table>
BREEDING HABITATS:

During twelve months (12) periods, mosquitoes larvae of fourteen (14) different species belonging to three genera were collected from seven different breeding habitats, during periods of Jan 2005 to Dec 2005 (Table XII and Fig. 14) table shows that only four species were found from catch-basin. Out of these four species only one belonged to *Culex* genus and three to genus *Anopheles*.

Table XII and Fig. 14 also shows that all 14 species were found from pools, and among all these species only *Cx. p. fatigans* was found in high density. In the same way 13 out of 14 species, the breeding habitat was pond, and among all these only 3 species like *Anopheles subpictus*, *Cx. p. fatigans* and *Cx. tritaeniorhynchus* were found significantly in higher densities.

During 2005, twelve (12) species out of 14 were collected from artificial container, but only two species like *Culex tritaeniorhynchus* and *Aedes aegypti* were in high density and the rest were non significant in distribution Fig. 14.

Among *Anopheles*, only *An. subpictus* was found from all habitats, and *An. stephensi* from all except ditches. *An. nigerrimus* species was not commonly collected.
TABLE XII
Breeding habitats of mosquito larvae
Jan 2005 - Dec 2005

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Mosquito Species</th>
<th>Habitats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pond</td>
</tr>
<tr>
<td>1</td>
<td><em>Anopheles annularis</em></td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td><em>Anopheles culicifacies</em></td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td><em>Anopheles nigerrimus</em></td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td><em>Anopheles pulcherrimus</em></td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td><em>Anopheles stephensi</em></td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td><em>Anopheles subpictus</em></td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td><em>Culex bitaeniorhynchus</em></td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td><em>Culex fuscocephalus</em></td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td><em>Culex p. fatigans</em></td>
<td>16</td>
</tr>
<tr>
<td>10</td>
<td><em>Culex pseudovishnui</em></td>
<td>7</td>
</tr>
<tr>
<td>11</td>
<td><em>Culex tritaeniorhynchus</em></td>
<td>18</td>
</tr>
<tr>
<td>12</td>
<td><em>Culex vagans</em></td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td><em>Aedes aegypti</em></td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td><em>Aedes albopictus</em></td>
<td>1</td>
</tr>
</tbody>
</table>
4.1.3. Results for the year 2006:

All mosquito species discussed in this section were collected during 2006. A total of 26826 mosquito larvae were collected and examining during the whole year, collectively from all six Districts. Out of 26826 larvae 3200 number of larvae were from genus *Anopheles*, 21686 mosquito larvae belong to genus *Culex*, and 1940 larvae were from *Aedes* genus (Table XIII). Table also shows that mosquito population belonging to all three genera mostly occurs in those areas which covers District Central and District East population of *Culex* genus was most prevalent among all the three genera.

*Anopheles* population:

An in depth investigation of the *Anopheles* mosquito larval abundance in all Districts of Karachi and Thatta, was conducted during 2006, shows that out of 26826 larvae 3200 (11.92%) were Anopheles. 82.41 percent (21686 larvae) were belong to Culex, and rest of 1940 larvae (7.23%) were *Aedes* genus (Table XIV). This table also shows the percentage population of all the genera. District Central and District East showed highest population of *Anopheles* and *Culex* genus, but population of *Anopheles* showed no significant differences in all Districts except District South. *Culex* population showed less in number in District West
(28.34) and District Thatta (2020) i.e. 13.07% and 9.31% respectively, but Aedes population showed highest number (535) in District Central and lowest number (35) in District Thatta i.e. 27.58% and 1.80% respectively. On the whole District Thatta showed less population of Aedes and Culex genus during 2006 period. But overall Culex mosquito population was much more higher than other two genus.

(Table XV) showed that during 2006 year a total of 3200 Anopheles larvae were collected, comprising six (6) different species out of six species Anopheles culicifacies, and Anopheles stephensi were the most common mosquito species in all the Districts i.e. 1190 and 790 out of 3200 (37.19% and 24.69%). Relative abundance of different species of Anopheles mosquito showed in Bar and pie (Fig. 15a and 15b), reflects percentage of different species belong to Anopheles during January 2006 to December 2006 period. An. annularis and An. nigerrimum showed very less percentage i.e. 6.88% and 7.50%. An. pulcherrimus and An. subpictus percentage was close to each other i.e. 10.94% and 12.81% respectively.

Graph and data (Fig. 16) Showed seasonal variation of different species belonging to genus Anopheles, during 12 months period i.e. period January 2006 to December 2006 (Fig. 16) shows that An. culicifacies species was essentially bimodal, increase in population depending on
seasonal changes. The first peak was during March-April and second highest peak position was during September – October 2006.

Fig. 16 also shows that *An. stephensi* species population was very high in November – December month 2006 (winter season) and showed low population in May, July and August 2006 (summer season), but *An. subpictus* mosquito was directly related to temperature.
TABLE XIII
District Wise Mosquitoes Larvae Collection Examined
(Jan 2006 - Dec 2006)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Collection Site</th>
<th>Total Mosquito Larvae Collected</th>
<th>Total Anopheles Mosquitoes</th>
<th>Total Culex Mosquitoes</th>
<th>Total Aedes Mosquitoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>District East</td>
<td>5875</td>
<td>639</td>
<td>4864</td>
<td>372</td>
</tr>
<tr>
<td>2</td>
<td>District West</td>
<td>3650</td>
<td>485</td>
<td>2834</td>
<td>331</td>
</tr>
<tr>
<td>3</td>
<td>District South</td>
<td>3775</td>
<td>351</td>
<td>3209</td>
<td>215</td>
</tr>
<tr>
<td>4</td>
<td>District Central</td>
<td>6175</td>
<td>690</td>
<td>4950</td>
<td>535</td>
</tr>
<tr>
<td>5</td>
<td>District Malir</td>
<td>4783</td>
<td>522</td>
<td>3809</td>
<td>452</td>
</tr>
<tr>
<td>6</td>
<td>District Thatta</td>
<td>2568</td>
<td>513</td>
<td>2020</td>
<td>35</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>26826</strong></td>
<td><strong>3200</strong></td>
<td><strong>21686</strong></td>
<td><strong>1940</strong></td>
</tr>
</tbody>
</table>
TABLE XIV
Percentage Wise Mosquitoes Larvae Collection Examined
(Jan 2006 - Dec 2006)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Collection Site</th>
<th>Total Mosquito Larvae Collected</th>
<th>Total Anopheles Mosquitoes</th>
<th>%</th>
<th>Total Culex Mosquitoes</th>
<th>%</th>
<th>Total Aedes Mosquitoes</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>District East</td>
<td>5875</td>
<td>639</td>
<td>19.97</td>
<td>4864</td>
<td>22.43</td>
<td>372</td>
<td>19.18</td>
</tr>
<tr>
<td>2</td>
<td>District West</td>
<td>3650</td>
<td>485</td>
<td>15.16</td>
<td>2834</td>
<td>13.07</td>
<td>331</td>
<td>17.06</td>
</tr>
<tr>
<td>3</td>
<td>District South</td>
<td>3775</td>
<td>351</td>
<td>10.97</td>
<td>3209</td>
<td>14.80</td>
<td>215</td>
<td>11.08</td>
</tr>
<tr>
<td>4</td>
<td>District Central</td>
<td>6175</td>
<td>690</td>
<td>21.56</td>
<td>4950</td>
<td>22.83</td>
<td>535</td>
<td>27.58</td>
</tr>
<tr>
<td>5</td>
<td>District Malir</td>
<td>4783</td>
<td>522</td>
<td>16.31</td>
<td>3809</td>
<td>17.56</td>
<td>452</td>
<td>23.30</td>
</tr>
<tr>
<td>6</td>
<td>District Thatta</td>
<td>2568</td>
<td>513</td>
<td>16.03</td>
<td>2020</td>
<td>9.31</td>
<td>35</td>
<td>1.80</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>26826</td>
<td>3200</td>
<td>11.93</td>
<td>21686</td>
<td>80.84</td>
<td>1940</td>
<td>7.23</td>
</tr>
<tr>
<td>S.No.</td>
<td>Species</td>
<td>Total</td>
<td>Relative abundance (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------</td>
<td>-------</td>
<td>------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>Anopheles annularis</em></td>
<td>220</td>
<td>6.88</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Anopheles culicifacies</em></td>
<td>1190</td>
<td>37.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>Anopheles nigerrimus</em></td>
<td>240</td>
<td>7.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Anopheles pulcherrimus</em></td>
<td>350</td>
<td>10.94</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>Anopheles stephensi</em></td>
<td>790</td>
<td>24.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><em>Anopheles subpictus</em></td>
<td>410</td>
<td>12.81</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>3200</strong></td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Culex mosquito:**

A total of 21686 *Culex* mosquito larvae were collected during January 2006 to December 2006, from all six Districts, comprising 6 different species of *Culex*. Among all species, *Cx. p. fatigans* was the most common and showed highest numbers (10250 out of 21686) and percentage i.e. 47.26. The second highest number (7910 out of 21686) and 36.47 percentage was of *Cx. tritaeniorhynchus*. Percent population of *Cx. fuscocephalus* and *Cx. pseudovishnui* was 7.37% and 4.14% simultaneously/respectively. The other two species like *Cx. bitaeniorhynchus* and *Cx. vagans* were found in a very minimum number (525 and 452 out of 21686) with lowest percentage i.e. 2.65% and 2.08% respectively (Table XVI).

Bar and pie (Fig. 17a and 17b) shows the relative abundance in percentage of different *Culex* mosquito species. Diagram shows all the six different species, recorded in 2006 year, in Histogram *Cx. p. fatigans* and *Cx. tritaeniorhynchus* both species showed high percentage than the rest of four species in pie diagram. It is cleared that *Cx. p. fatigans* and *Cx. tritaeniorhynchus* have 47.84% and 36.14% respectively, other four species showed very low percentage, as compared to these two species.
Graph and data (Fig. 18) represent seasonal fluctuation among 6 different species of *Culex* mosquito during January 2006 to December 2006 (Twelve months period). Graph indicated that *Cx. p. fatigans* population became very high in the months of January, November and December and low in May, June, and July month, mean more number of larvae/10 dips in winter season and less numbers of larvae/10 dips in summer season. While *Cx. tritaeniorhynchus* species population was very high in the months of June and July (summer season), population of *Cx. tritaeniorhynchus* increased gradually.

Up to the end of the April, then population became high with increase of temperature and then started to decrease. The other species like *Cx. fuscocephalus* also shows same growth pattern like *Cx. p. fatigans* but number of larvae/10 dips are very less, *Cx. pseudovishnui*, *Cx. bitaeniorhynchus* and *Cx. vagans* showed minimum numbers throughout the years.
TABLE XVI
Different Percentage of *Culex* Species
In All Districts
Jan 2006 - Dec 2006

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Total</th>
<th>Relative Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Culex</em> bitaeniorhynchus</td>
<td>575</td>
<td>2.65</td>
</tr>
<tr>
<td>2</td>
<td><em>Culex</em> p. fatigans</td>
<td>10250</td>
<td>47.27</td>
</tr>
<tr>
<td>3</td>
<td><em>Culex</em> pseudovishnui</td>
<td>899</td>
<td>4.15</td>
</tr>
<tr>
<td>4</td>
<td><em>Culex</em> tritaeniorhynchus</td>
<td>7910</td>
<td>36.48</td>
</tr>
<tr>
<td>5</td>
<td><em>Culex</em> fuscocephalus</td>
<td>1600</td>
<td>7.38</td>
</tr>
<tr>
<td>6</td>
<td><em>Culex</em> vagans</td>
<td>452</td>
<td>2.08</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>21686</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Aedes population:

Table-XVII shows that a total of 1940 Aedes larvae were collected during year 2006. Out of 1940 larvae 1325 were belong to *Ae. aegypti* and 615 were to *Ae. albopictus*. Bar and pie (Fig. 19a and 19b) shows relative abundance and percentage of two species of *Aedes*. *Ae. aegypti* showed very high i.e. 68.29% and *Ae. albopictus* which was 31.70%.

Graph and data (Fig. 20) shows seasonal fluctuation of two different species of *Aedes* during twelve months period. *Ae. albopictus* number of larvae/10 dips was very low as compared to *Ae. aegypti*. 
Table XVII
Different Percentage of *Aedes* Species In All Districts During
Jan 2006 - Dec 2006

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Species</th>
<th>Total</th>
<th>Relative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Aedes aegypti</em></td>
<td>1325</td>
<td>68.30</td>
</tr>
<tr>
<td>2</td>
<td><em>Aedes albopictus</em></td>
<td>615</td>
<td>31.70</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1940</td>
<td>100</td>
</tr>
</tbody>
</table>
**BREEDING HABITATS:**

With the analysis of mosquitoes larvae of fourteen (14) different species belonging to three genera and their breeding habitats during twelve months (12) periods of 2006. (Table XVIII). It shows that *Culex p. fatigans* were found from all seven different types of habitats. Another three species found from six habitats out of seven (7) habitats, like *Anopheles plucherrimus*, none from borrow pits, *Anopheles subpictus*, none from artificial containers and *Culex tritaeniorhynchus* but none from catch basin (Table XVIII). Table also showed that *Anopheles nigerrimus* and *Aedes albopictus* both were found from two or three habitats and is very less densities.

In the same way Table XVIII and Fig. 21 showed that all fourteen species were found from pool and twelve out of 14 founds. From ditches, borrow pits and seepage water ten (10), ten (10) and nine (9) species out of fourteen (14) were found respectively. *Aedes aegypti* found from 3/7 three out of habitats, significantly higher in densities from artificial containers.

Table XVIII and Fig. 21 shows that most common collected larval species during whole year 2006 were *Culex p. fatigans, Culex triaeniorhynchus* and *Anopheles subpictus*. Similar pool and pounds were very common habitats for *Anopheles* and *Culex* both genera.
# TABLE XVIII
Breeding habitats of mosquito larvae
Jan 2006 - Dec 2006

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Habitats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pond     Pool  Ditches Borrow Pits  Seepage H2O  Catch Basin  Artificial Container</td>
</tr>
<tr>
<td>1</td>
<td><em>Anopheles annularis</em></td>
<td>6 10 3 5 15 0 0 0</td>
</tr>
<tr>
<td>2</td>
<td><em>Anopheles culicifacies</em></td>
<td>3 6 15 3 9 0 0 0</td>
</tr>
<tr>
<td>3</td>
<td><em>Anopheles nigerrimus</em></td>
<td>0 2 0 6 0 0 0 0</td>
</tr>
<tr>
<td>4</td>
<td><em>Anopheles pulcherrimus</em></td>
<td>4 5 1 0 4 2 2 2</td>
</tr>
<tr>
<td>5</td>
<td><em>Anopheles stephensi</em></td>
<td>5 2 0 2 3 2 0 0</td>
</tr>
<tr>
<td>6</td>
<td><em>Anopheles subpictus</em></td>
<td>18 15 5 7 8 2 0 0</td>
</tr>
<tr>
<td>7</td>
<td><em>Culex bitaeniorhynchus</em></td>
<td>3 5 10 0 9 0 2 2</td>
</tr>
<tr>
<td>8</td>
<td><em>Culex fuscocephalus</em></td>
<td>4 8 4 0 0 0 0 0</td>
</tr>
<tr>
<td>9</td>
<td><em>Culex p. fatigans</em></td>
<td>18 35 16 4 4 9 10</td>
</tr>
<tr>
<td>10</td>
<td><em>Culex pseudovishnui</em></td>
<td>8 14 3 5 20 0 0 0</td>
</tr>
<tr>
<td>11</td>
<td><em>Culex tritaeniorhynchus</em></td>
<td>20 8 8 4 22 0 1 12</td>
</tr>
<tr>
<td>12</td>
<td><em>Culex vagans</em></td>
<td>0 3 2 0 0 2 1 1</td>
</tr>
<tr>
<td>13</td>
<td><em>Aedes aegypti</em></td>
<td>0 10 0 6 0 0 0 25</td>
</tr>
<tr>
<td>14</td>
<td><em>Aedes albopictus</em></td>
<td>2 1 0 1 0 0 0 10</td>
</tr>
</tbody>
</table>
4.1.4. Results for the year 2007:

All mosquito species collected during whole year period (2007) discussed in this section. A total of 25165 mosquito larvae were collected and examined during January 2007 to December 2007, collectively from all six Districts. Out of 25165 mosquito larvae 3367 larvae belong to genus *Anopheles*, 20714 numbers of larvae were from *Culex* genus and 1084 larvae belonged to *Aedes* genus. Table XIX. Table also shows that total number of larvae belongs to all three genera collected in maximum number from District East and secondly from District Central. But *Anopheles* mostly occurs in District Central, *Culex* in District East and *Aedes* were also in District East. Population of *Culex* genus was most prevalent in all Districts among all three genera

(Table XX) An in depth investigation of mosquito larval abundance in all six Districts, was initiated during January 2007-December 2007. It shows that 25165 mosquito larvae were examined, out of 25165 larvae 13.38% were *Anopheles*, 82.31% (20714) were *Culex* larvae and 4.31% (1084).

*Aedes* larvae (Table XX). It also shows that population (6075) of all three genera collectively was high percent in District East (18.83%, 24.90% and 26.20%). But *Anopheles* mosquito larvae showed no
significant difference in number of larvae collected (E-634, W-481, C-730, M-625, TL.-535). Except District South (360), *Culex* mosquito population percentage was maximum (24.90%) in District East, and minimum percentage (11.26%) was in District Malir (Table XX) also showed that *Aedes* mosquitoes population percentage was high (26.20%) i.e. 284 out of 1084, but over all *Culex* population was much more higher (82.31%) than *Anopheles* (13.38%) and *Aedes* (4.31%) mosquitoes.
TABLE XIX

District Wise Mosquitoes Larvae Collection Examined

(Jan 2007 - Dec 2007)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Collection Site</th>
<th>Total Mosquito Larvae Collected</th>
<th>Total Anopheles Mosquitoes</th>
<th>Total Culex Mosquitoes</th>
<th>Total Aedes Mosquitoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>District East</td>
<td>6075</td>
<td>634</td>
<td>5157</td>
<td>284</td>
</tr>
<tr>
<td>2</td>
<td>District West</td>
<td>3650</td>
<td>481</td>
<td>2981</td>
<td>188</td>
</tr>
<tr>
<td>3</td>
<td>District South</td>
<td>3390</td>
<td>360</td>
<td>2965</td>
<td>65</td>
</tr>
<tr>
<td>4</td>
<td>District Central</td>
<td>5450</td>
<td>730</td>
<td>4533</td>
<td>187</td>
</tr>
<tr>
<td>5</td>
<td>District Malir</td>
<td>3205</td>
<td>625</td>
<td>2333</td>
<td>247</td>
</tr>
<tr>
<td>6</td>
<td>District Thatta</td>
<td>3395</td>
<td>537</td>
<td>2745</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>25165</strong></td>
<td><strong>3367</strong></td>
<td><strong>20714</strong></td>
<td><strong>1084</strong></td>
</tr>
</tbody>
</table>
### TABLE XX

Percentage Wise Mosquitoes Larvae Collection Examined

(Jan 2007 - Dec 2007)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Collection Site</th>
<th>Total Mosquito Larvae Collected</th>
<th>Total <em>Anopheles</em> Mosquitoes</th>
<th>%</th>
<th>Total <em>Culex</em> Mosquitoes</th>
<th>%</th>
<th>Total <em>Aedes</em> Mosquitoes</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>District East</td>
<td>6075</td>
<td>634</td>
<td>18.83</td>
<td>5157</td>
<td>24.90</td>
<td>284</td>
<td>26.20</td>
</tr>
<tr>
<td>2</td>
<td>District West</td>
<td>3650</td>
<td>481</td>
<td>14.29</td>
<td>2981</td>
<td>14.39</td>
<td>188</td>
<td>17.34</td>
</tr>
<tr>
<td>3</td>
<td>District South</td>
<td>3390</td>
<td>360</td>
<td>10.69</td>
<td>2965</td>
<td>14.31</td>
<td>65</td>
<td>6.00</td>
</tr>
<tr>
<td>4</td>
<td>District Central</td>
<td>5450</td>
<td>730</td>
<td>21.68</td>
<td>4533</td>
<td>21.88</td>
<td>187</td>
<td>17.25</td>
</tr>
<tr>
<td>5</td>
<td>District Malir</td>
<td>3205</td>
<td>625</td>
<td>18.56</td>
<td>2333</td>
<td>11.26</td>
<td>247</td>
<td>22.79</td>
</tr>
<tr>
<td>6</td>
<td>District Thatta</td>
<td>3395</td>
<td>537</td>
<td>15.95</td>
<td>2745</td>
<td>13.25</td>
<td>113</td>
<td>10.42</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>25165</strong></td>
<td><strong>3367</strong></td>
<td><strong>13.38</strong></td>
<td><strong>20714</strong></td>
<td><strong>82.31</strong></td>
<td><strong>1084</strong></td>
<td><strong>4.31</strong></td>
</tr>
</tbody>
</table>
Anopheles Population:

(Table XXI) shows that during January 2007 to December 2007, a total of 3367 Anopheles larvae were collected from all Districts, comprising six (6) different species. Out of six species An. culicifacies, and An. stephensi were the most common mosquito species among in all Districts i.e. 1242 and 825 numbers out of 3367 (36.89% and 24.50%). Relative abundance of different species of Anopheles mosquito showed in Bar and pie (Fig. 22a and 22b) reflects percentage of different species belonging to genus Anopheles, during January 2007 to December 2007 period. An. annularis and An. nigerrimus showed very low percentage i.e. 6.98% and 7.28%. An. pulcherimus and An. subpictus percentage population was close to each other i.e. 11.73% and 12.67% respectively.

Graph and data (Fig. 23) shows seasonal fluctuation of different species of Anopheles mosquito, during 12 months period i.e. January 2007 to December 2007. It also shows that An. culicifacies species was bimodal in variation, the first peak of population was during March-April season, then start to decline then attained second highest peak population position was during September-October and early November period of 2007 (Fig. 23) also shows that An. stephensi species population was very high in the month of November and December (winter season) and very low
population was in the month of May, July and August 2007 (summer season). But *An. subpictus* species population was directly related to temperature i.e. as temperature increases population increased, and as temperature decreases population also decreased.

*An. annularis* and *An. pulcherimus* both shows similar pattern of population fluctuation i.e. more in January and February months then decline as temperature increases and again start increasing at the end of September 2007 upto December 2007 Graph and data (Fig. 23).
Table XXI

Different Percentage of *Anopheles* Species

In All Districts

Jan 2007 - Dec 2007

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Total</th>
<th>Relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Anopheles annularis</em></td>
<td>235</td>
<td>6.98</td>
</tr>
<tr>
<td>2</td>
<td><em>Anopheles culicifacies</em></td>
<td>1242</td>
<td>36.89</td>
</tr>
<tr>
<td>3</td>
<td><em>Anopheles nigerrimus</em></td>
<td>245</td>
<td>7.28</td>
</tr>
<tr>
<td>4</td>
<td><em>Anopheles pulcherrimus</em></td>
<td>395</td>
<td>11.73</td>
</tr>
<tr>
<td>5</td>
<td><em>Anopheles stephensi</em></td>
<td>825</td>
<td>24.50</td>
</tr>
<tr>
<td>6</td>
<td><em>Anopheles subpictus</em></td>
<td>425</td>
<td>12.62</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>3367</td>
<td>100</td>
</tr>
</tbody>
</table>
**Culex Population:**

A total of 20714 Culex mosquito larvae were collected during January 2007 to December 2007, from all six Districts, comparing six (6) different species of *Culex* genus. Among all species *Cx. p. fatigans* was the most common and showed highest numbers (9970 out of 20714) i.e. 48.13 percent. The second highest number was *Cx. tritaeniorhynchus* species (7665 out of 20714) i.e. 37.0 percent population. *Cx. fuscocephalus* and *Cx. pseudovishnui* species showed less numbers (1240 and 862 out of 20714) than *Cx. p. fatigans* and *Cx. tritaeniorhynchus*. These species were on 5.99% and 4.16% respectively. The rest of two species like *Cx. bitaeniorhynchus* and *Cx. vagans* were found in a very minimum number (575 and 402 out of 20414) with lowest percentage i.e. 2.78% and 1.94% respectively (Table XXII).

Bar and pie (Fig. 24a and 24b) shows the relative abundance in percentage of different *Culex* mosquito species. Figure shows all the six species, recorded in the year 2007. Histogram showed both *Cx. p. fatigans* and *Cx. tritaeniorhynchus* species high percentage than the other four species. In pie diagram, this percentage was more prominent that *Cx. p. fatigans* was 48.13% and *C. tritaeniorhynchus* was 37.00%. Other four
species showed very low and less significant in percent population i.e. 5.99%, 4.16%, 2.78% and 1.94%.

Graph and data (Fig. 25) represented seasonal fluctuation among six (6) different species of Culex mosquitoes during January 2007 to December 2007 (Twelve months period). Graph indicated that Cx. tritaeniorhynchus population was maximum in June and July months (975 and 1050 larvae/10 dips). Graph shows that population of Cx. tritaeniorhynchus species gradually increased become maximum in July and then from the end of month of August. It decreased with the decrease of temperature i.e. became low in December. On other hand Cx. p. fatigans population became very high in the months of January, February, November and December, 2007 and population became low in the months of May, June and July, 2007. It means more number of larvae/10 dips (1280, 1010, 1350 and 1450 larvae) collected in winter season, i.e. inversely related to the temperature. Cx. fuscocephalus also shows same variation like Cx. tritaeniorhynchus. Showed, but number of larvae/10 dips were low as compared with Cx. tritaeniorhynchus species. Rest of the three species i.e. Cx. bitaeniorhynchus, Cx. pseudovishnui and Cx. vagans showed very minimum number of larvae/10 dips throughout the years.
**TABLE XXII**

**Different Percentage of Culex Species In All Districts Jan 2007 - Dec 2007**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Total</th>
<th>Relative Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Culex bitaeniorhynchus</em></td>
<td>575</td>
<td>2.78</td>
</tr>
<tr>
<td>2</td>
<td><em>Culex p. fatigans</em></td>
<td>9970</td>
<td>48.13</td>
</tr>
<tr>
<td>3</td>
<td><em>Culex pseudovishnui</em></td>
<td>862</td>
<td>4.16</td>
</tr>
<tr>
<td>4</td>
<td><em>Culex tritaeniorhynchus</em></td>
<td>7665</td>
<td>37.00</td>
</tr>
<tr>
<td>5</td>
<td><em>Culex fuscoccephalus</em></td>
<td>1240</td>
<td>5.99</td>
</tr>
<tr>
<td>6</td>
<td><em>Culex vagans</em></td>
<td>402</td>
<td>1.94</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>20714</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
**Aedes Population:**

Table XXIII shows that a total of 1084 *Aedes* larvae were collected during 2007. Out of 1084 larvae 948 were belong to *Ae. aegypti* and 136 were to *Ae. albopictus* i.e. Bar and pie diagram (Fig. 26a and 26b) shows relative abundance and percentage of two species of *Aedes* mosquitoes, very high percentage (87.45%) of *Ae. aegypti* and only very low (12.55) percent was collected *Ae. albopictus* species during whole year period.

Graph and data (Fig. 27) shows seasonal variation of both species of *Aedes* mosquito during twelve months period of 2007. Table shows that the number of larvae/10 dips of *Ae. albopictus* was very low throughout the year as compared to *Ae. aegypti*. Graph also shows the great fluctuation of population in different months. Highest number show In October 2007, second highest peak was in July-August period. Minimum number was in the month of January, mid of March and mid of May 2007, but *Ae. albopictus* species showed minimum fluctuation, throughout the year, but it was maximum in the mid of June.
Table XXIII
Different percentage of *Aedes* species in all districts During Jan 2007 - Dec 2007

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Total</th>
<th>Relative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Aedes aegypti</em></td>
<td>948</td>
<td>87.45</td>
</tr>
<tr>
<td>2</td>
<td><em>Aedes albopictus</em></td>
<td>136</td>
<td>12.55</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>1084</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
BREEDING HABITATS:

Mosquitoes larvae of fourteen different species belonging to three different genera were collected from seven different breeding habitats (Table XXIV and Fig. 28). Table also shows that only four species out of fourteen were found from catch-basin. Out of these four species, three belonged to *Anopheles* genus and one from genus *Culex*.

From ponds and pools habitats more or less all fourteen species were found but *Aedes* species was found only once in a whole year from pond and pools (Fig. 28) also shows that only *Culex. p. fatigan* was found from all seven habitats. *Culex tritaeniorhynchus*, *Culex pseudovishnui* and *Anopheles annularis* were also collected from all habitats except catch-basin. Similarly *Anopheles subpictus* and *Anopheles stephensi* were found from six different habitats and out of seven habitat, both *Anopheles nigerrimus* and *Culex vagans* was found only from three habitat out of seven.

Larvae collected from artificial containers, mostly belong to genus *Aedes*. Table XXIV and Fig. 28 also showed that most of the species prefer two or three different habitats except *Aedes* mosquitoes because they prefer only artificial containers likes habitat.
### TABLE XXIV

Breeding habitats of mosquito larvae

Jan 2007 - Dec 2007

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Mosquito Species</th>
<th>Habitats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pond</td>
</tr>
<tr>
<td>1</td>
<td><em>Anopheles annularis</em></td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td><em>Anopheles culicifacies</em></td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td><em>Anopheles nigerrimus</em></td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td><em>Anopheles pulcherrimus</em></td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td><em>Anopheles stephensi</em></td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td><em>Anopheles subpictus</em></td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td><em>Culex bitaeniorhynchus</em></td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td><em>Culex fuscocephalus</em></td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td><em>Culex pipiens fatigans</em></td>
<td>18</td>
</tr>
<tr>
<td>10</td>
<td><em>Culex pseudoovishnai</em></td>
<td>8</td>
</tr>
<tr>
<td>11</td>
<td><em>Culex tritaeniorhynchus</em></td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td><em>Culex vagans</em></td>
<td>4</td>
</tr>
<tr>
<td>13</td>
<td><em>Aedes aegypti</em></td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td><em>Aedes albopictus</em></td>
<td>1</td>
</tr>
</tbody>
</table>
4.2. TOXICITY:

Toxicity of three different compounds (Biosal, Deltamethrin and *Acorus calamus*) were determined by using five different doses of each compound against 4th instars mosquito larvae (*Culex* genus) after 24 hours period.

The mortality rate gradually increased within the concentration of sample. It showed that mortality rate is directly proportional to dose. Determine mortality, range of sample extract was analyzed statistically. The standard deviation (± S.D), standard error (± S.E) and significance range of mortality at 95% confidence limit were calculated. Mortalities at different concentrations were calculated by using HP Laser Jet 30 Software.

**Toxicity Determination of Biosal, Deltamethrin and *Acorus calamus***

**Biosal:**

The 4th instar larvae of *Culex* mosquitoes treated with Biosal showed mortality after 24 hours which was found to be 25%, 41%, 56%, 80% and 95% for the concentration 1250, 1500, 1750, 2000, and 2250 ppm respectively with ± S.D value and ± S.E value as well as range of 95% confidence limit (Table XXV). By plotting the mean mortality values
against the log concentration of sample (Biosal). LC 50 was found as 1605.03 ppm (Fig. 29).

The maximum range of mortality of 4th instar larvae of mosquito at 95% confidence limit was 93.14-96.612 at 2250 ppm concentration whereas minimum range was 22.38-27.67 at 1250 ppm (Table XXV).

**Deltamethrin:**

Whereas for Deltamethrin the concentration selected for LC50 determination were 0.0937, 0.1875, 0.3750, 0.7500 and 1.5000 ppm. The mean mortalities % caused by these concentration in the case of Deltamethrin were 17%, 29%, 47% and 84% respectively mean mortality with ± S.D values, ± S.E values and range of 95% confidence limit also showed in (Table XXVI) By plotting the mean mortality values against log concentration of Deltamethrin the LC50 value was found as 0.61192 ppm (Fig. 30).

The maximum range of mortality of 4th instars mosquito larvae at 95% concentration limit was 79.89-88.11 at 1, 5000 ppm. Concentration where as minimum range was 13.85-20.15 at 0.0937 ppm concentration (Table XXVI)
**Acorus calamus:**

The concentration of A.C (*Acorus calamus*) was selected for LC50 value were 25, 50, 75,100, and 125 ppm. The mean mortalities % caused by these concentrations was 10%, 30%, 50%, 76% and 99% respectively (Table XXVII). The mean mortalities with ± S.D value, ± S.E values and range of 95% confidence limit also show in (Table XXVII). By plotting the mean mortality values against the log concentration of A.C the LC50 value was found as 70.6491 ppm (Fig. 31).

The maximum range of mortality of 4th instars mosquito larvae at 95% confidence limit was 94.73-103.27 at 125 ppm. Concentration where as minimum range was 7.77-12.23 at 25ppm concentration (Table XXVII).
Table XXV

Toxic effect of Biosal against 4th Instar larvae of *Culex* mosquito.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Converted concentration in ppm</th>
<th>Average mortality %</th>
<th>± S.D</th>
<th>± S.E</th>
<th>Average=−X ± 1.96xS.E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1250 ppm</td>
<td>25%</td>
<td>±3.0</td>
<td>±1.34</td>
<td>22.38—27.62</td>
</tr>
<tr>
<td>2</td>
<td>1500 ppm</td>
<td>41%</td>
<td>±2.74</td>
<td>±1.22</td>
<td>38.61—43.39</td>
</tr>
<tr>
<td>3</td>
<td>1750 ppm</td>
<td>56%</td>
<td>±2.91</td>
<td>±1.30</td>
<td>53.46—58.54</td>
</tr>
<tr>
<td>4</td>
<td>2000 ppm</td>
<td>80%</td>
<td>±2.91</td>
<td>±1.30</td>
<td>77.46—82.54</td>
</tr>
<tr>
<td>5</td>
<td>2250 ppm</td>
<td>95%</td>
<td>±2.12</td>
<td>±0.95</td>
<td>93.14—96.61</td>
</tr>
</tbody>
</table>
Fig. 29. Toxicity of Biosal (10 E.C.) against mosquito larvae of *Culex* species (4\textsuperscript{th} instar) with LC\textsubscript{50}=1605.03 ppm.
Table XXVI

Toxic effect of D.M (Deltamethrin) against 4th Instars larvae of (*Culex*) Mosquito.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Converted concentration in ppm</th>
<th>Average mortality %</th>
<th>± S.D</th>
<th>± S.E</th>
<th>Average=(X \pm 1.96\times S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.0937 ppm</td>
<td>17%</td>
<td>3.60</td>
<td>1.61</td>
<td>13.85—20.15</td>
</tr>
<tr>
<td>2</td>
<td>0.1875 ppm</td>
<td>29%</td>
<td>3.80</td>
<td>1.70</td>
<td>25.67—32.33</td>
</tr>
<tr>
<td>3</td>
<td>0.3750 ppm</td>
<td>47%</td>
<td>4.00</td>
<td>1.79</td>
<td>43.50—50.50</td>
</tr>
<tr>
<td>4</td>
<td>0.7500 ppm</td>
<td>61%</td>
<td>3.39</td>
<td>1.52</td>
<td>58.03—63.97</td>
</tr>
<tr>
<td>5</td>
<td>1.5000 ppm</td>
<td>84%</td>
<td>4.69</td>
<td>2.10</td>
<td>79.89—88.11</td>
</tr>
</tbody>
</table>
Fig. 30. Toxicity of Deltamethrin (DM) against mosquito larvae of *Culex* species (4th instar) with LC$_{50}$=0.61192 ppm.
Table XXVII

Toxic effect of *Acorus calamus* (AC) against 4th Instars larvae of *Culex* mosquitoes.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Converted concentration in ppm</th>
<th>Average mortality is %</th>
<th>±S.D</th>
<th>± S.E</th>
<th>Average= X ± 1.96xS.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25 ppm</td>
<td>10%</td>
<td>2.54</td>
<td>1.14</td>
<td>7.77—12.23</td>
</tr>
<tr>
<td>2</td>
<td>50 ppm</td>
<td>30%</td>
<td>3.67</td>
<td>1.64</td>
<td>26.79—33.21</td>
</tr>
<tr>
<td>3</td>
<td>75 ppm</td>
<td>50%</td>
<td>3.53</td>
<td>1.58</td>
<td>46.91—53.09</td>
</tr>
<tr>
<td>4</td>
<td>100 ppm</td>
<td>76%</td>
<td>3.39</td>
<td>1.52</td>
<td>73.03—78.97</td>
</tr>
<tr>
<td>5</td>
<td>125 ppm</td>
<td>99%</td>
<td>4.87</td>
<td>2.18</td>
<td>94.73—103.27</td>
</tr>
</tbody>
</table>
Fig. 31. Toxicity of *Acorus calamus* (A.C) against mosquito larvae of *Culex* species (4th instar) with LC$_{50}$=70.6491 ppm.
ENZYME ACTIVITY:

To assess the effect of pesticides on enzyme, the biochemical studies were carried out. For this purpose, the LC$_{50}$ concentrations of Biosal, Deltamethrin, and *Acorus calamus* used against 4$^{\text{th}}$ instars *Culex* larvae. The LC$_{50}$ Values for these compounds were 1605 ppm, 0.61192 ppm and 70.6491 ppm respectively. The 4$^{\text{th}}$ instars larvae of *Culex* mosquitoes were exposed 24 hours by each above compounds separately and then used for enzyme estimation for GOT, and GPT as described in “Materials and Methods”.

Effect of pesticide on glutamate oxaloacetate transaminase (GOT) activity in mosquito larvae:

The micromoles of glutamic acid obtained by the action of GOT, were calculated as per mg of tissue from each treated and control samples. The moles were converted into percentage while the activity of control was taken 100% and inhibition as zero % which were compared for each decrease in activity and increase inhibition by Bisol, Deltamethrin, and *Acorus calamus*.

The present inhibition of GOT caused by neem formulation Biosal (10 EC) was 5.78% with ± S.D 1.527, while percent inhibition caused by
Deltamethrin was found 26.66% with ± S.D 3.511 and *Acorus Calamus* was found 5.83% with ± S.D 2.30 (Table XXVIII and Fig.32).

**Effect of pesticide on glutamate pyruvate transaminase (GPT) activity in mosquito larvae:**

The micromoles of glutamic acid obtained by the action of GPT was calculated as per mg of tissues from each treated and control samples. The moles were converted into percentage. While the activity of control was taken as 100% and inhibition was supposed to be zero% which were compared for each decrease in activity and increase in inhibition. The % of inhibition of GPT by Biosal, Deltamehtrin and *Acorus calamus* are as below.

The percent inhibition of GPT caused by neem formulation Biosal (10 E.C) was 3.95% with ± S.D 2.51, while in Deltamethrin was found 19.65% with ± S.D 2.30 and *Acorus calamus* was found 13.77% with ± S.D 5.77 (Table XXIX and Fig.33)
TABLE XXVIII

EFFECT OF DIFFERENT PESTICIDES ON THE ACTIVITY OF
GOT IN TREATED MOSQUITO LARVAE

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>X µ/l</th>
<th>± S.D</th>
<th>± S.E</th>
<th>Range= $x\pm S.E \times 1.96$</th>
<th>% Activity</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biosal</td>
<td>24.33</td>
<td>1.52</td>
<td>0.88</td>
<td>26.05-22.60</td>
<td>105.78</td>
<td>5.78</td>
</tr>
<tr>
<td><em>Acorus calamus</em> (A.C)</td>
<td>21.66</td>
<td>2.30</td>
<td>1.33</td>
<td>19.05-21.66</td>
<td>94.17</td>
<td>5.83</td>
</tr>
<tr>
<td>Deltamethrine (D.M)</td>
<td>17.33</td>
<td>3.51</td>
<td>2.02</td>
<td>13.33-21.30</td>
<td>75.17</td>
<td>26.66</td>
</tr>
</tbody>
</table>

Control for all= 23 µ/l
### TABLE XXIX

**EFFECT OF DIFFERENT PESTICIDES ON THE ACTIVITY OF GPT IN TREATED MOSQUITO LARVAE**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>X ± S.D ± S.E</th>
<th>Range= x± S.E×1.96</th>
<th>% Activity</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biosal</td>
<td>16.33 ± 2.51 ± 1.45</td>
<td>13.49-19.17</td>
<td>96.05</td>
<td>3.95</td>
</tr>
<tr>
<td><em>Acorus calamus</em> (A.C)</td>
<td>14.66 ± 5.77 ± 3.33</td>
<td>8.14-21.18</td>
<td>86.23</td>
<td>13.77</td>
</tr>
<tr>
<td>Deltamethrine (D.M)</td>
<td>13.66 ± 2.30 ± 1.33</td>
<td>11.06-16.26</td>
<td>80.35</td>
<td>19.65</td>
</tr>
</tbody>
</table>

Control for all= 17.0 u/l
RESIDUE ANALYSIS BY HPLC:

Deltamethrin:

Deltamethrin was taken as standard synthetic pyrethroid, chromatogram was obtained by injecting 20 microliter samples in HPLC (Zorbax TM NH₂).

(Chromatogram 1 and Table XXX) gives the concentration of main component deltamethrin as 98.6884%. The sample of the 24 hours treated mosquito larvae indicates the concentration of main peak 98.6609% with minimum Biodegradation of the compounds (Chromatogram 2 and Table XXXI). The sample in which mosquito larvae were exposed for 48 hours indicates the concentration of main peak as 98.4981% showing slightly more Biodegradation (Chromatogram 3 and Table XXXII), as evident from chromatograms and Tables, biodegradation of deltamethrin takes place after 72 hours exposed so in (Chromatogram 4 and Table XXXIII) in addition to deltamethrin peak (conc. 70.4895), two minor peaks (Table XXXIII – 13.1190% and 8.6608%) were obtained. These may be biodegradation products, but are in minor quantity.

Acorus Calamus:

As far as Acorus calamus extract is concerned, biodegradation started from 48 hours exposure. In control (Chromatogram 5) major peak
of active ingredient (β-azarone) is evident as 96.8037% (Table XXXIV) after 24 hours exposure a very minor peak (Table XXXV – 2.6517%) appeared. However, after 48 hours exposure one major peak (Chromatogram 7 Table XXXVI) and two minor peaks (Table XXXVI in 13.4803 and 10.1092%) appeared, indicating biodegradation. After 72 hours exposure (Chromatogram 8 Table XXXVII) a major peak of β-azarone, with lesser concentration (66.2613 and two minor peaks with 16.7983% concentration and 13.4557%) can be seen showing lesser concentration of β. azarone and slightly more concentration of minor peaks.

**Biosal:**

In the case of another biopesticides Biosal also (Chromatogram 9), only one major peak (Table XXXVIII – 97.9274%) of azadirachtin is evident. After 24 hours exposure again a major peak (Chromatogram 10 and Table XXXIX) with 97.7975% can be seen. However after 48 hours in Chromatogram 11 one major peak (Table XL, 87.8744%) and one minor peak (Table XL – 11.6488%) can be seen. After 72 hours exposure (Chromatogram 12) one major peak (Table XLI – 78.1821%) can be seen with lesser concentration and 2 minor peaks (Table XLI – 17.8539% and 2.6728%) can be seen.
The result indicate that in deltamethrin degradation starts after 72 hours but in the case of biopesticides after 48 hours.

It was found that the synthetic pyretheroid which is the pure compound showed lesser degradation with increasing time exposure. The *Acorus calamus* extract which is a mixture of many individual compounds showed maximum Biodegradation with several minor peaks. Biosal which is also a plant product with one major compound Azadirachtin and some minor compounds showed medium Biodegradation, however in 72 hours exposure, all the three undertest samples showed maximum Biodegradation with more minor peaks. It was noted that with the increased exposure time the residues of the samples become lesser and lesser.
Chromatogram 1. HPLC chromatogram showing peak of deltamethrin (Control).

Table XXX. Concentration of Deltamethrin (control) obtained through HPLC.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Time</th>
<th>Area</th>
<th>Height</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.318</td>
<td>145509</td>
<td>108040 SV</td>
<td>98.6884</td>
</tr>
<tr>
<td>2</td>
<td>3.857</td>
<td>17766</td>
<td>1634 T</td>
<td>1.2046</td>
</tr>
<tr>
<td>3</td>
<td>4.526</td>
<td>1578</td>
<td>157 T</td>
<td>0.1070</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1474853</td>
<td>109831</td>
<td>100.0000</td>
</tr>
</tbody>
</table>
Chromatogram 2. HPLC chromatogram showing peak of deltamethrin residue after 24 hours exposure of mosquito larvae

Table XXXI. Concentration and other parameters of Deltamethrin residue in mosquito larvae exposed for 24 hours.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Time</th>
<th>Area</th>
<th>Height</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.150</td>
<td>1530</td>
<td>168</td>
<td>0.0234</td>
</tr>
<tr>
<td>2</td>
<td>3.534</td>
<td>11992</td>
<td>694 V</td>
<td>0.1836</td>
</tr>
<tr>
<td>3</td>
<td>3.589</td>
<td>6444563</td>
<td>423318 SV</td>
<td>98.6609</td>
</tr>
<tr>
<td>4</td>
<td>4.469</td>
<td>73949</td>
<td>6259 T</td>
<td>1.1321</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>6532034</td>
<td>430</td>
<td>100.0000</td>
</tr>
</tbody>
</table>
Chromatogram 3. HPLC chromatogram showing peak of deltamethrin residue after 48 hours exposure of mosquito larvae.

Table XXXII. Concentration and other parameters of Deltamethrin residue in mosquito larvae exposed for 48 hours.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Time</th>
<th>Area</th>
<th>Height</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.079</td>
<td>1115660</td>
<td>84462</td>
<td>98.4981</td>
</tr>
<tr>
<td>2</td>
<td>3.595</td>
<td>12274</td>
<td>1158 T</td>
<td>1.0813</td>
</tr>
<tr>
<td>3</td>
<td>4.183</td>
<td>4764</td>
<td>448 T</td>
<td>0.4206</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1132672</td>
<td>86068</td>
<td>100.0000</td>
</tr>
</tbody>
</table>
Chromatogram 4. HPLC chromatogram showing peak of deltamethrin residue after 72 hours exposure of mosquito larvae.

Table XXXIII. Concentration and other parameters of Deltamethrin residue in mosquito larvae exposed for 72 hours.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Time (min)</th>
<th>Area (a.u.)</th>
<th>Height (V)</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.479</td>
<td>18332</td>
<td>2205</td>
<td>2.0449</td>
</tr>
<tr>
<td>2</td>
<td>2.632</td>
<td>14855</td>
<td>1810 V</td>
<td>1.6570</td>
</tr>
<tr>
<td>3</td>
<td>2.852</td>
<td>631919</td>
<td>69257 V</td>
<td>70.4895</td>
</tr>
<tr>
<td>4</td>
<td>3.198</td>
<td>117608</td>
<td>7168 V</td>
<td>13.1190</td>
</tr>
<tr>
<td>5</td>
<td>3.671</td>
<td>77641</td>
<td>3943 V</td>
<td>8.6608</td>
</tr>
<tr>
<td>6</td>
<td>4.380</td>
<td>22169</td>
<td>590 V</td>
<td>2.4729</td>
</tr>
<tr>
<td>7</td>
<td>5.840</td>
<td>11128</td>
<td>497 V</td>
<td>1.2413</td>
</tr>
<tr>
<td>8</td>
<td>6.472</td>
<td>2820</td>
<td>133 V</td>
<td>0.3146</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>896472</td>
<td>85604</td>
<td>100.0000</td>
</tr>
</tbody>
</table>
Chromatogram 5. HPLC chromatogram showing peak of main component (β-Azarone) of *Acorus calamus* (Control).

Table XXXIV. Concentration of main compound (β-azarone) of *Acorus calamus* obtained through HPLC.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Time</th>
<th>Area</th>
<th>Height</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.358</td>
<td>11867</td>
<td>1275</td>
<td>2.2228</td>
</tr>
<tr>
<td>2</td>
<td>2.716</td>
<td>516828</td>
<td>48447 SV</td>
<td>96.8037</td>
</tr>
<tr>
<td>3</td>
<td>3.021</td>
<td>5198</td>
<td>872 T</td>
<td>0.9736</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>533893</td>
<td>50595</td>
<td>100.0000</td>
</tr>
</tbody>
</table>
Chromatogram 6. HPLC chromatogram showing peak of *Acorus calamus* (β-Azorone) residue after 24 hours exposure of mosquito larvae

![HPLC Chromatogram](image)

β-Azorone

Table XXXV. Concentration and other parameters of *Acorus calamus* extract (β-azarone) residue in mosquito larvae exposed for 24 hours.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Time</th>
<th>Area</th>
<th>Height</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.367</td>
<td>17773</td>
<td>1937</td>
<td>2.6517</td>
</tr>
<tr>
<td>2</td>
<td>2.735</td>
<td>634254</td>
<td>62537 SV</td>
<td>94.6308</td>
</tr>
<tr>
<td>3</td>
<td>3.033</td>
<td>6042</td>
<td>1008 T</td>
<td>0.9015</td>
</tr>
<tr>
<td>4</td>
<td>3.449</td>
<td>8768</td>
<td>1181 T</td>
<td>1.3081</td>
</tr>
<tr>
<td>5</td>
<td>4.088</td>
<td>1986</td>
<td>204 T</td>
<td>0.2963</td>
</tr>
<tr>
<td>6</td>
<td>5.466</td>
<td>1419</td>
<td>89</td>
<td>0.2117</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>670241</strong></td>
<td><strong>66955</strong></td>
<td><strong>100.0000</strong></td>
</tr>
</tbody>
</table>
Chromatogram 7. HPLC chromatogram showing peak of *Acorus calamus* (β-Azarone) residue after 48 hours exposure of mosquito larvae.

Table XXXVI. Concentration and other parameters of *Acorus calamus* extract (β-azarone) residue in mosquito larvae exposed for 48 hours.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Time</th>
<th>Area</th>
<th>Height</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.382</td>
<td>8474</td>
<td>927</td>
<td>2.0859</td>
</tr>
<tr>
<td>2</td>
<td>2.595</td>
<td>5276</td>
<td>831 V</td>
<td>1.2987</td>
</tr>
<tr>
<td>3</td>
<td>2.756</td>
<td>29112</td>
<td>33915 V</td>
<td>71.6561</td>
</tr>
<tr>
<td>4</td>
<td>3.061</td>
<td>54765</td>
<td>3434 V</td>
<td>13.4803</td>
</tr>
<tr>
<td>5</td>
<td>3.495</td>
<td>41070</td>
<td>2111 V</td>
<td>10.1092</td>
</tr>
<tr>
<td>6</td>
<td>4.282</td>
<td>5565</td>
<td>354 V</td>
<td>1.3697</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>406262</strong></td>
<td><strong>41571</strong></td>
<td><strong>100.0000</strong></td>
</tr>
</tbody>
</table>
Chromatogram 8. HPLC chromatogram showing peak of *Acorus calamus* (β-Azarone) residue after 72 hours exposure of mosquito larvae.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Time</th>
<th>Area</th>
<th>Height</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.405</td>
<td>7763</td>
<td>856</td>
<td>1.7799</td>
</tr>
<tr>
<td>2</td>
<td>2.779</td>
<td>288975</td>
<td>31824 V</td>
<td>66.2613</td>
</tr>
<tr>
<td>3</td>
<td>3.101</td>
<td>73260</td>
<td>3965 V</td>
<td>16.7983</td>
</tr>
<tr>
<td>4</td>
<td>3.519</td>
<td>58682</td>
<td>2921 V</td>
<td>13.4557</td>
</tr>
<tr>
<td>5</td>
<td>4.450</td>
<td>7435</td>
<td>422 V</td>
<td>1.7047</td>
</tr>
<tr>
<td>Total</td>
<td>436115</td>
<td>39988</td>
<td></td>
<td>100.0000</td>
</tr>
</tbody>
</table>
Chromatogram 9. HPLC chromatogram showing peak of Biosal (Azadirachtin) main compound (Control).

![HPLC Chromatogram]

Table XXXVIII. Concentration of main compound Biosal (Azadirachtin) obtained through HPLC.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Time</th>
<th>Area</th>
<th>Height</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.855</td>
<td>14791</td>
<td>971</td>
<td>0.1908</td>
</tr>
<tr>
<td>2</td>
<td>4.059</td>
<td>4980924</td>
<td>458552 SV</td>
<td>97.9274</td>
</tr>
<tr>
<td>3</td>
<td>4.636</td>
<td>90630</td>
<td>7592 T</td>
<td>1.7818</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>5086345</td>
<td>467115</td>
<td>100.0000</td>
</tr>
</tbody>
</table>
Chromatogram 10. HPLC chromatogram showing peak of Biosal (Azadirachtin) residue after 24 hours exposure of mosquito larvae.

Table IXL. Concentration and other parameters of Biosal (Azadirachtin) residue in mosquito larvae exposed for 24 hours.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Time</th>
<th>Area</th>
<th>Height</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.136</td>
<td>51650</td>
<td>1494</td>
<td>0.3354</td>
</tr>
<tr>
<td>2</td>
<td>7.554</td>
<td>15061334</td>
<td>671314 SV</td>
<td>97.7975</td>
</tr>
<tr>
<td>3</td>
<td>8.529</td>
<td>287551</td>
<td>11636 T</td>
<td>1.8672</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>15400535</td>
<td>684443</td>
<td>100.0000</td>
</tr>
</tbody>
</table>
Chromatogram 11. HPLC chromatogram showing peak of Biosal (Azadirachtin) residue after 48 hours exposure of mosquito larvae.

Table XL. Concentration and other parameters of Biosal (Azadirachtin) residue in mosquito larvae exposed for 48 hours.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Time</th>
<th>Area</th>
<th>Height</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.752</td>
<td>9736</td>
<td>438</td>
<td>0.3626</td>
</tr>
<tr>
<td>2</td>
<td>2.705</td>
<td>2359518</td>
<td>332224 V</td>
<td>87.8744</td>
</tr>
<tr>
<td>3</td>
<td>2.978</td>
<td>312783</td>
<td>25523 SV</td>
<td>11.6488</td>
</tr>
<tr>
<td>4</td>
<td>4.317</td>
<td>3066</td>
<td>193 T</td>
<td>0.1142</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>2685102</strong></td>
<td><strong>358379</strong></td>
<td><strong>100.0000</strong></td>
</tr>
</tbody>
</table>
Chromatogram 12. HPLC chromatogram showing peak of Biosal (Azadirachtin) residue after 72 hours exposure of mosquito larvae.

![HPLC Chromatogram](image)

Table XLI. Concentration and other parameters of Biosal (Azadirachtin) residue in mosquito larvae exposed for 72 hours.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Time</th>
<th>Area</th>
<th>Height</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.742</td>
<td>9077</td>
<td>493</td>
<td>0.0913</td>
</tr>
<tr>
<td>2</td>
<td>2.750</td>
<td>7772731</td>
<td>998357 VE</td>
<td>78.1821</td>
</tr>
<tr>
<td>3</td>
<td>3.048</td>
<td>1775002</td>
<td>179457 V</td>
<td>17.8539</td>
</tr>
<tr>
<td>4</td>
<td>3.407</td>
<td>265725</td>
<td>21111 V</td>
<td>2.6728</td>
</tr>
<tr>
<td>5</td>
<td>3.770</td>
<td>90896</td>
<td>4243 V</td>
<td>0.9143</td>
</tr>
<tr>
<td>6</td>
<td>4.657</td>
<td>12690</td>
<td>721 V</td>
<td>0.1276</td>
</tr>
<tr>
<td>7</td>
<td>5.125</td>
<td>15718</td>
<td>727 V</td>
<td>0.1581</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>9941838</strong></td>
<td><strong>1205109</strong></td>
<td><strong>100.0000</strong></td>
</tr>
</tbody>
</table>
DISCUSSION

The present survey, investigation, study and experiments were carried out to examine the immature stages (Larvae) of different mosquito species and their related ground water breeding habitats. The occurrence and abundance of mosquito larvae in different habitats, reflects both the ovipositor preferences of the females as well as the ability of the immature to survive in the condition under which they forced to exist themselves. Changes in physico-chemical and biotic characteristics of habitats may create the condition either favorable or unfavourable for their breeding success, depending upon the ranges of tolerances of different species (Singh et al., 1995).

During the present study and the experiments, the larval population of different mosquito species was examined and their related ground water breeding habitats which also noted. These studies cover the systematic details of different species, their density relation with habitats and seasonal fluctuation were also carried out in District of Karachi and Thatta.

The systematic details of determination were based on four years (2004-2007) consecutive research data, covering all the five (5) districts (East, West, Central, South and Malir) of Karachi city and Thatta. For convenience, the habitats have been classified into seven (7) categories as
ponds, pools, ditches, borrow pits, seepage water, catch basin and artificial containers. This survey for immature stages seems to be best the method for faunistic studies.

The present study showed that three important genera Anopheles, Culex, and Aedes were found throughout the four years research work. Among Anopheles 6 species like An. annularis, An. culicifacies, An. nigerremus, An. pulcherimus, An.stephensi, An. subpectus, were reported from Karachi and Thatta, Pakistan.

In the current investigation, all the data shows that among three genera, the most prevalent genus was Culex. A total of 94800 mosquito larvae were collected and examined during January 2004 to December 2007, collectively from all 6 districts (East, West, South, Central, Malir and Thatta). Out of 94800 larvae 12974(13.82%) belonged to genus Anopheles, while 77192 (81.03%) lavae were from Culex genus, and 4634(4.77%) belonged to Aedes genus. So the Culex was the most common and showed highest percentage, the second highest was Anopheles while Aedes was found in minimum percentage. Among Anopheles species An. annularis was frequently collected in lentic habitats chocked with submerged vegetation agreeing with previous observations throughout the Indo-Pakistan subcontinent (e.g. Russell and Rao, 1940; Ansari and Shah,
1950). This work indicated that *An. annularis* was collected in much colder water than suggested by Rao (1961) in India. This species is typically most abundant in the Punjab during winter (Ansari and Nasir, 1955). *An. nigerrimus* seemed to be a clear water species rarely found in turbid water. There was no significant physico-chemical relationships. *An. nigerrimus* normaly associated with *An. culicifacies* and *An. stephensi*.

*An. culicifacies* reportedly breeds in almost any available type of water (Bhatia and Krishna 1961). This species has been collected from high ammonia concentration Reisen *et al.*, 1981, but during present work larvae were collected from low concentration.

*An. pulcherrimus* was most prevalent in open, sunlight ground waters supporting dense growth of submerged vegetables (Ansari and Shah 1950). Present research work support the above deserription. *An. stephensi* breeds in a wide range of both urban and rural habitats throughout its distribution (Krishna 1961). In the present investigation the species was found from most of the samples but rarely found in highly polluted waters. *An. stephensi* was more prevalent during the cooler temperatures. In the Laboratory, Reisen and Siddqui (1978) have shown that this species preferentially oviposits in larval rearing water with high ammonia concentration. Ansari and Shah (1950) agreed that *An. subpictus* was able
to tolerate a wide range of physico-chemical conditions and was frequently collected in small drying pools, devoid of other mosquito species.

Zahar (1974) reported that *An. subpictus* was the common species of Lahore and Karachi. Present work shows that, not only *An. subpictus* but *An. stephensi*, *An. pulcherrimus* and *An. culicifacies* are very common and abundantly found. Among all of these only *An. culicifacies* was the commonest one, and *An. stephensi* shows inverse correlation with temperature i.e more prevalent during cold season i.e winter (November – December and January).

Riaz *et al.*, (1987) determined the significant effect of temperature, humidity and rainfall on mosquitoes population dynamics. In the present study (temperature and humidity) produced significant effects on larval population, but some species shows direct correlation e.g and some inversely correlated with temperature. Similarly species composition and their relative abundance may change from year to year at the same breeding places (site) of same districts.

Naqvi *et al.*, (1988) reported that among three genera i.e. *Anopheles*, *Culex* and *Aedes* in three different pools communities at Karachi University Campus area, *Culex* species are more prominent.
Suleman and Khan (1993) determined the highest population density of *An. stephensi*, *An. culicifacies* and *An. subpictus* during summer season from Abottabad area of Pakistan. Naqvi et al., (1997) reported high density of *Anopheles* in January from Karachi. The present investigation indicates that occurrence of over all *Anopheles* population was high during March to May and in the months of September to November during four years (2004 – 2007). i.e. Bimodal in nature.

Suleman (2000) reported seven species of *Anopheles* from Chitral i.e. *An. subpictus*, *An. stephensi*, *An. macularis*, *An. turkhendi*, *An. splendidus*, *An. fluviatalis* and *An. gigas*. According to present work, six different species of *Anopheles* from Karachi and Thatta were found i.e. *An. annularis*, *An. culicifacies*, *An. pulcherimus*, *An. nigerrimus*, *An. stephensi* and *An. subpictus*.

Suleman also reported that *An. maculatus* was the commonest among *Anopheles* from Chitral, Pakistan. In the present survey other species of *Anopheles* were found, but *An. maculatus* was not found from any where within Karachi districts. Suleman in the same year reported 10 species of *Anopheles* from Peshawar valley, Pakistan. In June, July and early of August mosquitoes activity was mostly suspended, probably due
to monsoon rain in August and temperature range 35 to 41ºC, after that larval population of mosquitoes began to increase.

Suleman and Khan (1993) reported 31 species of six (6) genera of mosquitoes from freshwater valley and adjoining areas. In the present work 14 species of three genera i.e. *Anopheles*, *Culex* and *Aedes* were found from Karachi. The species composition of different mosquito genera in freshwater valley is considerably very different from species composition of Karachi districts. More species found from Peshawar valley may be due to higher rainfall and more forest area.

Among *Culex* mosquitoes larvae Reuben (1971) described that *Cx. pseudovishnui* frequently associated with *cx. tritaeniorhynchus* and more prevalent in clean water habitats. During the present study this association was positive in summer season but not in winter.

Riaz *et al.*, (1987) determined the significant effect of temperature, humidity and rainfall on population of mosquito larvae. In the present study both temperature and humidity produced significant effects on larval population. Some species showed direct correlation with *Cx. tritaeniorhynchus* and some showed inverse correlation with temperature but *Cx. vagans* have no relationship with temperature. Similarly species
composition and their relative abundance may change from year to year at the same breeding places of same district.

Among *Culex* species, the *Cx. tritaeniorhynchus* which carry Japanese encephalitis was described all over Karachi city in low density (Kamimura *et al.*, 1986) but during present finding *Cx. tritaeniorhynchus* was found in high density from all breeding habitat all over Karachi districts, specially during summer, Similarly *Cx. p. fatigans* has shown abundance throughout the season.

Dadal and Kleinjan (1974) reported that immature were rarely collected during summer, when *Cx. p. fatigans* was replaced in polluted ground pool breeding sites by the *Cx. tritaeniorhynchus* in autumn season. During winter population densities frequently exceeded 500 larvae / dip in polluted ground pool habitats, which were typically devoid of other mosquito species. During present study similar results were found like *Cx. p. fetgans* showed inverse relation with temperature, as temperature decreases, population density increases.

Kamimura *et al.*, (1986) reported that the number of adult mosquitoes captured at the other localities in Karachi city. *Ae. aegypti*, the principal vector of dengue, was abundant in the summer season from July to September at the midtown area and their common breeding places were
water tanks. Now the weather condition of Karachi became changed and rainy season shifts to August – September therefore *Aedes* population showed abundance during October – November periods.

Two species of *Aedes* were found from Karachi city, both species continuously increased with time. One species, *Aedes albopictus* (Skuse), the Asian tiger mosquito (also known as forest day mosquito) and *Aedes aegypti* were the only mosquitoes found at a number of localities, surveyed in Karachi districts, but this genus is available only from specific habitat.

Suleman *et al.*, (1993) surveyed in Peshawar valley, indicated that immature stages of *An. albopictus* and *An. Armigeres, An. subalbatus* along with some other *Aedine* species from tree-holes at several localities including Peshawar University campus during summer and autumn seasons, while in the present study only two species *Aedes aegypti* and *Ae. albopictus* were found, not from tree-holes but from some specific habitats like small tub of tyre-puncture shops and from different artificial container from different areas of Karachi.

*Aedes aegypti* is apparently more common and widely distributed than *Ae. albopictus* as it was found breeding as the sole species in both species few habitats in Karachi area. Both species seems capable of utilizing a wide variety of oviposition sites as it is commonly found
breeding in domestic containers too. In a brief survey of *Aedes* mosquitoes at a Orangi and Liaqatabad area in the vicinity of a densely populated localities in Karachi.

*Aedes aegypti* and *Ae. albopictus* has a marked ability of establishing in areas where it was previously absent. For instance, upto 1985, it was restricted northern contined to Asia and many Island in the Pacific including Hawaii. In recent years its ranges has greatly expanded, presumably due to the shipments of used tires from northern Asia.

Suleman and Khan (1993) did not found a single speciemen of *Ae. aegypti* from Abbottabad or Peshawar, though it has previously been recorded from Kohat Hangu valley (Qutbuddin 1960), and from Karachi (Barraud 1934) while this research work showed that not only *Ae. aegypti* but *A. ablopictus* was also found during late September upto December, but it is to be noted that with every passing year, population of *Aedes* mosquitoes increased more and more that is why the number of dengue patients increases every year. According to Suleman and Khan (1993). *Aedes albopictus* was more common and widly distributed species in Abbottabad and Peshawar area but during presset survey in Karachi, during 2004 -2007 it was noted that *Aedes aegypti* was more common and
widely distributed than *Aedes albopictus* and also the frequency of occurrence and population density was very high.

In the Karachi city and Thatta of Sindh, Pakistan, definitely mosquito habitat associates were determined which were modified by increasing in temperature, salinity and pollution. Seasonal temporal shift of the fauna with the warm weather forms, e.g. *An. subpictus*, *Cx. pseudo* and *Cx. trittaeniorhynchos*, decreasing and the cold weather e.g *An. stephensi*, *Cx. p. fatigans* increasing in abundance. Temperature itself was not so significant in the present study.

Certain mosquitoes seemed associated with other environmental components e.g. *An. annularis* and *An. pulcherrimus* were usually collected among thick stands of submergent macrophytes. *An. culicifacies* was most prevalent in clean water. *Cx. bitaeniorhynchus* were always associated with filamentous green algae such as Spirogyra.

The other aspects of the present study, included the experiments for the estimation of biodegradation and residues in mosquito larvae treated with different pesticides (Biosal, Deltamethrin and *Acorus calamus* extract. Their effect on population densities and growth of mosquito larvae was also observed. The whole work was estimated on the bases of three parameters. (a) by evaluating the LC$_{50}$ values by contact method (b)
determination of the enzyme activation or inhibition after exposure; (c) pesticides residue estimation by using High Pressure Liquid Chromatography (HPLC).

Using the above three parameters the following results have been obtained which may be compared, with the results obtained by other researchers and workers. In the present work three compounds Biosal, Deltamethrin, and *Acorus calamus* extract were tested against *Culex* mosquito larvae collected from different habitats of Karachi district.

By plotting the average values on computer program (HP laser jet 30 software), the LC$_{50}$ value (median Lethal concentration) of Biosal against *Culex* mosquito larvae was found 1605.05 ppm, whereas the LC$_{50}$ value of Deltamethrin against *Culex* mosquito larvae was found as 0.6119 ppm, while the LC$_{50}$ value of *Acorus calamus* extract against *Culex* mosquito larvae was found as 70.6491 ppm.

The enzyme activity and inhibition produced due to the exposure of Biosal, *Acorus calamus* and deltamethrin extract were estimated by treating with the LC$_{50}$ values of undertest insecticides. The inhibition of GOT produced by Biosal, *Acorus calamus* and deltamethrin extract in *Culex* mosquito larvae was 5.37% 5.83% and 24.83% respectively. Whereas the inhibition of GPT by Biosal, *Acorus calamus* and
deltamethrine was 3.95%, 13.77% and 19.65% respectively. This indicate that inhibition by the pesticides in according their toxicities of the pesticide sample.

HPLC shows that 98.6884, 98.6609, 98.4981, and 70.4895% residue of deltamethrin were detected in control, after 24hours, 48hours and 72hours exposure respectively. This indicates that with time factor the biodegradation takes place. The results indicate that upto 48hours is nearer to control but after 72hours significant biodegradation takes place i.e 70.48% residue of the deltamethrin.

While 96.8037, 94.6308, 71.6561 and 66.2613% residue of *Acorus calamus* extract were detected in control, 24 hours, 48 hours and 72 hours exposed larval samples respectively. In this case the biodegradation starts from 48hours and maximum after 72 hours.

Whereas 97.9274%, 97.7975%, 87.8744% and 78.182% residue of Biosal were detected in control, 24 hours exposure, 48 hours exposure and 72 hours exposure of samples, respectively. The residue were directly related with exposure time i.e. 72 hours exposure time shows more biodegradation in all pesticides (Biosal, *Acorus calamus* extract and deltamethrin) But *Acorus calamus* extract and Biosal shows more degradation as compared to Deltamethrin. Which starts after 48hours
exposure. This indicates that the two biopesticides behave similarly, while synthetic pyrethroid shows more toxicity based on slow biodegradation, resulting higher residues and higher toxicity. When the effect of pesticides on enzymes is compared, then maximum inhibition was by deltamethrin, then Acorus extract and Biosal. The inhibition by the two biopesticides is near or high inhibition by deltamethrin may be due to higher toxicity at a very low dose (Table 27).

The LC50 value of Biosal, *Acorus calamus* extract and deltamethrin against *Culex* mosquito larvae was 1605.05ppm, 70.64ppm and 0.6119ppm respectively. These results also indicate same concept that the biopesticides are less toxic as compared to deltamethrin. The inhibition of GOT, and GPT by deltamethrin is higher than *Acorus calamus* extract and Biosal. This also indicate that less tolerance is present against deltamethrin as compared to *Acorus calamus* extract and Biosal.

Hussain *et al.*, (1995) have studied the efficacy of two pyrethrroids (cypermethrin and deltamethrin) against *Culex quinquefasciatus* Say larvae. They found LC50 of cypermethrin and Deltamethrine is 0.00064 and 0.00028ppm, respectively, i.e. deltamethrin is more toxic than cypermethrin, while in the present investigation also Deltamethrine
showed very high value of $LC_{50}$ (0.6119ppm) supporting findings of Hussain et. al., (1995).

Karmatak et al., (1991) found that Deltamethrin was significantly superior among all the synthetic pyrethroid insecticides against *Sitophilus oryzae* (L). Now it is found that mortality was found directly correlated with the concentration of insecticide.

Azmi et al., (1997) reported that deltamethrin is more toxic than neem products against the fish fry of *Cyprinus carpio*. $LC_{50}$ of “SDS” against fish fry was reported low in comparison to the present report i.e., $LC_{50}$ of Biosal in 1605.05ppm which may be due to the different formulation of SDS and Biosal.

Ahmad et al., (1998) studied the effect of cypermethrin (synthetic pyrethroid) and *Acorus calamus* extract (*Acorus calamus* Hex-1) against *Sitophilus oryzae* by using filter paper inpregnation method. The $LC_{50}$ values were found is to be 19 mg/cm$^2$ for cypermethrin and 3500mg/cm$^2$ for *Acorus calamus* extract, respectively. In the present work also $LC_{50}$ value of *Acorus calamus* extract is 70.64ppm and for biosal was 1605.05ppm against *Culex* mosquito larvae which may be due to different nature of synthetic pyrethroid and phytopesticides on the two different insects.
Azmi et al., (1998) reported the toxicity of Biosal (RB-a,) pyrethroid Coopex, along with their effects on GPT and GOT against *S.oryzae* (Karachi strain). They reported the LC$_{50}$ of Coopex as 6.128 mg/cm$^2$ and that of crude neem extract RB.a, on the highest does 1257 mg/cm$^2$. The coopex inhibited GPT 67.77% and no inhibition was recorded in GOT by coopex. Whereas RB-a, inhibited GPT, 57.47% and almost no inhibition was recorded in GOT, on the other hand, in the present work, the LC$_{50}$ of deltamethrin was recorded 0.6119ppm, for Biosal it was 1605.05ppm and for *Acorus calamus* LC$_{50}$ values was 70.6497ppm.

Biosal inhibited GPT 3.95% whereas GOT inhibition was 6.78 %. The precent inhibition by deltamethrine in GPT was 19.65% and for GOT it was 26.66 %. Azmi et al., (1998) recorded no inhibition by Biosal, and cypermethrin. Both results were quite different from each other, possibly due to different type pesticide or difference in insect species, or method of treatment.

Charleston et al., (2005) tested *Melia azadirachta* against *Plutella nylostella* L. Both extracts were found effective and at higher dose and the extract showed feeding deterrent effect was prominent. Treated insects oviposited lesser eggs, Biosal (neem product) was also effective at lower dose also, as evident during present study.
Bagavan et al., (2009) reported that the larvicidal activity of different solvent crude extracts of four plants were noted that the peel chloroformed extract of *C. sinensis* (LC$_{50}$=58.25 ppm), leaf ethyle acetate extracts of *O. canum* and *O. sanctum* (LC$_{50}$= 88.15 and 21.67 ppm) and chloroform extract of *R. nasutus* (LC$_{50}$= 4046 ppm) against the larvae of *An. subpictus* respectively. Similarly the researches observed against *cx. tritaeniorhynchus* mosquito larvae and also against the nymph of *A. gossypii*.

Their result suggest that leaf methanol extracts of *O. canum* and *R. nasutus* are repellent and more toxic to the nymph of *A. gossypii*, and the hexane fraction of *K. galanga* was found to exhibit the highest larvicidal effects with LC$_{50}$ of 42.33 ppm against *Cx. quinquefasciatus* (*Cx. fatigans* now) and possessed repellency against *Cx. tritaeniorhynchus* (choochote et. al., 1999). While in the present investigation deltamethrin (synthetic pyrethroid) showed very high value of LC$_{50}$ (0.6119 ppm) mean highest larvicidal effects as compared to *Acorus calamus* extract (β-azarone) and Biosal (azadirachtin).

The synthetic pyrethroid (deltamethrin) degradation started after 72 hours whereas the degradation of biopesticides started after 48 hours. This may be due to higher toxicity of pyrethroid and lesser toxicity of biopesticides (biosal and *Acorus calamus* extract).
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