5. DISCUSSION

Population of mosquitoes in a given area of region is a product of activities of environmental factors, genetic variation, mutation, and selection pressure. The existence and distribution of one or more species at a genetic designate suitability could interface with environment, which itself is dynamically changing due to climatic change all over the world.

Understanding relationship with habitats, environment and mosquitoes can decipher the out breaks of epidemics and in disease surveillance. This is significantly can input for public health care of communities, habitats of the region White (1926) care with preference of breeding habitats in India. Bhat (1975, 1990) Rajgopalan (1979) have been helping on to understand the triad. Mahesh and Jauhari, (2003), Devi and Jauhari (2004) conducted survey breeding sites to employed promising predictors of mosquitoes including vegetation and directly relation. Therefore, the any feature changes over the region. The semi-arid zone has classical environment in itself could also reveals new understanding as appeared during our study.

The distribution of mosquito species varies different geographical regions of the world. Therefore systematics study on semi-arid zone mosquitoes can be extremely significant. Subsequently, we have been able to identify the five the genera, Anopheles, Culex, Stegomyia, Armigeres, and Lutzia. After a careful screening on systematics and observation of the subgenera at species level, we were able to recorded subgenera, Cellia, Culex, Armigeres, Lutzia and species, An. stephensi, An. culicifacies, Cx. quinquefasciatus, Cx. gelidus, Ae. aegypti. Many of the study on the mosquito systematics globally have been focused on larval morphological variations, maxillary palpus and legs, wing spots variation. However, such types of studies were not carried out in semi-arid zone in last fifty years. Moreover, in this region, faster ecological changes have been recorded.
Therefore, we have in our study attempted study morphology and systematics larva, pupa, along with male, female, species specific characters. Devi and Jauhari (2007) have conducted a similar study in Himalayan region of India. They could collect thirty four species of mosquitoes across three phytogeographic zones, tropical (three hundred to one thousand meter), sub tropical (one thousand to two thousand meter) and temperate (two thousand to three thousand meter) in the Garhwal region of India, which is under mountain Himalayan region. They included five genera *Aedes, Anopheles, Armigeres, Culex* and *Uranotaenia*. The immature form twenty three species were recovered from different breeding habitats. The larval habitats were seepage pools, river beds, rice fields, tanks, forest pools, ditches, streams, rock holes, tree holes, intradomestic containers and shallow pits.

Similarly, in our study *Stegomyia* larvae were collected during an interception from an overseas vessel in Darwin, Northern Territory. In collected larvae, the median hair 1-VII was three, this was in Huang (1979) identification key states four in Lamche and Whelan (2003) descriptions. Whereas, larvae of the *Aedes* mosquitoes of the complex punctor was then compared to material preserved. In this study, presence and absence of accessory hairs on the surface of siphon and their parity have been recorded (Filippova, 1993). In the present investigation, we could successfully recorded variations in chaetotaxy of fourth instar of *Cx. quinquefasciatus* Say. The variations have been recorded in larval segment VII, VIII, and X. Similarly, in our study, we have similar type of variations in the seta segment X seta 2-X, 3-X, was with 2-4 branches, when compare with the work of Srivankaran and White (1979).

It was therefore evident that chaetotaxy variations have been in several mosquito species, from different habitats of the world. However, it is significant for the breeding habitat and ecological factors, like humidity, temperature, rainfall, which might be significant for the morphology of mosquitoes.

Moreover in the wing spots have a great significance to identify the species, generally in the case of *Anopheles* (Nagpal and Sharma, 1995). However, in case of *Culex* species,
wing scale characters were used in the identification keys. The wing vein scales were not
detailed out. During present study, we have detailed out the morphology of wing vein of
*Cx. quinquefasciatus* under the scanning electron microscope. This study reveals that the
veins were generally found to be covered with broad and narrow types of scales. Also,
the wings were covered with small setae, which were detected only under scanning
electron microscope. This observation gives the correct mosquito species identification
and can be better understanding for control *Cx. quinquefasciatus* in semi-arid zone with
unique ecological factors. Individual characters are considered here to enhance our
understanding.

Now it has been observed that the scales have to be considered as credentials for
taxonomic character of *Culex* also. It has been detailed but so far and described under
SEM technique. The narrow wing scale of *Melanoconion ocellatus* has comprises with 6
wing scale veins (Sirivankaran, 1982). Also, in *Chrysonotum* the clavate wing scales have
been with five wing scale veins and in *Spissipes* with nine wing scale veins in the wing
scale. In present study, *Cx. quinquefasciatus* Say which we have recorded under SEM.
The basic differences in wing scale disposition, morphology, and the types are recorded,
which is a distinct variation from the previous, disclosed species of the subgenus.
Therefore, we can conclude that an accurate taxonomy of species and biological data
are now significant.

We have also acknowledged habitat’s role which shows migration. However larva *Ae.
riversi* was collected primarily in tree holes and also found in bamboo stumps and
artificial containers in forest. Despite the abundance of adult *Ae. albopictus* in the forest,
larvae collected in tree holes less frequently than *Ae. riversi*. This has been frequently
exploited bamboo stumps, stone vases, and discarded tires outside the forest (Sota et
al. 1992). Whereas, in the longitudinal study on breeding habitats and variation in the
relative larval density of *Ae. aegypti* and *Ae. albopictus* were collected during October
1995 to September 1996 in cement pots, plant pots, molluscan shells of urban garden in
Calcutta City. The *Ae. albopictus* larvae were found in accumulated of water in bamboo
stumps and tree holes.
In our case of field survey, we have collected larvae of genus *Stegomyia* from tin containers and plastic containers which have not been reported as seen. The morphologically of the larvae pupa and adults, of this species was found new species specific morphological characters. The *St. agraensis* a new species thus can be well distinguished from other *Stegomyia* species by the following combination of characters. Scutum with four snowy white spots with a distant patch of border crescent-shaped white scales on fossal area. Foreleg femur has also been found completely covered with dark brown scales. Foreleg tibia has completely covered with very small light brown spot. Whereas the foretarsomere 4, 5 and midtarsomere 4, 5 have been covered with dark scales. In the present observation of *St. agraensis* 6-8 hairy comb teeth have been observed. In *St. aegypti* 8-12 comb scales have been recorded. *St. annandalei* 5-6 large and simple comb teeth were found. However, 5 comb scales were recorded in *St. mediopunctatus* from the posterior margin of a semicircular chitinised plate (Barraud, 1934).

Also, there were comparatively less than 20-25 hairy pecten spines in *St. agraensis* which can be easily this separated from *Stegomyia* species, where 20-34 pecten spines were found. However, the diagnostic for the separations of these two species as adult was the presence of four snowy white oval shaped spots on the mesonotum. The mesonotum of *Stegomyia* was marked with a pair of lateral curved white lines *aegypti* (Barraud, 1934, Rueda, 2004). While in case of *St. mediopunctatus* the white area on front of mesonotum were rounded and not continued back towards the scutellum. In *St. albopictus* these narrow, silvery white median line running nearly whole length of mesonotum. *Ae. aegypti*, the midfemur when viewed from the front, with a white longitudinal line, running from the base for nearly whole length, but not continued quite to knee. Tarsomere of foreleg and midleg have with narrow basal white bands to the first two or three segments.

This new species has now been distinguished easily from the foreleg femurs which were completely covered with dark brown scales and tibia with very small spot with pale scales. Except that the whole leg were covered with the dark brown scales. It could be
therefore differentiated from the other species of the genus *Stegomyia*. There were small pale bands on the mid leg and hind leg femur with pale bands. Whereas the midtarsus I, II, III with white bands and last IV and V tarsus were completely covered with dark brown, this can now be easily separated from the species of the genus *Stegomyia*. The philosophy behind this study was to ascertain that newly mutated species are fast increasing and harbinger of many new viral fevers in these areas, creating public health problems.

The container positively and relative larval density of both species was highest during monsoon from June 1996 to September 1996 and lowest during summer from February 1996 to May 1996 (Ray and Tandon, 1999). Similarly, *Ae. aegypti* and *Ae. albopictus* larvae were found breeding in almost all indoor and out door, temporary and permanent collection of water. These were either alone or in association with each other in residential areas of the city. Moreover, these larval indices of both the species were highest during monsoon and post monsoon (Tandon and Ray, 2000). According to Khan (1980) in one year study conducted on the outdoor breeding habitat and seasonal prevalence of larval population of *Ae. aegypti* and *Ae. albopictus* in ten different locations of Dacca City. Nine other locations were found infested with larvae of either one or both species during the rainy seasons from May to October of the year. *Ae. albopictus* larvae were present in nine including all four natural sites resulting in highest number. *Ae. aegypti* larvae were present in only four kind of artificial containers. The seasonal variations in the larval populations of both species closely followed the fluctuations in rainfall with zero population during driest three months.

In the present study, we could observe that the fourth instar of genus *Armigeres* were habitating from the rural site district Mainpuri of Agra region. This species was collected from different dwellings, permanent ponds, generally collected from shadow side water. The larvae, pupae and adults were when observed under compound microscope, we have not found exact species specific, when compared with previously reported species with the work of Barraud (1934) descriptions. However, *Ar. mainpuriensis* can be distinguished distinctly from all other species *Armigeres (Armigeres)* by combination of
the following characters. Seta 1-A and 1-S absent. Seta 1-X with 5-7 long branches. 2-X with 6-8 branches, 3-X with 6 long branches, 4aX, 4bX with 3, 4cX with 4-5, 4dX, 4eX with 4-6, 4fX, 4gX with 5 long branches. Whereas in case of Ar. theobaldi seta 1-A 0.38-0.52 mm from base, seta 1-X with 2 and seta 1-S single (Toma et al. 1994). Similarly in Ar. kinabaluensis seta 1-A at 0.49 from base, seta 1-S with 1,2 arising about 0.26 from apical and of siphon and Ar. kesseli seta 1-A single at 0.48 mm from base (Ramalingam, 1972, 1987). This new species Ar. mainpuriensis has also comparable with the Ar. obturbans (Walk) 1860, with short antennas, thick with a small hair at middle. This was been a common species in India (Barraud, 1934). The lateral hair were fairly well developed like Ar. obturbans. The number of branches in lateral tufts hair on the first abdominal segments were 5 instate of 7-10 as described in Ar. kuchigensis. Slightly to the tuft hair, there could be seen on segment II and I is usually fine and divided in to several branches in Ar. obturbans, which is in Ar. kuchigensis it is larger, either single or two branches. Which were in Ar. mainpuriensis always short and two branched.

As it depicts a close relationship with Ar. kuchigensis rather than to Ar. obturbans. Moreover, Ar. mainpuriensis has also been differentiated from legs, as hind femur with white with outer side from base to knee joint to Ar. obturbans. This was in case of Ar. mainpuriensis, the hind femur with pale on outer side from base to knee joint. Legs were recorded to cover with dark scales, from tibia to tarsus. The Ar. obturbans had all legs pale when seen from behind (Barraud, 1934).

The adults of Ar. kesseli were with elongated phallosome and bunch of about 5 large pointed teeth on the inner apical aspects (Ramlingam, 1987b). Whereas, in case of the male Ar. kinabaluensis we could very well recognize them by the very distinctive feature the phallosome, with the apical outer margin of which bearing 7-9 long curved teeth on each side (Ramlingan, 1972). After comparing the redescription of Ar. theobaldi produced by Toma et al. (1994) with features male genitalia, and Ramalingam (1987b) reported Ar. kesseli that was not found with variations but is specifically to be designated as a new species. This is probable that due to high diurnal variation noted throughout the year in semi-arid zone with added fluctuation in rain fall, humidity and
temperature might have produced stress in to few distinct new characters in genitalia of male mosquitoes. Moreover, we could in our present study noted that the ventrally phallosome were with three large teeth and two small. The inner apical aspect of the phallosome has a group of fairly large pointed teeth bunched together. This is unique distinction for a separate species formation.

It distinctly validates the significantly changing environmental parameters. However the geographical distribution and development rate of mosquitoes is closely related to temperature, humidity, and rainfall. We could record maximum diurnal fluctuation of humidity and temperature in semi-arid zone (Graph ).

Subsequently, at the same time we have also been collected predacious genus Lutzia from Dayalbagh of Agra region. This has so far not been reported earlier in this region. However, the mouthparts of Lutzia were modified for predacity, mouth brushes of a moderate number of strong curved rods. Cranium setae were single, siphon short, with a posteroro ventral row of hair tufts and with pecten, both extending along whole length of tube. The Anal segment was large, longer dorsally than ventrally (Barraud, 1934). In present study these characters were found in the collected larvae. The species-specific similarities were found in seta 5-C, 6-C, and 10-C, and the mentum was with 8 spines including middle one as Lutzia halifaxii. Whereas, Lutzia new species 40-45 comb scales were recorded. These were rounded at ends and covered with simple hair on both sides. The comb scale of Lutzia halifaxii was with pointed end, with bifurcated spines. In the pecten spines two teeth were recorded by us, which was found some time single. In our species 7-10 pecten spine were observed with always two spines one small and second long with pointed end. Moreover, the Gs and Gc were also differentiated as distribution pattern and size of setae (Fig.4.17).

On comparing the results of present study with other species of Lutzia with Barraud (1934) descriptions of larvae of Lutzia vorax, raptor, fusceanus this larva can be easily differentiated. These species-specific morphological structures were not recorded and are found in other species of Lutzia. Therefore these mark differences in Lutzia.
designated as a new species. The species-specific larval description is significant, especially in semi-arid zone of India.

Our data reveals that diurnal variation in the year with rainfall depicts highly significant deviation be validate further by Metrological Department India (Permission granted to produce this in scientific observation) validates that actual rainfall fluctuates much with the anticipated normal rainfall. Similarly, the cumulative rainfalls also having variation with the reference of normal rainfall in the zone. Mosquito systematics of this area encompasses a variety of ecological habitats such as moderately high to very high diurnal fluctuation of temperature and humidity ranges, and rainfall in semi-arid zone. Therefore, this subcontinent appears to be an ideal setting for study of the systematics of Culicidae as the records may be facilitate variations, species formation.

The mosquito diversity is becoming very essential now for controlling viral fevers in each country including India as they are emerging as health significance. They are environmentally interfacing with new viruses and becoming vectors of new diseases. It is paradox that in spite of such a great health implications the departments of public health as well as academic institutions could afford to ignore the basic cause of new emergence of diseases. We, now provides the microscopic details as well as the formation of new species along with detailed on mosquito systematics in semi-arid zone as they seem to be necessary.

The collected data therefore could be interpreted as new species as function of mutants and emergence of new mutants for the purpose of environment maturation and dynamical system. The system approach in this region can be justified. If we conclude that the larval variation in mosquitoes is function of environment system also.

The interaction of habitat, climate and mosquitoes could generate a model for predict abundance. When we compare our study with few of our previously reported studies the preference of the habitat is well marked phenomenon due to ovipositional preferences, physiological- chemical adaptations. The breeding sites of mosquitoes are sufficiently
limited in extent and easy to access, larval control that make significant contribution to mosquito control. However, in order to be effective as mosquito borne diseases control method, a high percentage of all productive breeding sites within flight range of the community. Furthermore, larval control reduces the density of local vector populations.

The development of insecticides over the past 60 years provides a relative simple tool for control of vectors of disease, especially in the vast rural areas of tropics. However, the emergence and spread of insecticide resistance in many species of vectors and occurrence of multiple resistances to organochlorine, organophosphate, carbamate and pyrethroid insecticides in several insects, increasing has been directed toward natural pathogens. Chemical control of the mosquito can be based on the larval stage of the mosquitoes. However, the larvae many be major disease vectors live in transient aquatic habitats produced during the rainy season or by agricultural irrigation. It is well known that resistance to insecticides is an emerging problem in many insect vectors of disease. Our knowledge of the basic mechanisms underlying resistance to commonly used insecticides is well established. The amount of resistance in insect vector populations is dependent both on the volume and frequency of applications of insecticides used against them and the inherent characteristics of the insect species involved.

Different approaches to detecting the emergence of resistance are now possible. However in present study we have employed WHO (1985, 1998, 2005) bioassay standard susceptibility tests in the laboratory. From these experiments the appropriate dosage required to kill 50% or 90% of populations can be calculated by Probit analysis (Finney 1971) and be able to detect any changes in percentage mortality over a period of time as well as occurrence of resistance. Probit analysis is a statistical method to assessment of the larvicides by means of the responses products, when dose are given to larvae. The estimation of median effective doses has been calculated. The experimental data on the relation between dose and mortality have been obtained graphical and used to estimate parameters.
Hackett (1937) has been suggested that the species identification clearly significant to any vector control programme that seeks to be efficient as well as effective. Green (1981) studied the benzene hexachloride insecticide resistance among the malaria vectors in Zimbabwe. This study suggested that most wild specimens of the *An. gambiae* species complex were susceptible to benzene hexachloride. However, when the specimens tested in these bioassays were subsequently identified to species by a diagnostic isoenzyme assay, it was found that only *An. quadriannulatus* was killed by benzene hexachloride constituting most of the test samples. The few *An. arabiensis* in the samples survived exposure to the insecticide. That was an important finding which indicated that the *An. arabiensis* vector in Zimbabwe, and *An. quadriannulatus* found highly zoophilic species that was not involved in transmission.

Braga *et al.* (2004) have been tested larval bioassays with the diagnostic temephos dose. The result of the study shows that the Northeast region, Sergipe and Alagoas, *Ae. aegypti* mortality levels varied from 35.3% of Arapiraca to 7.1% Itabaiana. In Rio de Janeiro, these levels ranged from 61.9% of Campos dos Goytacazes to 10.8% of São Gonçalo.

Sathantriphop *et al.* (2006) investigated in a field strain of *Cx. quinquefasciatus* from Baan Suan community, Nonthaburi province, to the resistance to various insecticides from organochlorine, organophosphate, carbamate and pyrethroid. The Baan Suan strain was found completely resistant to DDT and highly resistant to deltamethrin, permethrin, fenitrothion and propoxur but this strain was still found to be highly susceptible to malathion. This strain displayed high resistance to cypermethrin since the result revealed that the resistance ratio of the 50% lethal concentration value (RR50) between the field and the laboratory strains was sixteen. The study indicated that mosquitoes were resistant to almost all insecticide tested except malathion and this should be an alternative for *Cx. quinquefasciatus* control in this area. Moreover, *Ae. aegypti*, was a main dengue vector in Baan Suan community was also tested with deltamethrin, permethrin and fenitrothion. The results showed that dengue mosquitoes are clearly resistant to permethrin and tolerant to deltamethrin, but were 100%
susceptible to fenitrothion. The cause of insecticide resistance in Cx. quinquefasciatus may be due to the continuous use of insecticide for dengue vector control programme in Baan Suan community.

The various pathogens, the bacterium Bacillus thuringiensis H-14 and Bacillus sphaericus produce proteins that are toxic to mosquito and black flies larvae. The target site of the toxins is larval midgut cells, which is, in the presence of the toxin, undergo degradation and lysis, larvae undergo tremors, become sluggish, and eventually die (Rodcharoen and Mulla, 1994). The biological control of Cx. quinquefasciatus using Bacillus sphaericus was considered a practical solution because of its specific and prolonged killing against mosquito larvae. In this study the feasibility of in mosquito control multicentric trials were under taken. Initially, Bacillus sphaericus was very effective but with in a year, after twenty to twenty five rounds of application, field population of Cx. quinquefasciatus developed resistance up to 150 fold. Among the two bio-larvicidal agents, use of Bacillus sphaericus has resulted in the development of resistance in field populations of Culex quinquefasciatus (Adak et al., 1995). In the present investigation we have exposed the developmental stages of larvae of An. stephensi, Cx. quinquefasciatus, Ae. aegypti from lowest diagnostic concentration LC₉₀ 0.01mg/lit in the laboratory. This dose was effective on Cx. quinquefasciatus, and An. stephensi as per the WHO (1985). However, mortality was recorded at the selected concentrations. The result shows that the Bacillus sphaericus is now resistant in semiarid zone. This can be facilitate that resistance up to 150 fold may be due to different enzyme Adak (1995) have observed, while in our case the efficacy is declining but we were not able to observe in the study of esterase, monoxygenase, acetylcholinesterase. There study case in field population and in our case it was only in few generation of larvae in the laboratory itself.

The use of Bacillus sphaericus as a potential biolarvicide in India is limited due to development of resistance by the target mosquito species. Observations on the biological processes of development and resistance in the Bacillus sphaericus susceptible population of Cx. quinquefasciatus have provided good insight towards developing a
better control strategy for vector mosquitoes. In a laboratory evaluation, *Cx. quinquefasciatus* susceptible to *Bacillus sphaericus* attained a high resistance level (70 and 90.5 fold) at LC$_{50}$ and LC$_{95}$ levels, with several important underlying factors involving binding of *Bacillus sphaericus* toxic molecules to the receptor proteins at the site of action. The resistant larvae showed insignificant variation from susceptible larvae in biological features, especially pre-oviposition period, number of egg rafts laid, incubation period, hatching percentage, stadial period, adult longevity and mortality rate. As per the study they could get 90 fold increases in resistance and have conferred the variation with susceptible larvae with reference to Poopathi and Tyagi (2002).

Whereas, Skovmand and Bauduin (1997) studied and conferred this larvicide in two different formulations in which they found useful for studying efficacies but erratically it has not been extended up to resistance study. However, we could make some primary observations used to prove resistance but were unable to early generations of the selected formulations.

Susceptibility of the F1 and F2 larvae from individual mosquitoes against *Bacillus sphaericus* was checked by exposing the third instar at a discriminatory dose of 40 mg/lit of Spherix, which was determined by carrying out preliminary bioassays against *Bacillus sphaericus* susceptible and resistant strains. Exposure to third instars of *Bacillus sphaericus*-resistant strain of *An. stephensi* to a dose of 40 mg/lit. Spherix, did not induce any mortality among the larvae within 48 h, whereas all the larvae of susceptible strain were killed at this concentration. When *Bacillus sphaericus* susceptible strain was reciprocally crossed with *Bacillus sphaericus* resistant strain (crosses 3 and 4), larvae of F1 progeny from both these crosses were found to be completely susceptible to *Bacillus sphaericus* (Mittal, 2005). They could extrapolate this data from resistance population of F1 progeny with F2 selecting with susceptible study. This is significant for the environmental studies, whole theory remain always a possibility of crossing over strain/type of larvicides with another larvicides. However, we do not considered these studies to the formed which could defense the initialize the process of resistance development in first few generations. In case of *An. stephensi* mortality were started at
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concentration 5 mg/lit. At this concentration the 10% mortality in first and second instars and 8% in third and 5% in fourth instars were found. We have tested the developmental stage of larvae at different dose concentrations. The result of this study shows a specific dose was not showing mortality at all larval stages. The concentration of dose can be instar specific for larvae. This finding can be helpful for the resistance management against larvicides.

Moreover, in case of *Cx. quinquefasciatus* the mortality were recorded at concentration 0.01mg/lit in first and second instars and mortality was not found in third and fourth instars. Subsequently at the concentration 10mg/lit 100% mortality was found in first and second instars, 98% in case of third and fourth instars of *Cx. quinquefasciatus*. However in case of *An. stephensi* 12% mortality in first instars, 8% in second and third instars and 5% in fourth instars. Whereas the species of *Stegomyia* were less effective against *Bacillus sphaericus* (WHO, 1985). In present investigation we have also tested the new species *St. agraeinis*. This was found highly resistance against *Bacillus sphaericus* and this requires very high dose of concentrations.

Further Nielsen-Leroux (1995) has studied resistance to *Bacillus sphaericus* toxin, which consists of 51 and 42 kDa protein. It was reported to be due to a change in the receptors of larval midgut brush border membrane in the resistant strain of *Cx. quinquefasciatus*. They could designate the cause of these resistances and evaluate the site also, which was in mid gut brush border membrane of *Cx. quinquefasciatus*, this is true as a pure toxin structure was considered at molecular level, which we have avoided due to theoretically more intensive target.

In case of *An. stephensi* mortality were recorded at concentration of 5 mg/lit. At this concentration the 10% mortality in first and second instars and 8% in third and 5% in fourth instars were found. We have also tested the developmental stage of larvae at different dose concentrations. The result of this study shows a specific dose was not shows mortality at all larval stages. The concentration of dose can be instar specific. This can be helpful for the resistance management against larvicides. Moreover, in case
of *Cx. quinquefasciatus* the mortality were recorded at concentration 0.01mg/lit in first and second instars and mortality was not found in third and fourth instars. Subsequently at the concentration 10mg/lit, 100% mortality was found in first and second instars, 98% in case of third and fourth instars of *Cx. quinquefasciatus*. However in case of *An. stephensi* 12% mortality in first instars, 8% in second and third instars and 5% in fourth instars. Whereas the species of *Stegomyia* has not more effective against *Bacillus sphaericus* (WHO, 1985). A number of recent studies show that resistance development is possible in the laboratory. High and continuous exposures to *Bacillus sphaericus* lead to extremely high level of resistance in *Cx. quinquefasciatus* (Rodcharoen and Mulla, 1994, Nielsen-LeRoux and Charles, 1992, Georghiou *et al.*, 1992). For the first time the low-level resistance to *Bacillus sphaericus* in a field-treated population of *Cx. quinquefasciatus* reported in Brazil (Silva-filha *et al.*, 1995) and subsequently high level resistance (over 6000-fold at the LC50 level) in field population of *Cx. quinquefasciatus* in India.

In present investigation we have also test against the new species *St. agraensis*. This was found highly resistance against *Bacillus sphaericus* and this requires very high dose of concentrations.

The fungi and fungus-derived products are highly toxic on mosquitoes. This shows low toxicity to non target organisms. Accordingly, the use of entomophagus fungi and their derived products may be a promising approach for biological control of mosquitoes (Kirschbaum, 1985). In case of our study we have employed the crude metabolite of *Beauveria bassiana* on *Cx. quinquefasciatus* and *An. stephensi* only. This metabolite was found effective on all instars. In India Vijayan and Balaraman (1991) have been reported the metabolite of seventeen fungi to be highly larvicidal and their LC50 values against the third instar of *An. stephensi*, and *Cx. quinquefasciatus*. However, in our laboratory Mohanty and Prakash, 2004, Priyanka and Prakash, 2003, and Vyas *et al.*, 2007) we observe larvicidal activity of metabolite of *Trichophyton ajelloi*, *Chrysosporium tropicum* and *Lagenidium giganteum* against *Cx. quinquefasciatus*, and *An. stephensi*. The *Beauveria bassiana* could be better for larvae control if this apply in purified form.
However due to constraints of environmental food chain and food web can not employed these without considering non target species which will effect. To prevent this we suggest that metabolite less purified form could better approach to deal with larvae in aquatic system so that resistance development phenomenon can be delayed and debarred. In our study, we have applied to prevent localize the initiation where the scenario get changed. This in itself can provide better understanding in initial population control strategy rather then to generate resistance population. Our goal was to management of the resistance larvae in fields rather than allow these to develop resistance study and better attentive larvicides could be recommended measure resistance population.

Whereas, the increased development of mosquito resistance to chemical insecticides is of particular concern for many integrated mosquito control programs that utilize insecticide for mosquito control. Chemical control is the most important element in the integrated approach to control of mosquitoes of public health importance. However, the correct use of insecticides can be play an important global role in the prevention and mosquito control, as well as in sensitive ecological areas. The management of insecticide resistance has important impact on the availability of mosquito control tools.

Presently different formulations of synthetic chemical insecticides are in use for vector control. The development of resistance in mosquitoes has been reported to be due to selection by indoor sprays in the public health programs, and also by the use of pesticides In agricultures (Georghiou, 1990). The adulticide Dibrom and Trumpet were significantly more effective against the *Aedes* species complex than against *An. quadrimaculatus* and *Cx. quinquefasciatus* and no significance difference was found between Dibrom and Trumpet. Scourge caused greater than 97% mortality against all three species, and mortality did not differ significantly with species (Ham *et al.*, 1999).

Resistance of deltamethrin has been measured in five natural populations of *An. sinensiensis*. The median lethal concentrations LC$_{50}$ of deltamethrin in these populations were higher than those in susceptible strains originating from the same populations.
Whereas, the efficacy of insecticide mixture of permethrin and carbamate was tested by larval bioassay on two strains of *Cx. quinquefasciatus*, one resistant to pyrethroid and other resistant to carbamate (Wang, 2000, Corbel *et al.*, 2003). In present investigations we have tested a synthetic pyrethroid Gokilaht-S 5EC consisting of d,d-trans-cyphenothrin on all instars of *Cx. quinquefasciatus*, *An. stephensi*, and *Ae. aegypti* *St. agraensis* at six different concentrations. We have started our bioassay at lowest concentrations towards higher concentrations. The mortality was then tested at 0.0005 μl/lit, in all instars of *Cx. quinquefasciatus*, *An. stephensi*, and *St. agraensis*. However, the percentage of mortality was different in all instar of selected species (Table 4.09, 4.10). At concentration 0.05 μl/lit, 70% and 66% mortality were observed in third and fourth instars of *An. stephensi*. This species has been show resistance at LC$_{90}$-0.078 μl/lit in third instars and LC$_{90}$-0.08 μl/lit. The 100% mortality was than observed at 0.2 μl/lit in all instars of selected species. The doses concentrations have been based on fourth instar were and determine the toxicity (WHO, 2005, 2004, 1985). As per our observations the determined dose for fourth instars was not effective on early instars and pupae. In the second generation larvae do not shows mortality at selected doses. These types of strategies can be thus because of resistance against larvicides. Which we have again find out the initial pressure and concentration which could lead to resistance development in new species as well the local population of *Cx. quinquefasciatus* and *Ae. aegypti*.

Therefore, resistance study against larvicides can be an integral part of mosquito control. The early detection of resistance in a particular mosquito species of a particular area does not in itself justify an immediate change in policy for control strategies. The resistance of larvae can be ascertained before selection of larvicides and to provide baseline data for further resistance study. This can be significant for early detection of resistance.

In metabolic resistance, the metabolic pathways of the insect become modified in ways that detoxify the insecticide, or disallow metabolism of the applied compound into its toxic forms. Metabolic resistance to insecticides is mediated by qualitative and
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quantitative changes in proteins that can often be difficult to define precisely at the biochemical level. Three broad enzyme classes are involved in insecticide detoxication, the mixed function oxidases (MFO), esterases and glutathione S-transferases. Their involvement in resistance is commonly identified by increases in the characteristic metabolites they produce. All three classes exist in multiple forms within each species and it is often not known whether increased activity arises from qualitative or quantitative changes in these enzyme complexes.

However, the biochemical studies have been determine the resistance mechanisms for organophosphate and carbamate insecticides indicated non-involvement of insensitive acetyl cholinesterase conferring broad spectrum of resistance (ICMR, 2002). A high level of physiological resistance to deltamethrin in \textit{An. minimus} can be developed under selective pressure in the laboratory. Resistance could be shown by low mortalities of offspring from parents that survive selective pressure in preceding generations. The common insecticide resistance mechanism in insect has been reported elsewhere, including, three possible pyrethroid resistance mechanisms, namely mixed function oxydases (MFOs), elevated esterase, and reduced sensitivity of sodium channels (Georghiou, 1986, Roberts and Andre, 1994, Nelson \textit{et al.}, 1996, Scott \textit{et al.}, 1998, Feyereisen, 1999, Ganesh, \textit{et al.}, 2003, Karunaratne and Hemingway, 2000, Merryweather, 1990, Chareonviriyaphap \textit{et al.}, 2002).

Esterases and Monooxygenases have been associated with pyrethroid resistance in mosquitoes (Hemingway and Ranson, 2000). Monooxygenase are a chain of enzymes usually being cytochrome P450 and elevated monooxygenases have been responsible for degradation of pyrethroid in \textit{An. pseudopunctipennis} ((Nelson \textit{et al.}, 1996, Ocampo \textit{et al.}, 2000). Alternations in this rate limiting enzyme can dictate levels of resistance to pyrethroid, organophosphate, and carbamate insecticides using this metabolic mechanism. In our study the esterase has been detected in early stages of larvae of \textit{Cx. quinquefasciatus} by SDS/PAGE. However, the lower percentage content of esterases in fourth instar homogenate was also evident because a significant band was not observed on SDS/PAGE gels. The silver staining was used to compare total protein in resistant
and susceptible adults. In this study a 62 kDa band was present at high concentrations in the Dar strain, and trace quantities in the most strain. The protein was also expressed in Dar larvae and pupae (Karunaratne et al., 1993, Merryweather et al., 1990). In our study we have significantly detected esterase in third and fourth instar of *Cx. quinquefasciatus* in coomasie brilliant blue staining at 17 kDa band was observed by SDS/PAGE method. This method is most significant to detection of resistant enzyme specifically esterase. The acrylamide concentration was 7.5% and 10% same sample. This early detection of esterase significantly suggests that the dose determination for the larvae could be site specific and age specific. The detection of resistance in early stages can be helpful better strategy for larve control.

The electrophoresis is the study of movement of charged particles in electric field. The sample is applied on to the medium as a narrow zone or band the molecules with different mobalities travel as distinct zones which gradually separate from each other. However, SDS/PAGE gel electrophoresis is the most widely used technique for analysis and characterization of proteins. In this system proteins are dissociated into their constituent subunits using anionic detergent as SDS. The protein mixture is denatured by heating the sample 100 °C in the presence of excess SDS. Consequently SDS polypeptide complexes have essentially identical charge densities and these migrate in polyacrylamide gel of an appropriate porosity strictly according to polypeptide size or their molecular mass. The analysis of polypeptide composition of the sample, molecular weight markers of the polypeptide of the sample protein can also be determined by using standard molecular weight markers.

The *Cx. quinquefasciatus* PIN95 strain was also having resistance to fenitrothion, an insecticide that has never been applied by the mosquito control programs, indicating the occurrence of cross resistance. The major insecticide resistance mechanisms developed by the studied population seem to be the elevation of esterase activity that was determined to be about 7.4 and 9.9 higher than the values determined for the susceptible IAL strain for esterase (Bracco et al., 1995). These low values of esterase activity increments and RR at maximum 11.2 fold agree with previously reported data.
(Villani et al., 1983, Breeden et al. 1984, Brown, 1986, Bisset et al., 1990) that indicated a low elevation of esterase activity may be responsible for the establishment of low levels of resistance to a variety organophosphates. The significant increase in a esterase activity with the concomitant decrease of b esterase, suggest a selection for a esterase as a biochemical mechanism for insecticide resistance.

The ecological factors also affect the development of resistance in larvae. In the present study we could be suggested that the recorded highest diurnal fluctuations could be developed resistance against larvicides. We have detected resistant detection enzymes as, esterase but not differentiated it either a or b. Moreover, the considerable variations in esterase activity were observed with in strain. Several factors can be account for this variation. Other environmental factors such as nutritional or reproductive status have contributed to activity variation. The magnitude of environmental effects on enzyme activity was linked to be much greater in field population. However, in the early stages of the evolution of resistance, when the differences in esterase activity between susceptible and resistant individuals caused by gene amplification was small, such environmental effects make more difficult to detect individuals with genetically elevated esterase activity levels in samples taken directly from the field (Ferrari and Georghiou, 1990).

The increase detoxification is a common mechanism of resistance to larvicides. In Cx. pipiens such a mechanism is often involved in resistance to organophosphate. However the low level of organophosphate and pyrethroid resistance could be conferred by either elevated esterase or monooxygenase enzymes (Ganesh et al., 2003, Penilla et al., 1998).

In the microplate assay study it was considered as an excellent tool to identify resistance mechanism and to provide information on the frequency of resistant individuals present in a population (Brogdon, 1989). The detection of resistance and its mechanism even at low frequency by this method essentially makes monitoring for the early onset on insecticide resistance (Cordon-Rosales et al., 1990). It is well
documented that the microplate assay can detect the presence of elevated monoxygenase and acetylcholinesterase (WHO, 1998) and esterase also.

Successfully in present study we have detected the enzymes, acetylcholinesterase and monoxygenase by the microplate assay (WHO, 1998). These enzymes have been detected in early stage of third and fourth instars of *Cx. quinquefasciatus* at the dose 0.1μl/lit. The major mechanisms of organophosphate and carbamate resistance involve either change in their common target site, AChE, or increased rates of insecticide metabolism or sequestration. Esterases, GSTs and monoxygenases are involved in metabolism. Insecticides detoxifying enzyme activities and rates of AChE insensitivity were found to be similar in *Cx. tritaeniorhynchus* collected from the two sampling site, these were completely different elevations and climates. Limited biochemical data on *Cx. gelidus* revealed no particular differences between populations from these two localities However, monoxygenase can contribute to malathion resistance in two ways, by either increasing the rate of metabolism to non-toxic product, or decreasing the rate which the insecticidal malaoxon was produced from the malathion parent compound, with mobile phase the TLC, monoxygenase metabolites of malathion was eluted only slightly and located just above the point where the sample was applied (Hemingway and Ranson, 2000, Karunaratne and Hemingway, 2000, 2001).

The concept of resistance development and its initialization is significant and interesting scientifically rather than to just compare resistance developed by introducing different chemicals. Since the present study can be beneficial in understanding of initiation of resistance could provide the answers for the in early stage of resistance development itself. In our study the lethal dose concentrations could different on different species as well as instars specific. The results show the correct species identification is significant. Before applying any larvicide in the laboratory or field the species should be check. If species identification was not correct, in such conditions whole strategy can be failed for mosquito control. The mosquito systematics is significant with resistance study on larvicides, which provides mosquito control in early stages. This type of study can be carried out globally for local mosquito control.