EFFECT OF CERTAIN NEWER GENERATION INSECTICIDES ON THE DEVELOPMENTAL STAGES OF MOSQUITOES

By

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THESIS

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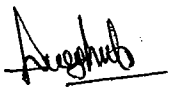
DECLARATION

I hereby declare that this thesis entitled **"EFFECT OF CERTAIN NEWER GENERATIN INSECTICIDES ON THE DEVELOPMENTAL STAGES OF MOSQUITOES"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or similar title, of any other University or Society.



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CERTIFICATE

Certified that the thesis, entitled **"EFFECT OF CERTAIN NEWER GENERATION INSECTICIDES ON THE DEVELOPMENTAL STAGES OF MOSQUITOES"** is a record of research work done independently by **Lucy Sabu**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the Advisory Committee of Lucy Sabu, 99-23-05, a candidate for the degree of **Doctor of Philosophy** in **Veterinary Parasitology**, agree that the thesis entitled "**Effect of certain newer generation insecticides on the developmental stages of mosquitoes**" may be submitted by Lucy Sabu in partial fulfillment of the requirement for the degree.

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Introduction

1. INTRODUCTION

Mosquitoes are found all over the world from the tropics to the artic. There are more than 3450 species in the Culicidae or mosquito family. In most parts of the world today, pest control of some kind is essential because crops, livestock and people live in a potentially hostile environment. Humans and livestock coexist with more than one million kinds of insects and other arthropods, which are pests (Bohmont, 1997).

Annoyance due to mosquitoes results from (1) abundance in flight near a person, (2) insistent singing as they hover above the resonating aural canal, (3) allergic reactions from bites and (4) being reservoirs for agents pathogenic to man and animals. The annoyance caused by mosquito feeding includes itching, restlessness, loss of sleep and nervous irritation in all people, pets and domestic animals that suffer from their attack. The saliva causes itching and this minor annoyance cannot be documented in terms of economic loss, but obviously there may be some major economic losses *e.g.* decreased recreation income and lower milk and beef production due to blood loss and irritation. The primary reason for controlling mosquitoes usually is to lessen the annoyance caused by their bites and then secondarily to reduce the transmission of diseases.

The need to reduce and if possible to eliminate altogether the ravages of insect pests and vectors has attracted the attention of scientists, national governments and international agencies for many decades. The term pesticide is an all-inclusive term that includes a number of individual chemicals designed specifically for the control of certain pests. As defined by federal and state laws of the United States of America it also includes

chemical compounds known as growth regulators that stimulate or retard the growth of plants/pests. It also applies to compounds used for repelling, attracting or sterilizing insects. The first generation insecticides are the naturally derived botanicals and materials like the arsenicals and petroleum products. The second-generation insecticides are exemplified by DDT and other chlorinated hydrocarbons, organophosphates, carbamates and synthetic pyrethroids. The period from 1940 to 1960 was characterized by major discoveries in the use of chemicals that secured the health and food crop of man, which later created problems of other kind.

Most of the conventional synthetic insecticides being broad spectrum in activity disrupt natural regulatory mechanisms. The drawbacks associated with the use of chemical pesticides namely pollution of the environment through accumulation in soil, air, water and agricultural products, the evolution of resistance in pests and destruction of beneficial insects and natural predators necessitated the development of environment friendly agents for the control of pests. Sole reliance upon chemicals for the control of ectoparasites is in jeopardy because of food safety, environmental concern, insecticide resistance and a lack of commercial interest in the development of new compounds. The problems with chemical pesticides cited above and subsequent development in biological sciences have led to renewed interest in biological control methods.

Biological methods for control of vectors suggest the use of insect predators, parasitoids, parasites and pathogens as alternatives to chemical agents. Among the above said agents, enteropathogenic spore-forming bacilli have been found to be a useful tool in controlling insects (Fast *et al.*,

1978). Later a potent biopestcide was developed from the endotoxin of *Bacillus thuringiensis israelensis* (*Bti*).

At present, integrated pest management (IPM) is the only option available for the control of vectors. IPM techniques in its simplest form is accepted as being a management strategy in which a variety of biological, chemical and cultural control measures are combined to give a stable long term pest control. Despite the problems imposed by insecticide resistance, there is no question that the judicious use of insecticides will remain an integral and essential component for most vector control programs in the foreseeable future. It is generally agreed among scientists that there is little, if any, chance that chemical pesticides can be abandoned until alternative control measures are perfected. Their use still provides in many instances the only economical and effective means of controlling the transmission of a number of mosquito borne diseases, particularly those for which neither a suitable chemotherapeutic agent nor an effective vaccine is available (Laird and Miles, 1983).

Alternative chemicals, which tend to be more compatible with biologicals include insect growth regulators (IGR) and pheromones. These newer alternatives came to be known as third generation insecticides. All compounds that regulate insect growth and development come under IGR (WHO, 1985). Juvenile hormone analogues instead of being directly toxic to target organism disrupt the metamorphosis resulting in larval-adult intermediaries or mosaics which die without gaining reproductive competence. Chitin synthesis inhibitors interfere with chitin biosynthesis and its deposition during ecdysis resulting in insect mortality due to cuticular malformation.

Since IGRs are specific and do not interrupt the natural regulatory mechanisms, they have become one of the integral component of IPM programs. It is important to realize that in IPM strategy, a species *per se* is not a pest but its high density. So the objective of IPM is not the eradication of a species but to limit their population density to a level that is acceptable as determined by economic factors and environmental concern. Despite the fears and real problems they create, pesticides clearly are responsible for part of the well being enjoyed by most people in the world over.

Hence the study on the effect of certain newer generation insecticides on the developmental stages of mosquitoes was taken up with the following objectives

1. to identify the various species of mosquitoes prevalent in and around Thrissur
2. to study the biology of the most commonly prevalent species of mosquitoes
3. to screen mosquitoes for the presence and identification of intermediary stages of animal parasites if any, and
4. to understand and compare the efficacy of certain newer generation insecticides against the developmental stages of mosquitoes.

Review of Literature

2. REVIEW OF LITERATURE

2. 1. PREVALENCE

Mosquitoes are common practically through out India and occur in enormous numbers in many parts. Christophers (1933) reported 38 species of *Anopheles* in British India including Ceylon and Burma, this being the first and still the authentic report on the tribe *Anophelini* from India.

Similarly, the first authentic report on the species of Culicines found in India by Barraud (1934) disclosed that they were collected at altitudes 14,000 feet or more in Kashmir and 3,760 feet below ground level in the Kolar Gold Mines in South India. The tribe *Culicini* comprised of fifteen genera and 239 species as furnished in the above report.

Reid (1968) defined mosquitoes as two-winged insects with many-segmented antennae, in which the wing veins and hind margins of the wings were clothed with scales and the mouth parts formed a long proboscis projecting forward. According to him 3000 species of mosquitoes were recorded till 1968 and further more being discovered constantly.

Fourteen species of *Culex* under the subgenus *Lophoceraomyia* were listed by Sirivanakarn (1977) from India.

In a mosquito survey conducted in Goa during August, 1983, 2096 adult mosquitoes belonging to 29 species were collected. *Culex tritaeniorhynchus* was the most abundant species forming 36 per cent of

the total collection. Henceforth, the total number of mosquitoes recorded from Goa surged to 51, comprising *Anopheles* – 25, *Armigeres*- 1, *Aedes*- 6, *Culex*- 14, *Heizmannia*- 1 and *Mansonia*- 4 species (Kulkarni *et al.*, 1986).

The checklist of mosquitoes of Bangladesh presented by Ahmed (1987) included 113 species coming under fifteen genera.

Malhotra *et al.* (1987) collected 48 species of mosquitoes belonging to nine genera such as *Anopheles, Aedes, Armigeres, Culex, Coquillettidia, Malaya, Mansonia, Toxorhynchites* and *Tripteroides,* in a survey carried out in Tirap and Subansiri Districts of Arunachal Pradesh. *Culex gelidus and Cx. tritaeniorhynchus* were found in large numbers in Tirap. In Sansuri District, *Cx. quinquefasciatus* formed 65 per cent of the larval collection followed by *An. maculates.*

Ten thousand two hundred mosquitoes collected by Nagpal and Sharma (1987) from 22 villages in northeastern region were identified into 61 species under eight genera viz. *Anopheles, Aedes, Armigeres, Coquittidia, Culex, Malaya, Mansonia* and *Toxorhynchites. Anopheles* was the most dominant genus followed by *Culex, Aedes* and *Mansonia.* The most prevalent species noted among these genera were *An.vagus, Cx. quinquefasciatus, Cx. vishnui, Ae. albopictus and Ma. annulifera.*

Twenty-three species of mosquitoes in Manipur belonging to *Anopheles, Aedes, Armigeres, Heizmania, Mansonia* and *Tripteroides genera* were found to have day biting habits (Rajput and Singh, 1987).

Rajput and Singh (1988), in an extensive survey of mosquito fauna in the state of Manipur during September 1983 to October 1985 recorded ninety species of mosquitoes under two subfamilies and ten genera, the latter being *Anopheles, Aedes, Armigeres, Heizmannia, Culex, Mimiyia, Coquiettida, Mansonia, Tripteroides* and *Uranotaenia.*

The population trends and behavioral attributes of adult mosquitoes associated with dairies in Southern California were studied by Schreiber *et al.* (1988). The most prevalent species caught in surveillance traps were *Cx. tritaeniorhynchus* followed by *Cx. erythrothorax* and *Cx. tarsalis.*

According to Dersie and Pradhan (1990), one hundred thirty species of mosquitoes under fourteen genera were known to occur in Nepal.

Two species of mosquitoes under the *Cx. vishnui* group (*Cx. pseudovishnui* and *Cx. tritaeniorhynchus*) were collected by Joshi and Bansal (1991) during an extensive three-month survey carried out in the desert district of Bikaner in Rajasthan. *Culex pseudovishnui* was found to be more dominant than Cx. *tritaeniorhynchus* during the entire period of study. Both the species preferred habitats with higher temperature and humidity. An increase in temperature and decrease in humidity brought a sharp reduction in density of both the species.

Naik *et al.* (1992) collected 89 species of mosquitoes belonging to ten genera from cattle sheds and human dwellings in Goa.

According to Alan Walker (1994), the family *Culicidae* had 3450 species in 37 genera. There were three subfamilies of mosquitoes;

Anophelinae consisting of *Anopheles* and two other rare genera (*Bironella* and *Chagasia)*; *Culicinae* comprising of nearly all other genera and *Toxorhynchitinae* with species that have a sharply bent proboscis and which were non-blood suckers. Mosquitoes of utmost importance to human and animal health belonged to eight genera and they were *Anopheles* in the subfamily *Anophelinae* and *Culex, Aedes, Haemogogus, Mansonia, Psorophora and Culiseta* in the subfamily *Culicinae.*

Nineteen genera of mosquitoes were reported to occur in Southeast Asia (Reuben *et al.,* 1994*).* They found that *Cx. quinquefasciatus* was strongly anthropophilic whereas other species of *Culex* fed extensively on cattle, pigs and birds. Keys for identifying the common sixteen species of *Culex* were provided. Simple identification keys to identify the three species coming under *Cx. vishnui* sub group *viz. Cx. tritaeniorhynchus, Cx. vishnui* and *Cx. pseudovishnui,* along with simple diagnostic keys for the various species of *Culex* fourth stage larvae were outlined. The fourth stage larvae of *Cx. tritaeniorhynchus* were identified by the apically rounded (fan-shaped) comb scales fringed with sub equal spicules and the weak siphonal tufts having 2 to 5 branches.

Aedes albopictus was reported as a biting nuisance in USA by Ali *et al.* (1995).

The study conducted in Soraipung village of Assam state revealed that the density of *Anopheles* species was significantly correlated with the monthly rainfall resulting in high prevalence in monsoon months and low in cool dry months (Prakash *et al.*, 1998). The mosquitoes identified

belonged to 30 species under six genera when 1037 mosquitoes were speciated.

Twenty-eight species of adult mosquitoes coming under eight genera were collected by Dutta *et al.* (1999) from the world's largest river island, Majuli in Assam

Armigeres joloensis, a rare mosquito was recorded for the first time from Assam by Bhattacharya *et al.* (2000). Of the forty-six species of *Armigeres* mosquitoes recorded worldwide, fifteen have been documented from India.

The subgenus *Ochlerotatus* under the genus *Aedes* was elevated to the rank of a genus by Reinert in 2000 based on the morphological peculiarity in the genitalia. The female genitalia had the insula lip-like, bearing well-developed setae laterally.

Three hundred and sixty nine species of mosquitoes under 43 subgenera and 21 genera were known to occur in India (Rajavel, 2004).

According to the Malaria Research Centre Report (2004), 58 species of *Anopheles* were known to occur in the Indian subcontinent, of which seven have been elucidated as vectors of Malaria viz., *Anopheles culicifascies, An. fluviatilis, An. stephensi, An. minimus, An. annularis, An. philippinensis- vivipes* and *An. subpictus*.

2. 1. 1. Kerala

No thorough work has been carried out so far on the species prevalence of mosquitoes in Kerala except for certain attempts made to identify the species involved in the particular locality during an outbreak of a mosquito borne disease in human beings. The transmission of *Brugia malayi* causing elephantiasis by *Mansonia* mosquitoes in Cherthallai, Kerala was recognized as early as 1932 by Iyengar. Later the vector species involved were identified as *Mansonia annulifera*, *Ma. uniformis* and *Ma. indiana* (Iyengar, 1938).

Daniel *et al.* (1986) conducted a study in Trivandrum city between August 1984 and July 1985 on the seasonal prevalence of mosquitoes. Twelve species coming under seven genera were collected from a cattle shed located very near to a canal in Kannammola. *Mansonia uniformis* constituted more than sixty per cent of the collection. Maximum number of *Ma. uniformis* was collected in February 1985 while minimum number in October 1984. One of the characteristic features of the collection was the presence of males of *Ma. uniformis* in fairly large numbers in the cattle shed whereas males of no other mosquito could be found. A decline in the number of mosquitoes visiting the host during the onset of monsoon was also noticed. The different species identified included *Ma. uniformis, Ma. annulifera, Cx. gelidus, Cx. sitiens, An. hyrcanus, An. jamesi, An. vagus, An. barbirostris. Ae. vexans, Ae. vittatus, Ar. subalbatus* and *Ficalbia chamberlaini.*

The vectors of brugian filariasis in the endemic zone of Cherthallai in Kerala, have been identified as various species of *Mansonia.* These

mosquito larvae and pupae were seen attached to the bulbs of aquatic plants such as *Pistia stratiotes, Eichornia crassipes* and *Salvinia molesta* (Kumar *et al.*, 1989).

Sabesan *et al.* (1991) studied the seasonal abundance and biting behavior of *Mansonia* species and their relative role in the transmission of brugian filariasis in Cherthallai. During this study 18 species of mosquitoes were identified namely, *Ma. annulifera, Ma. uniformis, Ma. indiana, Cx. sitiens, Cx. whitmorei, Cx. quinquefasciatus, Cx. vishnui, Cx. bitaeniorhynchus, Cx. fuscanus, Cx. minutissimus, Ae. vittatus, Ae. albopictus, Fi. chamberlaini, Ar. subalbatus, An. vagus, An. subpictus, An. pallidus* and *An. nigerrimus.*

Among the three species of *Mansonoides* mosquitoes that were prevalent in Mararikulam village in Cherthallai taluk, Krishnamoorthy *et al.* (1993) found that *Ma. annulifera* was the predominant species followed by *Ma. uniformis* and *Ma. indiana.*

In a study conducted in Vypeen Island, Cochin, Mariappan *et al.* (1996) could identify 14 species of mosquitoes under four genera *viz., Aedes, Anopheles, Armigeres* and *Culex. Culex sitiens* was found to be the predominant mosquito species. The various species identified were *Ae. aegypti, Ae. albopictus, An. barbirostris, An. nigerrimus, An. subpictus, An. vagus, Ar. subalbatus, Cx. bitaeniorhynchus, Cx. brevipalpis, Cx. fuscanus, Cx. gelidus, Cx. quinquefasciatus, Cx. sitiens* and *Cx. whitmorei.*

In yet another study, Mariappan *et al.* (1997) identified 35 species of mosquitoes belonging to eight genera from Kochi. The mosquitoes of utmost public health importance were identified as *Cx. quinquefasciatus* and *Ar. subalbatus* was the predominant nuisance mosquito.

Mosquito fauna was studied during an explosive insular outbreak of Japanese encephalitis, which occurred for the first time in 1996 in Kuttanad, Kerala (Hiriyan *et al.* 2003). A total of twenty-six species belonging to six genera of mosquitoes viz., *Aedes, Anopheles*, *Armigeres, Culex, Coquillettidia* and *Mansonia* were collected. *Culex* species represented 77 per cent of total collection with *Cx. tritaeniorhynchus* as the most dominant among them representing 63 per cent. The six species of medical importance were identified as *Cx. gelidus, Cx. quinquefasciatus, Cx. tritaeniorhynchus, Mn. annulifera, Mn. indiana* and *Mn. uniformis.*

In a two-year entomological survey carried out in the Kuttanadu region of Kerala state, Arunachalam *et al.* (2004) identified 18 species belonging to five genera from collections of adult mosquitoes from vegetation surrounding cattle sheds and pigsties at dusk. *Culex tritaeniorhynchus* was the most abundant species, with an increase in number in areas associated with rice cultivation. The species identified were *Ae. aegypti, An. barbirosrtis, An. jamesi, An. pallidus, An. peditaeniatus, An. subpictus, An. tesellatus, Ar. subalbatus, Cx. fuscanus, Cx. fuscocephala, Cx. Gelidus, Cx. quinquefasiatus, Cx. infula, Cx. tritaeniorhynchus, Cx. vishnui, Ma. annulifera, Ma. indiana* and *Ma. uniformis.*

Blood meal identification of individual mosquitoes of the species of *Cx. quinquefasciatus* and *Ma. annulifera* carried out in the rural areas of Kuttanadu, Kerala, revealed that both these species were highly anthropophilic and feeding on cattle accounted for only 1.5 and 2.1 per cent respectively (Samuel *et al.*, 2004).

2. 2. BREEDING AND MAINTENANCE OF MOSQUITOES IN THE LABORATORY

Mosquitoes were colonized in the laboratory according to WHO (1975).

2. 2. 1. Eggs

According to Horsfall (1972), mosquitoes like all Diptera, underwent complete metamorphosis during their development. The female mosquitoes laid about 30 to 300 eggs at any one oviposition. The Culicine eggs were brown, elongate or approximately ovoid. The eggs were laid directly on the surface of water as a raft, which floated on the surface. In the tropics, eggs hatched within two to three days but in cooler temperate countries they did not hatch until after about 7- 14 days or longer.

2. 2. 2. Larvae

According to Service (1980), mosquito larvae could be distinguished from all other aquatic insects by being legless and having a bulbous thorax that was wider than both the head and the abdomen. There were four larval instars, all of which being aquatic. The larvae had a well

developed mobile head that had a pair of antennae and a pair of compound eyes. Prominent mouth brushes were present to sweep water containing minute food particles into the mouth. The thorax was globular in outline and had various simple or branched hairs. Nine abdominal segments were present, and the last segment had a sclerotised plate called the saddle, which completely encircled the segment and two pairs of transparent sausage shaped gills. These gills were concerned with osmoregulation.

Mosquito larvae fed on yeast, bacteria, protozoa and numerous other plant and animal microorganisms found in water. In tropical countries larval development, that is the time from egg hatching to pupation, could be as short as five to seven days but many species required 7-14 days. In temperate areas the larval period might last several weeks or months and several species over wintered as larvae. Almost any collection of temporary or permanent water could constitute a mosquito larval habitat, but larvae were usually absent from large expanses of uninterrupted water such as lakes especially if they had large numbers of fish or other predators. They were also absent from large rivers and fast flowing waters except that they might occur in marshy areas and isolated pools and puddles formed at the edges of flowing water. All Culicine larvae possessed a siphon that might be long or short. They hung upside down and at an angle from the water surface, when they were getting air. There were no abdominal palmate hairs on tergal plates of Culicine larvae (Service, 1980).

2. 2. 3. Pupae

All mosquito pupae were aquatic and comma shaped. The head and thorax were combined to form the cephalothorax that had dorsally a pair of

respiratory trumpets. The abdomen consisted of ten segments. Each segment had numerous short hairs and the last segment terminated in a pair of oval and flattened structures termed the paddles. In between the paddles was a small pouch like projection containing the developing external genital processes of the adults; in female pupae this consisted of the cerci and the pouch was quite small but in male pupae the pouch was bigger because it contained the claspers of the male genitalia. Some of the developing structures of the adult mosquitoes could be seen through the integument of the cephalothorax, the most conspicuous features being a pair of dark compound eyes, folded wings, legs and the proboscis (Service, 1980).

Service (1980) further reported that pupae did not feed but spent most of their time at the water surface taking in air through the respiratory trumpets. They were capable of active movement unlike the pupae of most other Diptera. If disturbed, they alternately flexed and extended the abdomen and this movement aided by their paddles enabled them to swim up and down in the water in a jerking fashion. The respiratory trumpets of the Culicine mosquitoes were generally longer, more cylindrical and their openings narrower than in *Anopheles*. Abdominal segments two to seven had numerous setae.

2. 2. 4. Culicine Adults

According to Reid (1968), adult mosquitoes rested on surfaces with the thorax and abdomen more or less parallel to the surface, with only the proboscis forming a slight angle with the surface. The whole surface from the proboscis to the legs was covered with scales of a uniform brown

colour. In females the palps were shorter than the proboscis; in males the palps were as long as the proboscis but were not swollen distally. The scutellum was trilobed and the scutellar setae were restricted to those lobes. Adults were recognized more by their lack of ornamentation. The tip of the abdomen of females was blunt and the cerci were retracted. The claws of all tarsi were simple and those of the hind tarsi were very small, and all tarsi had well developed fleshy pulvilli. Narrow fringe scales were present on alula of the wings. Postspiracular bristles and bristles in the spiracular area were absent.

In general, females seemed to outlive males, but even their longevity varied widely according to climate, season and species. The females surviving winter lived for several months during the colder part of the year and a few weeks only in midsummer (Horsfall, 1972).

2. 2. 4.1. *Culex tritaeniorhynchus*

Giles established this species in 1901 for the first time. Later Barraud (1934) described them as very similar in appearance to *Cx. vishnui, Cx. barraudi*, and *Cx. whitei*, but usually smaller. Mesonotum uniformly clothed with dark brown scales. Femora and tibiae dark brown, except underside of femora. Pale band on proboscis of female often extended towards base on underside.

According to Reuben *et al.* (1994) *Cx. tritaeniorhynchus* were distinguished by the presence of erect scales on the vertex, accessory pale patch on the ventral surface of proboscis proximal to median pale ring and the distinct narrow dark ring distally in the hind femur.

2. 3. MOSQUITOES AS VECTORS OF HELMINTH PARASITES

Medical and Veterinary Entomology dates back to 1878 when Patrick Manson discovered that filarial worms were transmitted by mosquitoes. The first animal species of helminth which was found to require mosquito as an intermediate host was *Dirofilaria immitis*. This was established by Grassi and Noe in 1900 (Morgan and Hawkins, 1949).

In India, filariasis due to *Brugia malayi* was recognized as a health problem mainly of the coastal region of Kerala state, extending from Karunagaplly in the south to Ponnani in the north. Cherthallai in the endemic belt was considered as the hot bed of the disease. The vectors of brugian filariasis in Kerala were identified as *Ma. annulifera, Ma. uniformis* and *Ma. indiana* (Iyengar, 1938). The more prevalent filarial species infecting man in other parts of the country was *Wucheraria bancrofti*.

Culex quinquefasciatus, the urban house mosquito has been incriminated as a good vector for *D. immitis* by Coluzzi and Trabucchi (1968).

Ludlam *et al.* (1970) opined that over 36 neartic mosquitoes acted as potential vectors of dirofilariasis and the primary vector appeared to vary greatly in relation to geographical distribution.

Verma *et al.* (1971) reported that species of *Armigeres* could transmit *Setaria digitata*, the cattle filarial worm.

Christensen (1977) studied the development and transmission of *D. immitis* by *Ae. trivittatus* and suggested it to be a natural vector in central Iowa.

Aedes sticticus and *An. punctipennis* were found to be the major vectors of *D. immitis* in Lee County, Alabama. (Buxton and Mullen, 1980)

Tolbert and Johnson (1982) dissected live mosquitoes to determine the presence of immature stages of heartworms in Macon County, Alabama. A total of 2549 mosquitoes representing sixteen species and five genera were examined. Two per cent of the mosquitoes collected were naturally infected with larvae of *Dirofilaria.* The infective larvae were found in the abdomen/thorax or the distal ends of the Malpighian tubules. Although *Cx. quinquefasciatus* consisted of 20 per cent of the mosquitoes collected, only 2.7 per cent were naturally infected with *D. immitis*.

Hendrix *et al.* (1986) documented over 72 species of mosquitoes as potential intermediate hosts for *D. immitis*

Parker (1986) examined 2885 mosquitoes comprising of ten species in four genera for filarial developmental stages in North Carolina, USA. Nineteen mosquitoes (0.7%) coming under four species were found to be naturally infected.

Involvement of *Cx. quinquefasciatus* in the transmission of human filariasis due to *Wucheraria bancrofti* was established in an endemic area of Orissa state in India by dissecting mosquitoes. Average L_3 load per infective mosquito ranged from 1.0 to 7.2. (Dash *et al.*, 1998).

Culex erraticus, a largely ornithophilic species was found to support *D. immitis* development (Afolabi *et al.*1989). The apparent tolerance of this species for *D. immitis* infection and its abundance during hot dry months when most other mosquito species failed to thrive marked its potential as a vector.

According to Wright *et al.* (1989) the microfilariae developed to the third larval stage in the Malpighian tubules of mosquitoes and subsequently migrated to the proboscis through the body cavity. Animals got infected through the wounds inflicted by mosquitoes.

A mosquito sampling programme was carried out by Aranda *et al.* (1998) in Barcelona, Spain with CO_2 light traps. Two thousand one female mosquitoes belonging to three genera and five species were dissected to find the developmental stages of helminth parasites. Twenty per cent of the dogs sampled from the same area were detected to contain microfilariae, but none of the mosquitoes sampled were found to harbour any developmental stage of filarid worms. *Culex quinquefasciatus*, the most abundant species (94%) collected was incriminated as a good vector for *D. immitis*.

Singh *et al.* (2000) dissected 339 *Cx. quinquefasciatus* and failed to find any developmental stage of filarial nematodes in Pathancot town of Punjab.

2. 3. 1. ***Dirofilaria repens*** Railliet and Henry, 1911

The species was described by Railliet and Henry in 1911 from the subdermal tissues of dogs in Italy (Sonin, 1985). It is a common parasite of dogs in Europe and Asia. Development of *D. repens* up to the infective stage in *Aedes* mosquitoes was first studied by Bernard and Bauche in 1913. The first moulting occurred in the Malpighian tubules and infective stage was reached by 10-13 days. Mosquitoes of the genera of *Aedes, Anopheles, Culex, Armigeres* and *Mansonia* have been considered as potential vectors of *D.repens*. It was found that the duration of development depended on species of the intermediate host and temperature. Microifilariae reaching the gut of mosquitoes migrated to the body cavity within 36 hours and settled in the Malpighian tubules for further development. After attaining infective stage, the larvae migrated to the head and mouthparts of mosquitoes. Infective larvae were 995± 20 microns long and 28± 2 microns wide (Sonin, 1985).

Anyanwu *et al.* (2000) established that out of the six common species of mosquitoes occurring in Zaria, Nigeria, *Ae. aegypti* was the only species susceptible to *D. repens*. It was observed that the rate of development to infective larvae was faster in mosquitoes infected in July when the environmental temperature was high than in November when it was delayed for four more days. They further stated that the factors that determined the vector species as a good transmitter of disease/parasite included the vast availability of vectors, longer lifespan of vectors, and susceptibility of vectors to parasites and the ability of vector to feed on vertebrate host.

The incidence of microfilariosis in dogs by examination of the peripheral blood was found to be 13. 04 per cent in Calicut (Valsala and Bhaskaran, 1974) and 24.2 per cent in Thrissur (Saseendranath *et al.,* 1986). No specific staining procedure was adopted to identify the species involved. But in a later study conducted in dogs of Ernakulam and Thrissur districts for a period of one year (Radhika, 1997) found that the prevalence of microfilariosis was 7.59 percent. Histochemical staining method was employed to identify the species involved as *D. repens*.

Dirofilaria repens has been recorded from man from various parts of the world including 12 cases reported from Kerala (Sabu *et al.*, 2005).

2. 4. EFFECT OF INSECTICIDES ON THE DEVELOPMENTAL STAGES OF MOSQUITOES

Many of the new groups of pesticides being used in the 1990s were so biologically active that they were applied at the rates of grams or ounces per acre. Most of these compounds offered greater safety to the user and the environment. According to Bohmont (1997), there are approximately 750 active chemical ingredients being formulated into about 25,000 commercial preparations.

2. 4. 1. Insect Growth Regulators

All compounds that regulate insect growth and development come under insect growth regulators (WHO, 1985). Juvenile hormones (JH) and JH analogues instead of being directly toxic to target organisms disrupt the metamorphosis resulting in larval adult intermediaries that die without gaining reproductive competence. Chitin synthesis inhibitors interfere with

the chitin biosynthesis and its deposition during ecdysis resulting in insect mortality due to cuticular malformation. IGRs are specific and do not interrupt the natural regulatory mechanisms, they have become one of the integral parts of IPM programme. IPM technique in its simplest form is accepted as being a control strategy in which a variety of biological, chemical and cultural control measures are combined to give stable long term pest control. The three components of IPM include maximizing natural control, monitoring the concentration of pests and natural control factors in a given area and selecting the appropriate techniques to suppress the pest only when necessary.

2. 4. 1.1. Diflubenzuron (Dimilin) and Methoprene (Altosid)

On testing insect development inhibitors, Schaefer and Wilder (1972), observed much variation in mortality between replicate tests run at different times than among those for classical insecticides. The sensitivity of larvae to a given compound varied with age in the fourth stage. It was further reported that the earlier larval stages were less sensitive to insect development inhibitors than the fourth stage.

Post and Vincent (1973) reported Dimilin as effective against all developmental stages of mosquitoes in low concentrations and relatively safe to other organisms associated with mosquito breeding habitats.

Hsich and Steelman (1974) conducted a study to determine the comparative susceptibility of five insect development inhibitors against 12 species of mosquitoes. The susceptibility of each species within a genus relative to the type of compound varied to such a degree that no

comparative generalizations of genera susceptibility could be made. Some species difference in susceptibility varied as high as 10,000 times among compounds. *Aedes sollicitans* was the most susceptible species to all the five compounds tested. The morphogenic effect of Altosid included fully formed dead adults with hard cuticle, with appendages that remained within the pupal case and abdomen that extended to the position normally held during ecdysis. All five compounds induced death of larvae, pupae and pupal-adult intermediaries. Abnormal adults were also spotted. Failure to successfully moult to the next larval instar was ascribed to a defect in the process of cuticle deposition. When all species were considered collectively, Altosid killed 74 per cent of treated mosquitoes as pupae, 19 per cent as larvae and two per cent each as intermediates and adults.

When Mulla *et al.* (1974) evaluated four insect growth regulating compounds against the developmental stages of *Cx. quinquefasciatus* and *An. albimanus*, bulk of the mortality was found to occur interstadially indicating the action of moulting toxins.

According to Mulla and Darwazeh (1975), insect growth regulators induced a variety of responses such as inhibition of egg hatching, delayed development in the larvae and pupae, discolouration of cuticle of immatures and inhibition of adult emergence in the target organisms unlike chemical larvicides which caused instant mortality on application.

Two formulations of Dimilin were applied at the rate of 0.08 and 0.016 ppm respectively to experimental ponds and the mosquito larvae and some non-target organisms were sampled later (Mulla *et al.,* 1975). Decline in the population of 3rd and 4th instar mosquito larvae was apparent

from two to eight days after treatment. Adult emergence was almost completely inhibited up to at least eleven days post treatment. The non-target organism population also was depressed slightly, but recovered to normal levels soon after treatment.

Miura *et al.* (1976) observed that exposure of egg rafts of *Cx. quinquefasciatus* to diflubenzuron treated water induced abnormal side hatching as well as ovicidal action. They further reported that during normal hatching, a portion of the anterior end of the eggshell was forced open transversely at a line of dehiscence, thus forming an egg cap. The egg-cap was not commonly detached but hinged midventrally to the remaining eggshell.

The ovicidal effect of diflubenzuron was first reported by Ascher and Nemny in 1974. When mosquito larvae were exposed to diflubenzuron, mortality occurred during a moult, in the pupal stage at concentrations lower than those resulting in larval mortality and in the partial emergence of abnormal adults at still lower concentrations (Schaefer *et al.*, 1978).

Methoprene at the rate of 1.0 ppm was very effective in drains and ditches where 80 per cent inhibition in adult mosquito emergence was obtained (Self *et al.*, 1978).

Methoprene was evaluated against three species of mosquitoes at the Vector Control Research Centre, Pondicherry (Das *et al.,* 1981). At a concentration of 2 ppm on larval instars, near total or total inhibition of adult emergence was observed in all the three species. However, treatment

with the same dose at the pupal stage could produce only six to fifty per cent inhibition in adult emergence.

Bhakshi *et al.* (1982) evaluated the chitin synthesis inhibitors diflubenzuron and penfluron against four species of mosquitoes. Diflubenzuron was found more effective against Anophelines than Culicines. The LC_{50} values of diflubenzuron on 4^{th} stage larvae ranged from 0.00016 to 0.1 ppm. The treatment did not cause much mortality among the pupae. When fourth instar larvae were treated, larval and adult mortality contributed equally to the total mortality. Mortality occurring at the intermoult period was attributed to the chitin synthesis inhibition during moulting stage.

Despite large number of insect growth regulators being available for pest control, only juvenoid methoprene and chitin synthesis inhibitor diflubenzuron have been cleared for use in mosquito control (Amalraj *et al.*, 1988).

Prakash (1992) found that 0.0075 ppm diflubenzuron could inhibit 50 per cent hatchability in 0-6 hr old Cx. *quinquefasciatus* eggs while, older eggs needed five times more diflubenzuron to produce the same effect.

The LC_{50} values of methoprene and diflubenzuron against *Ae. albopictus* were found to be 0.0011 and 0.0003 ppm respectively in a laboratory study conducted by Kawada (1993).

In a study conducted by Krishnamoorthy *et al.* (1993) against the immatures of *Mansonoides* mosquitoes in Alleppy District, Kerala using methoprene (Altosid), a decrease in larval density was observed in treated ponds and the adult emergence was inhibited by 14, 21 and 28 days post treatment at 0.5, 1.0 and 2 ppm of methoprene respectively.

The differential susceptibility of various life stages of mosquitoes to certain chitin synthesis inhibitors *viz.*, diflubenzuron, penfluron, ethylpenfluron and Bay SIR 8514 was studied by Prakash (1993). All these compounds caused moderate to high mortality in various life stages. The mortality occurred during the larval or pupal moults and also at adult emergence, indicating that the compounds inhibited chitin synthesis during each moulting stage. The LC_{50} values of diflubenzuron (ppm) on the fourth stage larvae of four mosquito species tested were as shown below.

Species	**LC_{50} in ppm**
Cx.quinquefasciatus	0. 03466
Ae. aegypti	0. 00289
An. stephensi	0. 00122
An. culicifacies	0. 00214

The LC_{50} values on the pupae of the above species ranged from 0.89 to 2.1 ppm. Fecundity of the females emerged from treated pupae was considerably reduced, though no significant effect was noticed on the egg hatchability or larval survival of the F1 generation.

Ali *et al.* (1995) observed that the LC_{50} values of diflubenzuron and methoprene were 0.00045 and 0.0022 ppm respectively against a

laboratory population of *Ae. albopictus*. They further observed that the LC_{90} values for the same were 0.00084 and 0.0081 ppm respectively. They ranked IGRs superior to pyrethroids, organophosphates and microbials in their toxicity to mosquito larvae.

When methoprene pellets were applied to a *Culiseta* breeding swamp, the inhibitory effect on pupae exceeded 81 per cent over a five-week treatment period as per the study by Woodrow *et al.* (1995).

Methoprene and diflubenzuron were evaluated against mosquito larvae in the laboratory as well as in different breeding habitats in Tezpur, Assam by Baruah and Das (1996). The LC_{50} values of both the compounds were almost the same (0.0009 and 0.0011 ppm respectively) against *Ae. albopictus* and *Cx.quinquefasiatus*. The LC_{90} values of diflubenzuron against the two species of mosquitoes were 0.0022 and 0.0027 ppm and those of methoprene were 0.0027 and 0.0022 ppm respectively. In a field study, at 0.2 ppm, both the compounds were found to eliminate 92 to 96 per cent of *Culex* and *Anopheles* larvae.

Becnel *et al.* (1996) remarked that slow release pellet formulation of methoprene (Altosid) provided almost complete control of *Ae. albopictus* adult emergence for 116 days. It was found superior to *Bti* (*Bacillus thuringiensis israelensis*)

Ali *et al.* (1999) evaluated different larvicides and insect growth regulators against field collected *Cx. quinquefasciatus* larvae from urban Dhaka, Bangladesh and noted the LC_{90} values of diflubenzuron and methoprene to be 0.0034 ppm and 0.052 ppm respectively.

2. 4. 2. *Bacillus thuringiensis israelensis* (*Bti*)

Fast *et al.* (1978) found that the spore-forming bacillus, *Bacillus thuringiensis* (*Bt*) was capable of producing proteinaceous inclusions during sporulation known as protoxins. They were insecticidal crystal proteins (ICPs) requiring solubilization and activation in the insect mid gut. When susceptible larvae ingested ICPs, the protoxins were solubilized in the mid gut and bound to specific receptors on the surface of mid gut epithelial cells to initiate the pathological changes.

The first isolation of *Bt* was by Ishiwata in 1902. However, the development of *Bt* as a biopesticide stood in the shadow of successive chemical insecticides for a long period. In 1956, Angus, proved that the insecticidal activity was located in the parasporal inclusion. This initiated the lookout for *Bt* strains with a potent endotoxin (Sudharani, 1996).

The development of resistance against most of the chemical insecticides and their well known environmental hazards favoured intensive research on *Bt* during 1970 to 1980. With the isolation of a new strain of *Bt* in Israel by Goldberg and Margalit in 1977, a new chapter was opened. Later, Barjac (1978) identified it as a new serotype (H14) and named it after its origin, serovar *israelensis*.

The first report on the larvicidal effect caused by the toxin of *Bacillus thuringiensis* on diptera was by Korzh *et al.* (1977), who observed that the first stage larvae of *Musca domestica* and *Stomoxys calcitrans* did not develop further in manure heaps containing 100 to 500 ppm of *Bt* toxin.

Mulligan *et al.* (1978) evaluated *Bacillus sphaericus*, Neide strain against larval stages of mosquitoes in the laboratory and field and found it to be effective against *Cx. tarsalis* and *Cx. quinquefasciatus* without any deleterious effect on non target organisms.

Larget and Barjac (1981) reported the extremely toxic effects of *Bti* suspension on mosquitoes, simulids and sand flies.

In a preliminary study conducted to assess the effect of *Bti* on mosquito larvae, Narsaiah and Jamil (1986) found that the sites of action of the bacteria were between the respiratory siphon and alimentary canal. The bacterial strains multiplied in the alimentary canal and made their way to the head region.

Significant variation in the response to *Bti* (Bactimos) has been reported against three species of Culicine mosquitoes by Farghal (1987). The LC_{50} values for the three species viz. *Cx. pipiens, Culiseta longiarcolata and Ae. caspius* were 4.5, 44 and 100 ppm respectively.

Ali *et al.* (1995), while comparing pyrethroids, OPs and IGRs against a laboratory population of *Ae. albopictus* claimed that *Bti* was more effective economically.

A liquid formulation of *Bti* (Acrobe) provided significant control of *Ae. albopictus* for 47 days in North Central Florida (Becnel *et al.,* 1996).

Skovmand *et al.* (1998) found that significant variations were seen in the potency of *Bti* as a larvicide between laboratories. They tested and compared similar *Bti* products formulated by six different companies from four different countries against the international standard powder IPS-82. They attributed that variation to be associated with factors such as age, stage and strain of larvae used as well as the amount and type of food provided to the larvae.

Ponce *et al.* (2002) conducted laboratory bioassays to determine the susceptibility of larvae of *Ae. aegypti* to Vectobac^{R} containing *Bti* (600 ITU/mg). The LC_{50} and LC_{95} values were found to be 0.0104 ppm and 0.18 ppm respectively. The field dose was calculated at four times the LC_{50} values and in the subsequent field study the larval populations were reduced to zero during the two week treatment period.

2. 4. 3. Azadirachtin

Since mosquitoes and many other insects had become resistant to pesticides, heavy and frequent applications were required leading to problems of toxic residues contaminating the environment and adversely affecting non target organisms. This dictated the need to develop safe, less expensive and preferably locally available materials for pest control.

Although the use of plant species to control insect pests had been in practice for centuries to a limited extent, it was only recently that interest has been renewed in the pest management potential of natural products. These products were the compounds that had evolved in plants for defence

against phytophagous insects. Berenbaum (1982) interestingly established that these secondary plant products were actually coevolved with insects that would have exploited them as a food source. The modern researcher now has the technology to exploit the properties of some of these compounds and use them against potential pests.

One group of compounds harbouring significant toxic effects on pests has been discovered in the neem tree (*Azadirachta indica*). The most active constituent, azadirachtin, a triterpenoid, was shown to have properties including feeding and oviposition deterrence, repellency, growth disruption, reduced fitness and sterility in a number of species of insects (Schmutterer, 1990).

Mong Ting Tan and Sudderuddin (1978) detected profound ovicidal and larvicidal effects of neem extracts on diamond black fly. They noticed inhibition of pupal development and presence of deformed wings in the surviving adults.

Attri and Prasad (1980) demonstrated that neem oil extract, a waste byproduct of neem was an effective mosquito larvicide, causing complete inhibition of adult emergence when applied to first stage larvae of *Cx. fatigans* at a concentration of 0.005 per cent.

Bioassays against larvae of *Ae. aegypti* were conducted with neem seed kernel extracts in water and organic solvents (Zebitz, 1984). Exposure of 4^{th} stage larvae resulted in a conspicuous growth disrupting effect, mainly during imago development.

Neem Azal a proprietary product derived from neem seed kernel was evaluated as a potential means of control of *Ae. aegypti* (Boschitz and Grunewald, 1994). Second, third and fourth stage larvae were reared in water containing different concentrations of Neem Azal. Continuous exposure to treated water induced moulting inhibition and mortality in all the larval instars tested.

Various neem products were tested by Su and Mulla (1998) to assess their ovicidal properties in different species of mosquitoes. When the egg rafts were deposited directly in fresh neem suspension and left there for four hours before being transferred to untreated water, one ppm of Azadirachtin produced almost cent per cent mortality of eggs. Based on this study, they demonstrated the potential of neem products as possible ovicides against *Culex* larvae.

Elhang *et al.* (2001) studied the effect of methanolic extracts of neem seeds on the egg hatchability and larval development of *Cx. quinquefasciatus*. It was found to cause reduction of egg hatchability, suppression of larval development, pupation and adult emergence in concentrations as low as 0.02 per cent. A remarkable effect was observed on larvae, which suffered 90 per cent mortality in neem extract of 0.1 per cent. Only 6.7 per cent of the larvae reared in 0.02 per cent managed to reach pupal stage and none of the pupae emerged as adults.

2. 4. 4. Ethofenprox

Ethofenprox is a non ester pyrethroid insecticide with comparable toxicity and a similar mode of action to other pyrethroids. Yoshimoto

et al. (1989) investigated the insecticidal properties of ethofenprox, a compound developed as an insecticide. It was found to possess a number of favourable properties as low mammalian toxicity, high compatibility with other insecticides, low dermal, and eye irritation and no cross resistance to carbamates and organophosphates.

Baktharatchagan and David (1991) evaluated the efficacy of ethofenprox (Trebon®) against three species of mosquito larvae in the laboratory. *Culex quinquefasciatus* was more susceptible than *Ae. aegypti* and *An. stephensi*. The residual activity against culicine larvae was found to persist in rice agro ecosystem even beyond five weeks.

Putsintseva *et al.* (1992) found ethofenprox as effective as permethrin against cockroaches and bedbugs. When studied against *Ae. aegypti*, the residual activity was found to prolong for one month. They further reported that the 10 per cent flow concentration was officially permitted only for the control of mosquito larvae in water not used for domestic purposes.

The LC_{50} values for Trebon® (ethofenprox) against *Cx. quinquefasciatus* was 0. 00579 mg/l according to Vasuki *et al.* (1995). No appreciable residual activity was noticed for this compound on the surfaces treated at the rate of 1 to 100 mg/sq.m against any mosquito species.

2. 4. 5. Deltamethrin

Photostable synthetic pyrethroids such as permethrin and deltamethrin were recognized as potent alternative insecticides to replace organochlor and organophosphate insecticides in mosquito abatement programs due to their excellent larvicidal, pupicidal and adulticidal properties and remarkably low mammalian toxicity.

In a study conducted by Chakraborti *et al.* (1993) in Maharashtra, it was found that the LC_{50} values of deltamethrin against five populations of *Ae. albopictus* varied from 0.0007 to 0.002 and the LC_{90} values ranged from 0.0022 to 0.01 ppm.

Sahgal and Pillai (1993) treated eggs of *Ae. aegypti, Cx quinquefasciatus* and *An. stephensi* with permethrin and deltamethrin and found that they caused moderate ovicidal activity, but inflicted delayed effects such as high larval and low pupal and adult mortality. Deltamethrin was found to be ten times more toxic as with permethrin against eggs of these mosquitoes. Egg mortality did not exceed 50 per cent even at the highest concentration (1 ppm) tried in the experiment. The eggs were found to be more tolerant to the action of insecticides compared to larval stages. Insect eggs were covered with shell, which differed biochemically from the integument of the larvae, which could be ascribed to the difference in the penetration of the insecticide through the eggshell and larval integument.

Ali *et al.* (1995) conducted bioassay of three pyrethroids, *viz.* permethrin, cypermethrin and bifenthrin against a laboratory colony of field collected *Ae. albopictus* mosquitoes in Florida and found the LC_{50}

values as 0.00095, 0.0026 and 0.0052 and the LC_{90} values as 0.0031, 0.0079 and 0.0175 ppm respectively.

Generally pyrethroids were not recommended for use as mosquito larvicides due to their effects on non target organisms. However, pyrethroids as mosquito larvicides might be utilized in some highly polluted habitats where non-target organisms were of minimal or of no concern (Ali *et al.*, 1999). Three pyrethroids were evaluated against field collected *Cx. quinquefasciatus* larvae in urban Dhaka, Bangladesh and the LC_{50} values ranged from 0.0001 to 0. 009 ppm while the LC_{90} values were from 0.00061 to 0.021 ppm.

In a study to assess the insecticide resistance in six strains of *Cx. quinquefasciatus* mosquitoes from Mumbai city, India by Mainkar *et al.* (1999) it was observed that both the larval and adult stages were resistant to organochlor and organophosphate compounds. None of the strains showed resistance to deltamethrin.

Ansari and Razdan (2001) found that deltamethrin treated window and door curtains of houses could reduce *An. stephensi* and *Ae. aegypti* population by 88 to 93 per cent in Motibagh area of New Delhi.

To avoid selection pressure on immature stages of mosquitoes (larvae and pupae) pyrethroid insecticides should never be used for larviciding, as they are invaluable adulticides (WHO, 2002). Adult *Culex* mosquitoes are relatively more tolerant than other types of mosquitoes against most insecticidal applications, making adulticidal control of *Culex* ineffective. In situations where *Culex* mosquitoes breed prolifically in

flooded drains and sites that could not be readily treated, larviciding could not be expected to have sufficient impact to reduce the population, particularly where monsoon climate and periodic flooding cause extensive breeding sites to be unmanageable.

Ganesh *et al.* (2003) studied pyrethroid susceptibility and enzyme activity in two malaria vectors, *An. stephensi* and *An. culicifacies* from Mysore, India. They were found to have increased enzyme activity indicating tolerance to deltamethrin and permethrin. The LC_{50} and LC_{90} values of *An. stephensi* were 0. 00418 and 0.03358 ppm respectively while that of *An. culicifacies* were 0. 00204 and 0.00893 ppm respectively.

Materials and Methods

3. MATERIALS AND METHODS

3. 1. PREVALENCE OF MOSQUITOES

3. 1. 1. Collection of Mosquitoes

3. 1. 1. 1. Location and Periodicity of Collection

The mosquitoes resting on cattle and walls of cattle sheds from five locations noted below were collected between 7.30 and 8.30 p.m at monthly intervals

1. K A U Livestock Farm, Mannuthy
2. Osho Farm, Pattikkad
3. Vinpi Nagar Farm, Nellikkunnu
4. City Farm, Dhanalakshmi Bank Road, Thrissur
5. Surya Farm, Ayyanthole.

Mosquitoes were also collected from human dwellings in the vicinity of dog houses. Data on temperature and humidity during the period of study were collected from the Department of Meteorology, Kerala Agricultural University, Vellanikkara.

3. 1. 1. 2. Method of Collection

Resting mosquitoes were collected using test tubes (WHO, 1975). The mouth of the test tube was applied perpendicularly over the wings of the resting mosquitoes in such a way that when the mosquito was disturbed and attempted to fly, it flew directly into the tube. A thin card board was applied to the mouth of the test tube before the tube was detached from the surface and plugged with a piece of cotton wool. Different types of

mosquitoes resting on the walls and body of animals were collected. The mosquitoes were brought to the laboratory and anesthetized with ether or chloroform. They were examined under a dissecting microscope for the different morphological peculiarities enabling their identity (Christophers, 1933; Barraud, 1934; Reuben *et al.*, 1994). Pooled samples of mosquitoes from each location were also sent to Vector Control Research Centre, Pondicherry, for specific identification.

3. 1. 1. 3. Preservation of Specimens

Specimens were preserved in the dry state after pinning. Mosquitoes collected individually in test tubes were killed with ether or chloroform. They were pinned with minuten entomological pins on the thorax. The pins were fixed on cork piece and enclosed in specimen bottle taking care to avoid contact of the specimen with the sides of the bottle. A small grain of thymol was placed in the bottle to inhibit fungal growth.

3. 1. 1. 4. Selection of Mosquitoes as the Predominant Species

This was done using three criteria as suggested by Anyanwu *et al.* (2000).

1. The most abundant species in terms of number
2. The mosquito species repeatedly collected in substantial numbers in each collection and
3. The most abundant species during a specific period or throughout the year.

3. 2. BREEDING AND MAINTENANCE OF MOSQUITOES UNDER LABORATORY CONDITIONS.

The engorged female mosquitoes collected were brought to the laboratory and released into rearing cages (Fig.1 and 2). The mosquitoes were fed on 5 to10 percent glucose solution contained in small bottles plugged with cotton wool wicks. About 2 cm of cotton wool was left sticking out of the solution on which the male and female mosquitoes readily fed. The glucose solution was refreshed on alternate days. When egg laying was desired, pigeon enclosed in a small compartment was placed inside the cage for the females to feed on. Cotton wool soaked in water was also placed in the cage to maintain adequate humidity.

3. 2. 1. Collection of Eggs

Enamel trays filled with water were kept in the mosquito cages. Eggs were collected every day with camel hairbrushes.

3. 2. 2. Preparation of Artificial rearing pans

Hay infusion was used as the rearing medium. It was prepared by keeping hay in a bucket of water for about ten days in the open. Later the water was filtered and stored in closed pet jars till use (Anyanwu, 2003). The egg rafts were transferred to the rearing pans (Fig. 3) using fine haired brush. The larvae were fed daily or on alternate days with small grains of yeast according to requirement, taking care to avoid the development of excess turbidity. The medium was usually changed two times in the course

of a lifecycle. At L_{II} stage and at L_{IV} stage, the larvae were washed and transferred to fresh medium.

3. 2. 3. Biology of the Most Commonly Prevalent Species

The eggs, larval stages I, II, III and IV and pupae obtained by rearing mosquitoes as described earlier were collected, observed under the microscope for morphological and morphometric studies. They were allowed to grow in hay infusion and the emerged adults were let out in small cages. The mosquitoes were fed on sugar solution and blood as described earlier. Selected number of females were kept in rearing cages to observe their egg laying capacity.

3.3. SCREENING MOSQUITOES FOR THE PRESENCE OF INTERMEDIARY STAGES OF HELMINTH PARASITES.

3. 3.1. Dissection of Mosquitoes

3.3. 1. 1. Preparation of Mosquitoes for Dissection

Mosquitoes were dissected to find out the presence of helminth larvae, if any. Dissection was performed on live mosquitoes collected the previous night from cattle sheds and human dwellings in the vicinity of kennels. They were immobilized by placing the collection tube containing mosquito in the chill tray of the refrigerator for a few minutes. After immobilization the insect was held by one wing and the legs were pulled off one by one followed by the second wing. Then the specimen was placed on a slide and the remaining wing was cut off with the dissecting needle.

3. 3. 2. Dissection Proper

After preparing the mosquito as described above, the specimen was placed on a microscope slide with the apex of the abdomen to the right. A drop of physiological saline was placed at three spots on the slide and the abdomen, thorax and head were separated into each. Each part was teased and dissected thoroughly with the help of a pair of dissecting needles and examined under low and then high power of the microscope to detect the presence of helminth larvae in the contents. The helminth larvae when present were identified based on morphology (Yamaguti, 1961; Sonin, 1985; Anderson, 2000).

3. 4. EVALUATION OF NEWER GENERATION INSECTICIDES ON THE DEVELOPMENTAL STAGES OF MOSQUITOES

3. 4. 1. Insecticides Used in the Study

3. 4. 1. 1. Insect Growth Regulators

3. 4. 1. 1.1 Diflubenzuron (HILMILIN 25% WP) is an insect growth regulator and is a product of Hindustan Insecticides Ltd.

3. 4. 1.1. 2. Methoprene (Altosid 5% aqueous suspension) is a mosquito growth regulator and a product of Wellmark International, United States of America.

3. 4.1.2. Other Insecticides Used

3. 4.1.2.1. ***Bacillus thuringiensis var israelensis*** (HIL *Bti* 1.2% aqueous suspension manufactured by Becker Microbial product, Inc. USA and imported and marketed by Hindustan Insecticides Ltd.

3. **4.1.2.2. Azadirachtin** (Neem Azal 1% Emulsifiable Concentrate) is marketed by EID Parry (India) Ltd.

3. **4.1.2.3. Ethofenprox** (Primo 10% Emulsifiable Concentrate) is a product of SEARL Agrochemicals, India (Now, RPG Life Sciences Ltd.).

3. **4.1.2.4. Deltamethrin** (Butox 1.25% Emulsifiable Concentrate) is a product of Intervet Laboratories, India, Ltd.

3. 4. 2. Developmental Stages of Mosquitoes Used in the Study

Culex tritaeniorhynchus mosquitoes were colonized in the laboratory as mentioned previously and the eggs, L_{IV} and pupae were collected from the colony for the study. Each experiment was repeated four times and the mean values were subjected to statistical analysis.

3. 4. 2. 1. Eggs

The freshly laid eggs not older than twelve hours were used for the study. To obtain such eggs, pans were kept in the rearing cages in the evening and the eggs laid there were used for the study in the next morning. Each raft of eggs was dissociated carefully with a fine haired brush on a piece of filter paper and transferred to water in a cavity block. The eggs were counted and pipetted out under a dissection microscope and transferred to plastic disposable cups in which the various insecticides were added at different concentrations. After twelve hours the eggs were repeatedly washed and placed in an equal volume of fresh water. They

were observed for hatching after twelve hours. Each treatment with a water control was duplicated and the test was repeated thrice.

3. 4. 2. 2. Fourth Stage Larvae

The larvae were pipetted out from the larval pools. Ten larvae each were counted and transferred to disposable cups containing the various insecticides at different concentrations for further observation. Each treatment with a water control was duplicated and the test was repeated four times.

3. 4. 2. 3. Pupae

The freshly formed pupae were collected from the larval pools for the study. In each concentration of insecticide, ten pupae were used. Each treatment with a water control was duplicated and the test was repeated thrice.

3. 4. 3. Experimental Medium

The medium used was 100 ml of stored tap water. When the experiments were with larvae, hay infusion was also used. Small quantities of dog biscuits and yeast at the ratio of 3:1 was added into the medium according to requirement taking care to avoid excess turbidity of the medium.

3. 4. 4. Observation of Results

3. 4. 4. 1. Eggs

The medium containing the eggs was pipetted out after twelve hours and the eggs were repeatedly washed with fresh water to remove any trace of the insecticide and the medium was replaced to the original level. The number of eggs hatched was counted from the next morning onwards. The hatched eggshells were examined for any abnormality in the type of hatch. Unhatched eggs were counted as dead. The number of eggs exhibiting abnormal hatching (partial hatch, side hatch etc.) was also noted.

3. 4. 4. 2. Fourth Stage Larvae and Pupae

The larval stages were observed for any mortality from the next morning onwards. The dead larvae and pupae were removed. The mouth of the plastic cup was covered with thin muslin cloth to prevent the flying away of emerged adults and later they were released into small rearing cages to observe their survival. They were fed on 6 per cent sugar solution initially and later on pigeon blood. The number of adults emerged were also noted by counting the pupal exuviae. Emerged adults unable to leave the cups were also counted as dead. Percentage of successful pupation and adult emergence were determined until all adults had emerged or all larvae were dead.

3. 4. 4. 3. *Statistical Analysis*

3. 4. 4. 3. 1. Correction for Control Mortality

In each experiment, controls were also kept and the corrected mortality was obtained by using Abbot's formula (Busvine, 1971).

$$Pt = \frac{Po - Pc \times 100}{100 - Pc} \quad \text{where}$$

Pt = Corrected mortality
Po= Observed mortality
Pc= Control mortality, all in percentages.

All experiments in which the control mortalities exceeded 20 per cent, the data were rejected. For each insecticide the concentrations giving at least two values each below and above 50 percent, mortalities were obtained by repeated experiments. LC_{50} and LC_{90} were estimated from the mortality data by probit analysis of log dosage, using SPSS (Statistical Package for Social Sciences).

$p = a+bx$ where, p = probit
a = intercept
b = regression coefficient
x = log dose

3. 4. 5. Cost of Application in Unit Volume

The field dosages were calculated as four times the LC_{50} on 4^{th} stage larvae (Ponce *et al.*, 2002). Based on this, the quantity of the preparations required for applying in one lakh litres of water and its cost was calculated.

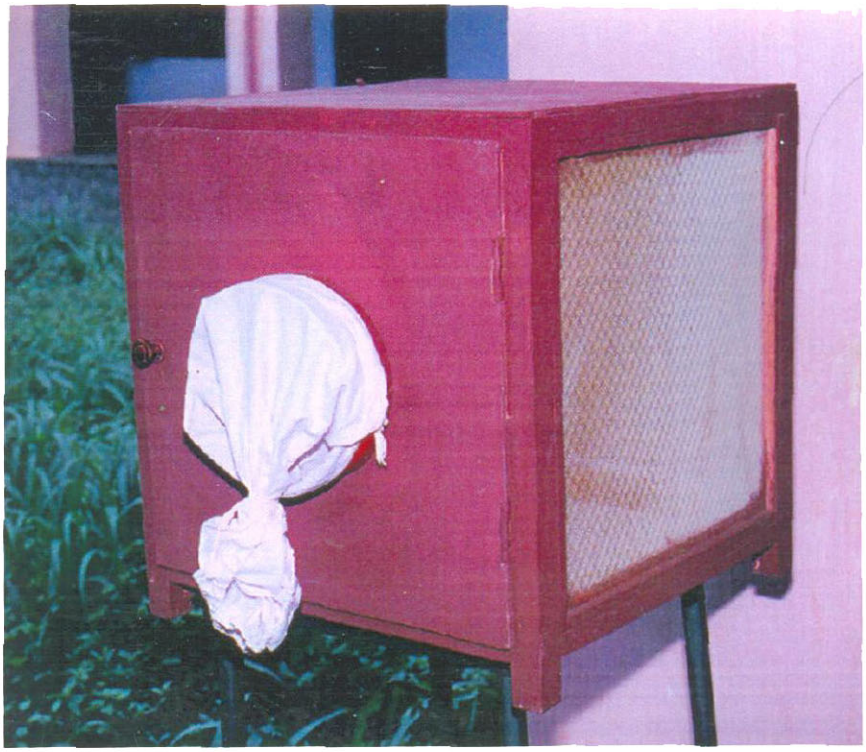


Fig. 1. Large Rearing Cage

Fig. 2. Small Rearing Cage

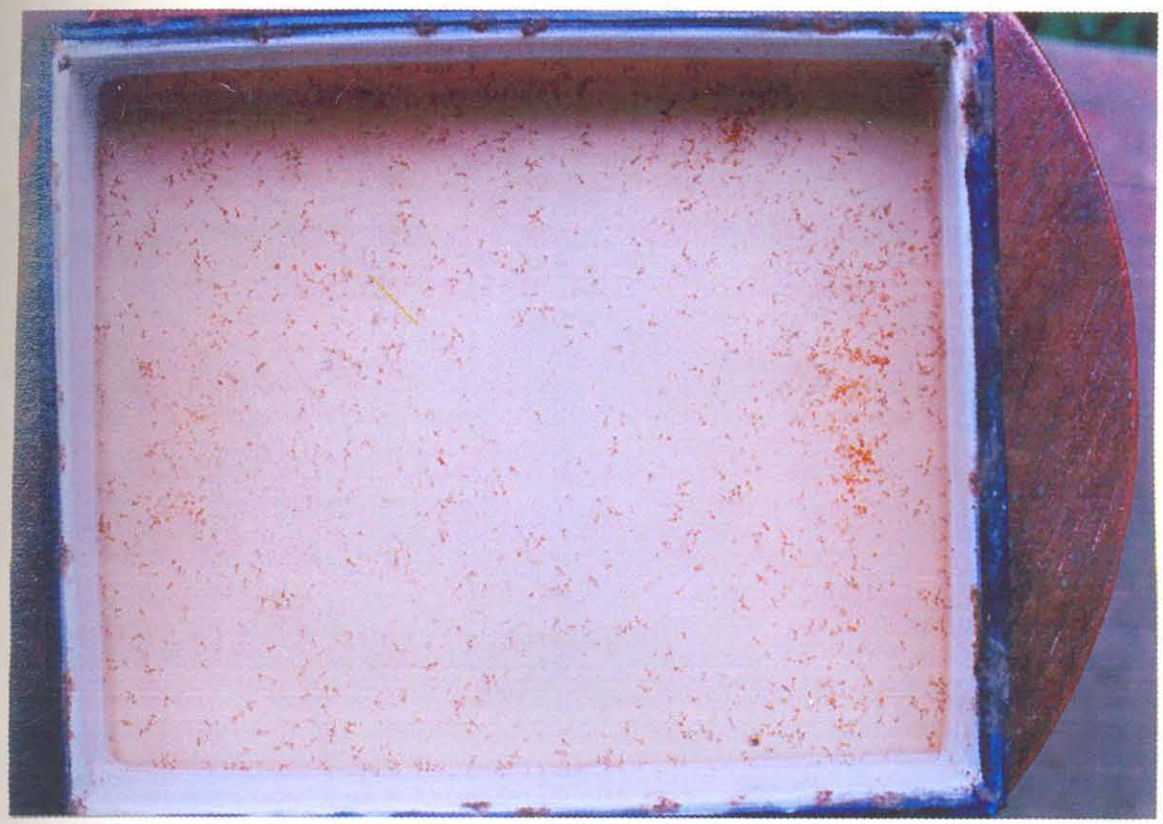


Fig.3. Mosquito larval rearing pan

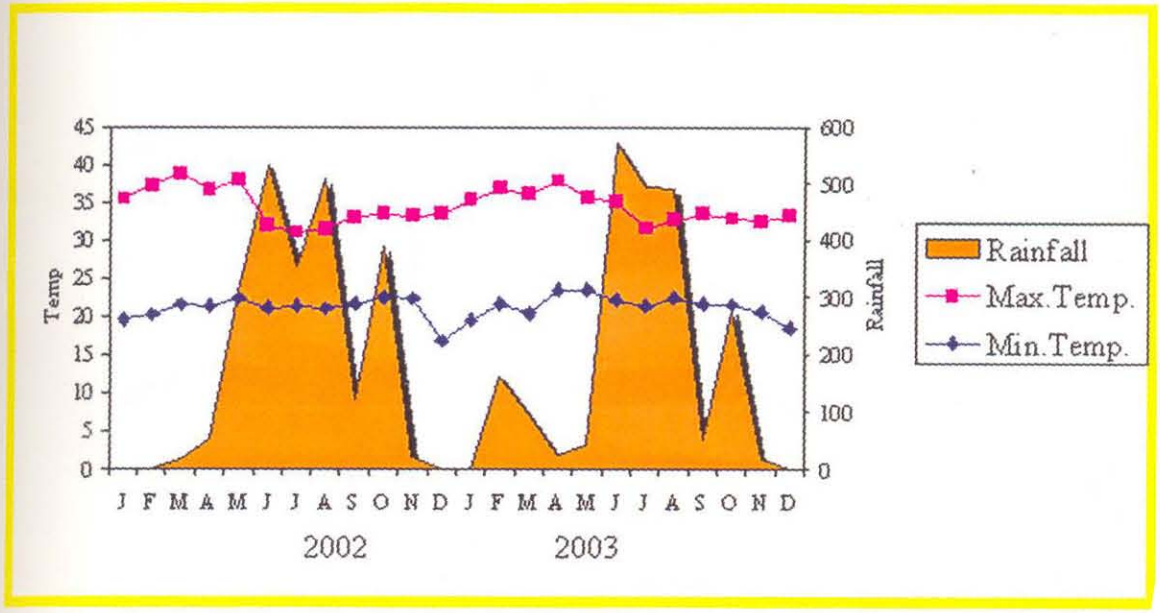


Fig. 4. Climatic conditions recorded at Vellanikkara

Results

4. RESULTS

4. 1. PREVALENCE

The area of the study was warm and humid with temperatures ranging from 17 to 38° C. The average annual rainfall during 2002 and 2003 was 2263 mm from both the Southwest and Northeast monsoons. Maximum number of rainy days occurred from May to October with two to three dry months between December and February (Fig. 4).

The different species of mosquitoes collected at monthly intervals from the six locations during the calendar year 2002 are shown in Table. 1.

A total of 1087 mosquitoes were collected for the identification of species. They were identified into 27 species under seven genera. The different genera in the order of prevalence were *Culex* (67.1 %), *Mansonia* (14.3%), *Anopheles* (8.9%), *Armigeres* (6.9%), *Aedes* (2.5%), *Ochlerotatus* (0.3%) and *Toxorhynchites* (0.1%).

The farm wise prevalence of various species is shown in Table 2 to 6. Maximum number of species were obtained from Osho Farm, Pattikkad (15) followed by Vinpi Nagar (14), KAU Livestock Farm, Mannuthy (11), Surya Farm (8) and City Farm (7). The genus *Culex* predominated (67.1%) followed by *Mansonia* (14.3%) and *Anopheles* (8.9%).

The species of mosquitoes identified from human dwellings is given in Table 7. While 36.4 per cent of the collection from human dwellings constituted *Cx. quinquefasciatus*, 34.5 per cent were *Ar. subalbatus*.

Table 1. The overall prevalence of mosquitoes collected from Thrissur

	Genus	No	Percentage
	A. *Culex*		
1	*Cx. tritaeniorhynchus*	468	43.1
2	*Cx. gelidus*	153	14.1
3	*Cx. pseudovishnui*	63	5.8
4	*Cx. bitaeniorhynchus*	10	0.9
5	*Cx. sitiens*	4	0.4
6	*Cx. sinensis*	4	0.4
7	*Cx. infula*	4	0.4
8	*Cx. vishnui*	1	0.1
9	*Cx. fuscocephala*	1	0.1
10	*Cx. brevipalpis*	1	0.1
11	*Cx. quinquefasiatus*	20	1.8
	Total	**729**	**67.1**
	B. *Mansonia*		
1	*Ma. uniformis*	137	12.6
2	*Ma. annulifera*	12	1.1
3	*Ma. indiana*	6	0.6
	Total	**155**	**14.3**
	C. Aedes		
1	*Ae. albopictus*	10	0.9
2	*Ae. vittatus*	15	1.4
3	*Ae. vexans*	1	0.1
4	*Ae. lineatopennis*	1	0.1
	Total	**27**	**2.5**
	D. *Anopheles*		
1	*An. peditaeniatus*	65	6
2	*An. nigerrimus*	17	1.6
3	*An. jamesi*	7	0.6
4	*An. tesellatus*	4	0.4
5	*An. jeyporensis*	3	0.3
6	*An. barbirostris*	1	0.1
	Total	**97**	**8.9**
	E. *Armigeres*		
1	*Ar. subalbatus*	75	6.9
	Total	**75**	**6.9**
	F. Ochlerotatus		
1	*Oc. pulchriventer*	3	0.3
	Total	**3**	**0.3**
	G. *Toxorhynchites*		
1	*To. splendens*	1	0.1
	Total	**1**	**0.1**
	Grand total	**1087**	**100**

Table 2. Mosquito species collected from K.A.U Livestock Farm, Mannuthy

	Species	No.	Percentage
1	*Culex tritaeniorhynchus*	164	64.3
2	*Cx. gelidus*	20	7.8
3	*Cx. pseudovishnui*	13	5
4	*Cx. infula*	2	0.8
5	*Cx. fuscocephala*	1	0.4
6	*Armigeres subalbatus*	15	5.9
7	*Anopheles peditaeniatus*	17	6.7
8	*An. nigerrimus*	6	2.4
9	*Mansonia uniformis*	13	5.1
10	*Aedes vittatus*	3	1.2
11	*Ae. lineatopennis*	1	0.4
	Total	**255**	**100**

Table 3. Mosquito species collected from Osho Farm, Pattikkad

	Species	No.	Percentage
1	*Culex tritaeniorhynchus*	83	43
2	*Cx. gelidus*	28	14.5
3	*Cx. pseudovishnui*	14	7.3
4	*Cx. sitiens*	4	2.1
5	*Cx. infula*	2	1
6	*Cx. sinensis*	4	2.1
7	*Armigeres subalbatus*	6	3.1
8	*Anopheles peditaeniatus*	15	7.8
9	*An. jamesi*	4	2.1
10	*An. jeyporensis*	3	1.6
11	*An. tesellatus*	1	0.5
12	*Mansonia uniformis*	22	11.4
13	*Ma.indiana*	2	1
14	*Aedes vittatus*	2	1
15	*Ochlerotatus pulchriventer*	3	1.6
	Total	**193**	**100**

Table 4. Mosquito species collected from Surya Farm, Ayyanthole

	Species	No.	Percentage
1	*Culex tritaeniorhynchus*	99	49.5
2	*Cx. gelidus*	41	20.5
3	*Cx. pseudovishnui*	14	7
4	*Armigeres subalbatus*	3	1.5
5	*Anopheles nigerrimus*	4	2
6	*Mansonia annulifera*	12	6
7	*Ma. uniformis*	23	11.5
8	*Mn. indiana*	4	2
	Total	**200**	**100**

Table. 5. Mosquito species collected from Vinpi Nagar, Nellikkunnu

	Species	No.	Percentage
1	*Culex tritaeniorhynchus*	56	27.1
2	*Cx. gelidus*	24	11.6
3	*Cx. pseudovishnui*	17	8.2
4	*Cx. bitaeniorhynchus*	10	4.8
5	*Cx. vishnui*	1	0.5
6	*Armigeres subalbatus*	17	8.2
7	*Anopheles peditaeniatus*	17	8.2
8	*An. nigerrimus*	6	2.9
9	*An. jamesi*	3	1.4
10	*An. barbirostris*	1	0.5
11	*Mansonia uniformis*	38	18.4
12	*Aedes albopictus*	6	2.9
13	*Aedes vittatus*	10	4.8
14	*Ae. vexans*	1	0.5
	Total	**207**	**100**

Table 6. Mosquito species collected from City Farm, Thrissur

	Species	No.	Percentage
1	*Culex tritaeniorhynchus*	66	37.3
2	*Cx. gelidus*	36	20.3
3	*Cx. pseudovishnui*	2	1.1
4	*Armigeres subalbatus*	15	8.5
5	*Anopheles peditaeniatus*	16	9
6	*An. tesellatus*	3	1.7
7	*Mansonia uniformis*	39	22
	Total	**177**	**100**

Table 7. Mosquito species identified from human dwellings

	Species	No.	Percentage
1	*Culex quinquefasciatus*	20	36.4
2	*Cx. pseudovishnui*	3	5.5
3	*Cx. brevipalpis*	1	1.8
4	*Cx. gelidus*	4	7.3
5	*Armigeres subalbatus*	19	34.5
6	*An. nigerrimus*	1	1.8
7	*Aedes albopictus*	4	7.3
8	*Mansonia uniformis*	2	5.5
9	*Toxorhynchites splendens*	1	1.8
	Total	**55**	100

The generic prevalence of mosquitoes collected during the survey is given in Fig. 5. The 27 different species of mosquitoes identified belonged to seven genera as shown in Table 8. *Culex quinquefasciatus,* the southern house mosquito, very abundantly present in human dwellings was virtually absent in all cattle farms. *Armigeres subalbatus* was equally distributed in cattle farms as well as human dwellings. A single specimen of *To. splendens* alone could be collected from a house in the present study.

Table 8. Number of species of mosquitoes identified under different genera

Generic name	Number of species detected
Aedes	4
Anopheles	6
Armigeres	1
Culex	11
Mansonia	3
Ochlerotatus	1
Toxorhynchites	1
Total	**27**

4. 1. 1. Family *Culicidae*

All mosquitoes came under the family *Culicidae.* They were very slender flies with elongate piercing proboscis and with no ocelli. Legs were long and antennae were densely plumose in the males and pilose in the females. The whole body, legs and wings (Fig. 6) were in part, or entirely, clothed with scales. Mosquitoes belonging to three subfamilies could be collected *viz. Toxorhynchitinae, Anophelinae* and *Culicinae.*

4. 1. 1. 1. Subfamily *Toxorhynchitinae*

4. 1. 1. 1. 1. Female *Toxorhynchites* (Fig. 7 & 8)

Only a single female fly was collected. It was a large colourful mosquito and was easily recognized by the possession of a proboscis that was recurved. The antennae were pilose. The species was identified as *Toxorhynchites splendens* based on the descriptions of Barraud, 1934. This was the largest mosquito collected and was identified by the presence of well developed lateral tufts of yellow, black and orange hairs on terminal abdominal segments. The terminal tarsal segments of the fore and hind legs were without white markings. Mesonotum was without a continuous pale border; abdomen with conspicuous lateral tufts of hairs on VI to VIII. They were incapable of sucking blood.

4. 1. 1. 2. Subfamily *Anophelinae*

4. 1. 1. 2. 1. Female *Anopheles* (Fig. 9)

The abdomen was entirely or mostly devoid of scales. They rested with the body at an angle to the surface, that is with proboscis and abdomen in a straight line. They had dark and pale scales on the wing veins arranged in blocks or specific areas to form distinctive spotted pattern (Fig.10 A & B). The antennae were pilose. The palps were as long as the proboscis and lay close to it. The scutellum was rounded posteriorly and had setae along the edge. The six species of *Anopheles* mosquitoes collected were *An. peditaeniatus, An. nigerrimus, An. jamesi, An. tesellates, An. jeyporensis* and *An. barbirostris* in the order of prevalence.

4. 1. 1. 3. Subfamily *Culicinae*

They were mosquitoes of variable size. Proboscis flexible and nearly always uniformly slender and not hooked. Palps of females were much shorter than proboscis. Scutellum more or less trilobed and with three separate groups of bristles. Abdomen with tergites and sternites covered with scales that usually lay flat on the surface. All the other species of mosquitoes (20) collected belonged to this subfamily.

4. 1. 1. 3. 1. *Culex* female

The scales on the body were narrow and not metallic coloured. There were no distinct patterns on the body. The abdomen was blunt ended. Pre spiracular and post spiracular areas were without bristles. The tarsi had well developed pulvilli. The maximum number of species collected in this survey (11) belonged to this genus.

4. 1. 1. 3. 2. *Aedes* female (Fig. 11 A & B)

The scales were narrow. There were usually silver coloured patterns on the body. The abdomen was pointed at the end. Tarsi appeared banded due to the presence of silver coloured scales. Post spiracular bristles were present. On the hind legs the first segment of the tarsus was shorter than the tibia. The stem vein of the wing had bristles on the ventral surface.

4. 1. 1. 3. 3. *Armigeres* female (Fig.12 A)

Moderate in size and robustly built. Not usually highly ornamented, but otherwise resembled *Aedes* in external morphology and structure.

Scales of head were broad and flat. Proboscis rather stout, slightly curved downwards towards tip, the curved part somewhat laterally flattened (Fig. 12 B). Abdomen somewhat tapering, segment VIII partially retractile. *Armigeres subalbatus* was the only species that could be collected.

4. 1. 1. 3. 4. *Mansonia* female (Fig. 13)

Adults frequently had the legs, palps, wings and body covered with a mixture of dark and pale scales giving the insects a rather dusty appearance. This speckled pattern of scales gave the appearance of pepper and salt. The scales on the wing veins were rather broad and often asymmetrical giving them an almost heart shaped appearance (Fig. 14 A and B). Tarsal claws were simple and pulvilli were absent.

4.1.1. 3. 5. *Ochlerotatus* female

This was a subgenus under the genus *Aedes* till 2000 when, Reinert elevated it to the rank of a genus based on the morphological peculiarities of the genitalia. The female genitalia had the insula lip like, bearing well developed setae laterally.

4. 1. 2. Most Commonly Prevalent Species

4. 1. 2. 1. *Culex tritaeniorhynchus* (Fig. 15 A & B)

Culex tritaeniorhynchus was identified as the most commonly prevalent species in cattle sheds based on the parameters suggested by Anyanwu (2000). Of the 1032 mosquitoes collected from all the farms during the survey, 468 (45%) were *Cx. tritaeniorhynchus*. In the farm wise prevalence of various species of mosquitoes also, this species was more

prevalent than all other species (Table 1 to 6). The percentage prevalence varied from 27.1 to 64.3 whereas for other species of mosquitoes the highest was 20.5 per cent.

4. 1. 2. 2. Morphology

They were relatively small sized mosquitoes and were identified by the dark brown scaling on the vertex and scutum and the accessory pale patch basal to the pale band on the ventral surface of the proboscis (Fig. 16 A). There was a narrow apical dark ring on the hind femur (Fig. 16 B). The male mosquitoes measured 4.13 mm (4 to 4.32) long and the proboscis was 1.51 mm (1.46 to 1.59). The females were slightly shorter measuring 4.09 mm (3.78 to 4.23) long and the proboscis was 1.47 mm (1.37 to 1.55).

4. 2. BREEDING AND MAINTENANCE OF MOSQUITOES IN THE LABORATORY

Culex tritaeniorhynchus, identified as the most commonly prevalent species in cattle sheds was successfully colonized in the laboratory. The blood fed females collected individually in tubes were released into the rearing cages. The cages were kept in wooden cupboards to maintain temperature and humidity. They started laying eggs within two to three days. The egg rafts were carefully removed with fine haired brushes. Each raft contained 77 to 166 eggs with an average of 117. The average number of eggs laid in the laboratory by a female after a full blood meal was calculated as 42. It took 36 to 72 hours for the eggs to hatch. The larvae were reared in hay infusion and adequately provided with larval feed. There were four larval instars and a pupal stage. The time for development to the next stage was almost equal ranging from 24 to 48 hours. The total

time for adult emergence from eggs ranged from seven to eighteen days. The measurements of the various larval stages are as shown in Table 9.

Table 9. Measurements of the larval stages of *Cx. tritaeniorhynchus*

Larval stage	Length in microns				
	Head	Thorax	Abdomen	Total length	Siphon
L1	205	176	884	1268	293
L2	326	308	1443	2077	512
L3	476	433	1884	2789	774
L4	642	728	3367	4732	1447

4. 2. 1. Eggs (Fig. 18 A & B)

Eggs were brownish, long and cylindrical, laid upright on the water surface and placed together to form an egg raft that comprised of 77 to 166 eggs with an average of 117 eggs per raft. The operculated end of the eggs was placed upright in the raft. The eggs measured 566-595/137-156 (Av. 585/145) microns.

4. 2. 2. Larvae (Fig. 17 A, B, C & D)

The larvae were legless having a bulbous thorax wider than both the head and the abdomen. The head was well developed with a pair of antennae and a pair of compound eyes. Prominent mouth brushes were present to sweep in food particles to the mouth. The thorax was round in outline and had many conspicuous simple and branched hairs. The abdomen was distinctly segmented and had nine visible segments. Each segment had simple and branched hairs. The last segment had two paired groups of long hairs forming the caudal setae and a larger group of hairs forming the ventral brush. There were no abdominal palmate hairs or tergal plates. Four anal gills were present at the tip of the last segment, which was

at an angle to the long and slender respiratory siphon. There was a four to five times increase in the size of the larvae as it moulted to the 4th stage. The larvae were identified by the presence of four siphon tufts each with two to five branches (Fig. 19 A & B). The comb scales were apically rounded (fan shaped) and fringed with sub equal spicules (Fig. 20 A & B).

4. 2. 3. Pupae (Fig. 21)

Pupae were comma shaped. The head and thorax were combined to form the cephalothorax measuring 1.37–1.55/1.59–1.73 mm (Av. 1.44/1.62), which had a pair of respiratory trumpets. The abdomen measured 2.78 – 3.19 mm (Av. 2.99 mm). The abdomen had ten segments. Each segment had numerous short hairs and the last segment terminated in a pair of oval and flattened structures called paddles. In between the paddles, was a small pouch like projection containing the developing external genital process of the adult. The female pupae had smaller pouch containing the cerci, while in the male pupae the pouch was bigger as it contained the claspers of the male genitalia (Fig. 22 A & B). The developing structures of the adult mosquitoes such as a pair of compound eyes, folded wings, legs and proboscis could be seen through the integument of the cephalothorax. The pupal period lasted for two to three days. Male mosquitoes always emerged first.

4. 3. DISSECTION OF MOSQUITOES

The number of mosquitoes collected exclusively from cattle sheds and dissected during the calendar years 2002 and 2003 are presented in Table No. 11. No helminth larvae could be noticed in any of the mosquitoes dissected.

Details regarding the dissection of mosquitoes collected from human dwellings/in the vicinity of dog houses are presented in Table No. 12. Larval stages of helminths were found in two mosquitoes. One *Armigeres* mosquito was found to harbour 56 sausage shaped larvae in the Malpighian tubules (Fig. 23 A & B). The average length of the larva was 246 microns and maximum width was 29.5 microns. One *Culex* mosquito was also found to harbour five third stage (infective) larvae. The average length was 1075 microns and maximum width was 44 microns. They were identified as second stage and third stage larvae of *D. repens* (Fig. 24 A & B) based on morphology (Yamaguti, 1961; Sonin, 1985; Anderson, 2000).

Table 10. Details of mosquitoes dissected from Cattle sheds

Genera	Mosquitoes dissected	Results
Culex	749	No helminth larvae detected
Anopheles	166	,,
Armigeres	11	,,
Mansonia	35	,,
Aedes	40	,,
Total	**1001**	Nil

Table 11. Details of mosquitoes dissected from human dwellings/in the vicinity of dog houses

Genera	Mosquitoes dissected	Results
Culex	454	1 positive for helminth larvae
Anopheles	1	No helminth larvae detected
Armigeres	506	1 positive for helminth larvae
Mansonia	10	No helminth larvae detected
Aedes	48	No helminth larvae detected
Total	**1019**	**2 positive cases detected**

4. 4. EVALUATION OF NEWER GENERATION INSECTICIDES ON THE DEVELOPMENTAL STAGES OF MOSQUITOES

4. 4. 1. Eggs

The response of eggs exposed to various concentrations of the insecticides are shown in Tables 12 to 16. The probit curves (ld-p lines) are presented in Figs. 30 to 35. The LC_{50} and LC_{90} values of various insecticides on the eggs of *Cx. tritaeniorhynchus* is given in Table 17.

4. 4. 1. 1. Insect Growth Regulators

4. 4. 1. 1. 1. Diflubenzuron (Hilmilin)

One hundred per cent non-hatchability was observed at a concentration of 300 ppm. While at 10 ppm, it was 21.6 percent. Hatching abnormalities included non-hatch, side-hatch and various types of partial hatch. The LC_{50} was found to be 38. 2658 while the LC_{90} was 197. 9877 ppm.

4. 4. 1. 1. 2. Methoprene (Altosid)

Methoprene was found to have no action on the hatchability of eggs. A concentration of 500 ppm (10000 times the LC_{100} on 4^{th} stage larvae) was found to induce no influence on hatchability.

4. 4. 1. 2. *Bacillus thuringiensis israelensis* (Hil *Bti*)

Cent percent non hatchability was noticed at a concentration of 200 ppm. At 5 ppm the egg mortality was 18.3 per cent. Hatching abnormalities included non hatch, side hatch, and various types of partial hatch. The LD_{50} was 21. 7252 while the LD_{90} was 110. 5554 ppm.

Table 12. Effect of diflubenzuron on the eggs of *Cx. tritaeniorhynchus*

Concentration (ppm)	Number of eggs		Percent non-hatchability	Corrected non-hatchability
	Hatched	Not hatched		
10	47	13	21.6	21.6
20	44	16	26.6	26.6
40	31	29	48.3	48.3
80	23	37	61.6	61.6
100	11	49	81.6	81.6
200	7	53	88.3	88.3
300	Nil	60	100	100
Control	60	Nil	-	-

Table 13. Effect of *Bti* on the eggs of *Cx. tritaeniorhynchus*

Concentration (ppm)	Number of eggs		Percent non-hatchability	Corrected non-hatchability
	Hatched	Not hatched		
5	49	11	18.3	18.3
10	45	15	25	25
20	34	26	43	43
50	20	40	33.3	33.3
100	6	54	90	90
200	Nil	60	100	100
Control	60	Nil	-	-

Table 14. Effect of azadirachtin on the eggs of *Cx. tritaeniorhynchus*

Concentration (ppm)	Number of eggs		Percent non-hatchability	Corrected non-hatchability
	Hatched	Not hatched		
50	53	7	11.6	11.6
80	43	17	28.3	28.3
100	26	34	56.6	56.6
150	8	52	86.6	86.6
200	2	58	96.6	96.6
300	Nil	60	100	100
Control	60	Nil	-	-

Table No. 15. Effect of ethofenprox on the eggs of *Cx. tritaeniorhynchus*

Concentration (ppm)	Number of eggs		Percent non-hatchability	Corrected non-hatchability
	Hatched	Not hatched		
0.05	55	5	8.3	8.3
0.10	49	11	18.3	18.3
0.20	37	23	38.3	38.3
0.40	24	36	60.0	60.0
0.50	10	50	83.3	83.3
1.00	1	59	98.3	98.3
2.00	Nil	60	100	100
Control	60	Nil	-	-

Table 16. Effect of deltamethrin on the eggs of *Cx. tritaeniorhynchus*

Concentration (ppm)	Number of eggs		Percent non-hatchability	Corrected non-hatchability
	Hatched	Not hatched		
0.005	47	13	21.6	21.6
0.010	39	21	35.0	35.0
0.050	34	26	43.3	43.3
0.100	25	35	58.0	58.0
0.500	17	43	71.6	71.6
1.000	8	52	86.6	86.6
2.000	Nil	60	100	100
Control	60	Nil	-	-

Table 17. LC_{50} and LC_{90} values of various insecticides on the eggs of *Cx. tritaeniorhynchus*

Insecticide	LC_{50} in ppm	Range	LC_{90} in ppm	Range
Diflubenzuron	38.26575	25-53	197.98773	126-434
Methoprene	--	--	--	--
Bti	21.72520	14-31	110.55540	67-267
Azadirachtin	93.37866	86-101	163.50408	147-188
Ethofenprox	0.23482	0.18-0.31	0.76112	0.54-1.32
Deltamethrin	0.04814	0.02-0.10	1.57149	0.56-11.28

Table 18. Effect of diflubenzuron on fourth stage larvae of *Cx. tritaeniorhynchus* (Mean values)

Sl. No.	Concentration in ppm	Larval mortality %	Pupation %	Pupal mortality %	Malformed adults %	Total mortality %	Longivity adults in days	No. of eggs laid by female (mean) during lifetime	Percentage of eggs hatched
1	0.00001	6	94	4	1	11	25	11	87
2	0.00005	21	79	2	2	25	23	9	34
3	0.0001	26	74	3	1	30	15	0	0
4	0.0005	49	51	6	7	62.5	11	0	0
5	0.001	68	32	6	5	79	6	0	0
6	0.005	78	22	4	3	85	5	0	0
7	0.01	97.5	0	0	5	97.5	5	0	0
8	0.02	100	0	0	0	100	0	0	0
9	Control	Nil	100	Nil	Nil	Nil	40	47	95

Table19. Effect of methoprene on fourth stage larvae *Cx. tritaeniorhynchus* (Mean values)

Sl. No.	Concentration in ppm	Larval mortality %	Pupation %	Pupal mortality %	Malformed adults %	Total mortality %	Longevity adults in days	No. of eggs laid by female during lifetime	Percentage of eggs hatched
1	0.00005	1.25	98.75	Nil	Nil	1.25	30	20	75
2	0.0001	3.75	96.25	2.5	Nil	6.25	25	12	67
3	0.0002	10.25	89.75	14.75	Nil	25	17	0	0
4	0.0005	6	94	42	8	56	12	0	0
5	0.001	5	95	45	14	64	5	0	0
6	0.005	2	98	60	11	73	1	0	0
7	0.01	1	99	70	12	83	0	0	0
8	0.05	2	98	83	15	100	0	0	0
9	Control	Nil	100	Nil	Nil	Nil	41	51	87

Table 20. Effect of *Bti* on fourth stage larvae of *Cx. tritaeniorhynchus* (Mean values)									
Sl. No.	Concentration in ppm	Larval mortality %	Pupation %	Pupal mortality %	Malformed adults %	Total mortality %	Longevity adults in days	No. of eggs laid by female during lifetime	Percentage of eggs hatched
1	0.6	3	97	2	0	5	30	8	0
2	1.2	5	95	2.5	0	7.5	28	7	0
3	2.4	5	95	4	1	10	25	9	0
4	3.6	7	93	6	2	15	20	0	0
5	4.8	20	80	7	4	31	15	0	0
6	6	35.5	64.5	18	4	58	17	0	0
7	12	40	60	29	4	73	20	0	0
8	24	82	18	16	2	100	0	0	0
9	Control	2.5	97.5	0	0	2.5	43	37	96

Table 21. Effect of azadirachtin on fourth stage larvae of *Cx. tritaeniorhynchus* (Mean values)									
Sl. No.	Concentration in ppm	Larval mortality %	Pupation %	Pupal mortality %	Malformed adults %	Total mortality %	Longevity adults in days	No. of eggs laid by female during lifetime	Percentage of eggs hatched
1	3	17	83	3	0	20	15	10	0
2	4	32	68	9	2	43	18	6	0
3	5	35	65	12	1	48	11	0	0
4	7	62	42	3	0	65	7	0	0
5	8	93	7	2	0	95	5	0	0
6	10	99	1	1	0	100	0	0	0
7	Control	0	100	0	0	Nil	38	18	97

Table 22. Effect of ethofenprox on fourth stage larvae of *Cx. tritaeniorhynchus* (Mean values)									
Sl. No.	Concentration in ppm	Larval mortality %	Pupation %	Pupal mortality %	Malformed adults %	Total mortality %	Longevity adults in days	No. of eggs laid by female during lifetime	Percentage of eggs hatched
1	0.002	12	88	2	0	14	5	0	0
2	0.003	13	87	1	1	15	6	0	0
3	0.005	40	60	6	1	47	4	0	0
4	0.01	38	62	3	1	42	6	0	0
5	0.02	65	35	6	3	74	0	0	0
6	0.03	77	23	3	3	83	0	0	0
7	0.05	94	6	4	2	100	0	0	0
8	Control	0	100	0	0	0	40	41	97

Table 23. Effect of deltamethrinin on fourth stage larvae of *Cx. tritaeniorhynchus* (Mean values)									
Sl. No.	Concentration in ppm	Larval mortality %	Pupation %	Pupal mortality %	Malformed adults %	Total mortality %	Longevity adults in days	No. of eggs laid by female during lifetime	Percentage of eggs hatched
1	0.0005	5	95	0	0	5	11	7	0
2	0.001	22	78	3	0	25	13	11	0
3	0.002	43	57	5	1	49	9	0	0
4	0.004	61	39	4	0	65	2	0	0
5	0.006	87	13	1	2	90	3	0	0
6	0.01	96	4	3	1	100	0	0	0
7	Control	0	100	0	0	Nil	37	44	96

4. 4. 1. 3. Azadirachtin (Neem Azal)

At a concentration of 300 ppm, 100 percent non hatchability was observed. At 50 ppm the egg mortality was 11.6 percent. Hatching abnormalities noticed included non hatch, side hatch and various forms of partial hatch. The LC_{50} was 93.3787 while the LC_{90} was 163.5041 ppm.

4. 4. 1. 4. Ethofenprox (Primo)

When the eggs were exposed to ethofenprox at a concentration of 2 ppm, 100 percent non hatchability was observed. At 0.05 ppm the per cent mortality was 8.3. Hatching abnormalities were minimum. The LC_{50} was 0.2348 while the LC_{90} was 0.7611 ppm

4. 4. 1. 5. Deltamethrin (Butox)

Cent percent non hatchability was observed at 2 ppm while, 0.005 ppm deltamethrin induced 21.6 percent mortality. Hatching abnormalities were minimum.

Typical symptoms of toxicity on eggs in general were observed as

1. Unhatch – failed to hatch, apparently embryo died before hatching (Fig. 25 C).
2. Abnormal hatch – larvae eclosed from a longitudinal line of weakness at the mesal dorsum of the eggshell (Fig. 26 A).
3. Partial side hatch – larvae died during eclosion (Fig. 26 B & 27)

Such eggs had

a. Head capsule free, but caudal end still in the shell
b. Caudal end free, but head capsule still in the egg shell, or
c. Thorax and abdomen free, but head and caudal end in eggshell.

Unhatched eggs were found to contain fully developed embryos that failed to hatch, apparently died just before hatching. Segmentation, eyespots and setae were visible through the eggshell. In some cases, larvae eclosed from a longitudinal line of weakness at the mesal dorsum of eggshell, which was different from normal hatch. All the control eggs completed hatching by 36 hours while the exposed eggs required more time for hatching. In normal hatch, a portion of the anterior end of the egg shell was forced open transversely at a line of dehiscence forming an egg cap that was not completely detached but hinged to the remaining shell (Fig. 25 A & B).

4. 4. 2. Fourth stage Larvae

The effect of various insecticides in the 4^{th} stage larvae of *Cx. tritaeniorhynchus* is given in Tables 18 to 23. The LC_{50} and LC_{90} values are presented in table 24. The probit curves (ld-p lines) are represented in Figs. 30 to 35.

Table 24. LC_{50} and LC_{90} values various insecticides on the 4th stage larvae of *Cx. tritaeniorhynchus*

Insecticide	LC_{50} (ppm)	Range (ppm)	LC_{90} (ppm)	Range (ppm)
Diflubenzuron	0.00023	0.00014-0.00036	0.00407	0.002-0.009
Bti	6.22577	4.15-10.74	20.81060	11.7-90.07
Methoprene	0.00068	0.00034-0.0014	0.00673	0.003-0.039
Azadirachtin	4.68681	3.37-5.87	8.49508	6.58-18.15
Ethofenprox	0.00819	0.0053-0.012	0.03931	0.023-0.117
Deltamethrin	0.00209	0.0015-0.003	0.00654	0.005-0.012

4. 4. 2. 1. Effect of Diflubenzuron

The effect of diflubenzuron on the 4th stage larvae is shown in Table 18. Cent per cent emergence inhibition was noticed at a concentration of 0.02 ppm of diflubenzuron while, 0.00001 ppm produced 11 percent mortality. The LC_{50} was calculated as 0.00023 ppm while the LC_{90} was 0.00407 ppm.

Pupation was considerably delayed in the treatment groups while in the control groups, adults started emerging from the 3rd day onwards. In all concentrations of diflubenzuron above 0.01 ppm, no pupa was formed even on the 7th day of exposure. In the control groups, emergence was completed by the 7th day with majority of the adult eclosion occurring on the 4th, 5th and 6th days. The larvae and pupae were found to be generally dechitinized and deformed. The partially eclosed adults were seen trapped in the pupal skin in varying forms. The successfully emerged adults survived for few days than the control mosquitoes. In general the treatment caused significant mortality during the larval stage than the pupal stage. The

females emerged from larvae exposed to a concentration of 0.0001 ppm or more of diflubenzuron did not lay eggs. The eggs laid by females eclosed from larvae exposed to lesser concentrations exhibited lower hatchability when compared to the controls.

4. 4. 2. 2. Effect of Methoprene

Complete inhibition of emergence was produced at a concentration of 0. 05 ppm of methoprene. The LC_{50} was calculated as 0.00068 ppm while the LC_{90} was 0.00673 ppm based on the probit analysis of data obtained. Though mortality occurred at the larval stage, pupal stage or at eclosion to the adult stage, bulk of the mortality was recorded at the pupal stage when larvae were exposed to methoprene. The period taken for pupation was relatively long when compared to the control. The mosquitoes unable to fly away were also recorded as dead. Different types of deformities were noticed like decolorization, failure of eclosion or partial eclosion. While all the control larvae moulted to the pupal stage, some of the methoprene treated larvae remained as larvae for a longer time depending on the concentration of the insecticide. In certain cases, the treated larvae remained as such without moulting up to the 8^{th} day. It was also observed that some of the larvae and pupae had turned pale or whitish. Some of the mosquitoes died while emerging from pupal exuviae and such half emerged adults were trapped in different conditions (Fig. 33 A & B). When the larvae were exposed to a concentration of more than 0.0001 ppm of methoprene, the adults which emerged did not lay eggs. The eggs laid by females emerged from larvae exposed to lesser concentrations, exhibited lower hatchability than the control.

4. 4. 2. 3. Effect of *Bti*

The effect of *Bti* on the 4th stage larvae are presented in table 20. Total emergence inhibition was noticed at a concentration of 24 ppm of *Bti* and 5 percent mortality was noticed at 0.6 ppm. The LC_{50} was assessed as 6.2258 ppm while, the LC_{90} was 20.8106 ppm. Mortality occurred at the larval or pupal stage depending upon the concentration. That is more larvae were dead when the concentration was high and vice versa. There was no difference in the longevity of the successfully emerged adults in the treatment and control groups. The longevity of females emerged from larvae exposed to various concentrations of *Bti* was lesser when compared to the controls. Very few eggs were laid by them. When the concentration of *Bti* was 3.6 ppm or more, no egg laying was observed.

4. 4. 2. 4. Effect of Azadirachtin

The effect of azadirachtin on the 4th stage larvae are presented in Table 21. One hundred per cent mortality was effected at a concentration of 10 ppm. At 3 ppm of azadirachtin, 20 percent mortality was seen. From the probit analysis data, the LC_{50} was found to be 4.6868 ppm while the LC_{90} was 8.4851 ppm. Pupation was delayed for more than 3 days while in the control group pupation was over by 3rd day. Mortality occurred at the larval stage or pupal stage. But much of the mortality occurred in the larval stage. The successfully emerged adults in the treated groups survived for lesser period when compared to the adults emerged from the control group. When the larvae were exposed to a concentration of 5 ppm or more of azadirachtin, the females emerged from them did not lay eggs. The hatchability of eggs from the other experimental groups exposed to lower concentrations was zero.

4. 4. 2. 5. Effect of Ethofenprox

The effect of ethofenprox on the 4th stage larvae is shown in Table 22. One hundred percent emergence inhibition was recorded at 0.05 ppm while, while 0.002 ppm ethofenprox produced 14 per cent mortality. The LC_{50} was calculated as 0.0082 ppm and the LC 90 was 0.0393 ppm. Mortality occurred at the larval or pupal stage depending on the concentration of ethofenprox used. The adults emerged from larvae exposed to various concentrations of ethofenprox survived for lesser periods when compared to the controls. Such females did not lay eggs.

4. 4. 2. 6. Effect of Deltamethrin

The effect of deltamethrin on the 4th stage larvae are shown in Table 23. The probit analysis data are presented in Fig. 40. Cent percent mortality was recorded at a concentration of 0.01 ppm while at 0.0005 ppm five percent mortality was recorded. The LC_{50} and LC_{90} values from probit analysis data were 0.00209 and 0. 00654 ppm respectively. Mortality occurred at the larval or pupal stage depending on the concentration of deltamethrin used. The survival of adults in the treated and control group did not show much variation. Females emerged from larvae exposed to a concentration of 0.002 ppm or more did not lay eggs.

4. 4. 3. Effect on pupa

The pupal stages of mosquitoes do not feed though they move actively. All the six insecticides were tried for their effect on pupae. The various concentrations producing mortality rates between 10 and 100 percent were found out for each insecticide and presented in Tables 25 to 30. These values were further subjected to probit analysis to compute LC_{50}

and LC_{90} values and are shown in Table 31. The probit curves (ld-p lines) are represented in Figs. 30 to 35.

4. 4. 3. 1. Insect Growth Regulators

The insect growth regulators exhibited varying degrees of activity against pupae. The exposure time to the IGR before eclosion was minimum and hence larger concentrations compared to 4^{th} stage larvae were required to produce mortality in the pupal stage. Consequently the LC_{50} and LC_{90} were relatively much higher than those obtained for 4^{th} stage larvae.

4. 4. 3. 1. 1. Effect of Diflubenzuron

A concentration of 5 ppm produced 30 per cent mortality while, 400 ppm of diflubenzuron was required to effect cent per cent mortality. The LC_{50} and LC_{90} were found to be 28. 77 ppm and 489. 73 ppm respectively.

4. 4. 3. 1. 2. Effect of Methoprene

Cent per cent mortality in pupae was produced at a concentration of 20 ppm while 0.05 ppm caused 18 per cent mortality. The LC_{50} and LC_{90} obtained through probit analysis were 0.2286 and 6.2518 ppm respectively.

4. 4. 3. 2. Effect of *Bti*

Though *Bti* is a known endotoxin producing its effect by the consumption of the toxin, when pupae were exposed to *Bti,* a concentration of 24 ppm produced 17 per cent mortality, while 200 ppm was required to produce cent per cent mortality. Through probit analysis, the LC_{50} and LC_{90} arrived at were 66. 68872 and 187. 3660 ppm respectively.

4. 4. 3. 3. Effect of Azadirachtin

Twenty per cent mortality among pupae was caused by 2 ppm of azadirachtin while cent per cent mortality was produced by a concentration of 12 ppm. The LC_{50} and LC_{90} obtained through probit analysis were 4.5036 and 11. 3119 respectively.

4. 4. 3. 4. Effect of Ethofenprox

When pupae were exposed to 0.02 ppm of ethofenprox, 10 per cent mortality was produced, while 5 ppm could effect cent percent mortality. The LC_{50} and LC_{90} values on pupae were 0.26044 and 1.91214 ppm respectively.

4. 4. 2. 5. Effect of Deltamethrin

When pupae were exposed to a concentration of 0.001 ppm of deltamethrin, 19 per cent mortality was produced while, 0.05 ppm could kill the entire population. Based on probit analysis the LC_{50} and LC_{90} were computed as 0.00209 and 1.9121 ppm respectively.

In general, at higher concentrations of the insecticides, the pupae died within the pupal exuviae or in still lesser concentrations the eclosed adults were unable to fly away as they were caught attached to the pupal exuviae (Fig. 29 A & B). The longevity and the other biological parameters of the adults emerged were much higher than those obtained for the 4^{th} stage larvae.

Table 25. Effect of diflubenzuron on pupae of *Cx. tritaeniorhynchus* (Mean values)

Sl. No	Con. in ppm	Pupal mortality	Malformed adults	Total mortality	Longevity adults emerged in days	No. of eggs laid by female (mean) during lifetime	Percentage of eggs hatched
1	5	29	1	30	24	7	69
2	10	31	2	33	21	15	75
3	20	39	1	40	18	11	78
4	50	50	3	53	20	13	71
5	100	54	3	57	15	21	73
6	200	73	4	77	14	15	74
7	300	87	3	90	12	11	54
8	400	95	5	100	5	0	0
9	Control	Nil	Nil	Nil	48	53	100

Table 26. Effect of methoprene on pupae of *Cx. tritaeniorhynchus* (Mean values)

Sl. No	Con. in ppm	Pupal mortality	Malformed adults	Total mortality	Longevity adults emerged in days	No. of eggs laid by female (mean) during lifetime	Percentage of eggs hatched
1	0.05	17	1	18	25	15	75
2	0.1	43	2	45	28	14	73
3	0.5	67	3	70	21	15	68
2	1	72	3	75	15	10	71
5	2	74	2	76	16	11	67
6	5	78	3	81	10	9	71
7	10	82	8	90	3	0	0
8	20	93	7	100	3	0	0
9	Control	Nil	Nil	Nil	41	55	78

Table 27. Effect of Bti on pupae of *Cx. tritaeniorhynchus* (Mean values)

Sl. No	Con. in ppm	Pupal mortality	Malformed adults	Total mortality	Longevity adults emerged in days	No. of eggs laid by female (mean) during lifetime	Percentage of eggs hatched
1	24	16	1	17	25	10	67
2	48	24	3	27	24	11	75
3	100	60	3	63	21	13	73
4	150	73	7	80	17	12	63
5	200	95	5	100	12	0	0
6	Control	Nil	Nil	Nil	48	38	100

Table 28. Effect of azadirachtin on pupae of *Cx. tritaeniorhynchus* (Mean values)

Sl. No	Con. in ppm	Pupal mortality	Malformed adults	Total mortality	Longevity of adults in days	No. of eggs by female	Eggs Hatched %
1	2	18	2	20	16	14	0
2	3	23	2	25	13	15	0
3	5	42	5	47	0	0	0
4	8	70	7	77	0	0	0
5	10	76	7	83	0	0	0
6	12	96	4	100	0	0	0
7	Control	Nil	Nil	Nil	46	31	100

Table 29. Effect of ethofenprox on pupae of *Cx. tritaeniorhynchus* (Mean values)

Sl. No	Con. in ppm	Pupal mortality	Malformed adults	Total mortality	Longevity adults emerged in days	No. of eggs laid by female (mean) during lifetime	Percentage of eggs hatched
1	0.02	10	0	10	13	15	80
2	0.05	17	0	17	12	14	78
3	0.1	20	0	20	14	14	76
4	0.2	47	0	47	11	12	68
5	0.5	48	0	48	8	0	0
6	1	81	2	83	3	0	0
7	2	93	2	95	5	0	0
8	5	97	3	100	0	0	0
9	Control	Nil	Nil	Nil	39	43	100

Table 30. Effect of deltamethrin on pupae of *Cx. tritaeniorhynchus* (Mean values)

Sl. No	Con. in ppm	Pupal mortality	Malformed adults	Total mortality	Longivity adults emerged in days	No. of eggs laid by female (mean) during lifetime	Percentage of eggs hatched
1	0.001	17	2	19	14	14	67
2	0.002	34	0	34	13	16	73
3	0.004	49	0	49	14	13	68
4	0.005	56	0	56	10	14	47
5	0.01	68	0	68	10	10	41
6	0.02	94	0	94	0	0	0
7	0.05	100	0	100	0	0	0
8	Control	Nil	Nil	Nil	42	37	100

Table 31. LC_{50} and LC_{90} values of various insecticides on the pupae of *Cx. tritaeniorhynchus*

Insecticide	LC_{50} (ppm)	Range (ppm)	LC_{90} (ppm)	Range (ppm)
Diflubenzuron	28.77353	12.33-53.81	489.73381	201-3478
Methoprene	0.22858	0.09-0.43	6.25179	2.89-23.5
Bti	66.68765	34.83-107.86	187.36604	114-907
Azadirachtin	4.50358	3.25-5.88	11.31192	8.16-22.51
Ethofenprox	0.26044	0.16-0.42	1.91214	1.02-5.57
Deltamethrin	0.00382	0.0031-0.005	0.02016	0.015-0.0298

4. 4. 4. Comparison of Effective Values of Insecticides on Different Stages of *Cx. tritaeniorhynchus*

The LC_{50} and LC_{90} values of each insecticide on the different stages are compared and presented in Tables 32 and 33.

From Table No. 32 it can be seen that the least concentration of insecticide to produce 50 per cent mortality was when they were applied against 4^{th} stage larvae. The concentrations of IGRs to produce 50 per cent mortality in larvae were minimum while several thousand multiples of these insecticide concentrations were needed to produce the same effect on pupae and eggs.

The LC_{50} values on larvae and pupae were almost the same in case of azadirachtin. That is the same concentration when used could produce similar mortality in larvae as well as pupae. But 20 times of the LC_{50} was needed to cause 50 per cent non-hatchability in eggs.

In case of deltamethrin, slightly higher concentrations than LC_{50} on larvae could produce the same effect on pupae while a 23 times concentration was found to produce 50 per cent non-hatchability of eggs.

While methoprene had no action on the eggs of *Cx. tritaeniorhynchus*, three hundred and thirty nine times concentration of LC_{50} on larvae was needed to produce the same effect on pupae.

Table 32. Comparison of LC_{50} values of various insecticides on eggs, larvae and pupae of *Cx. tritaeniorhynchus*

Insecticide	**LC_{50} values in ppm**				
	Eggs		4th stage Larvae	Pupae	
		*X			*X
Diflubenzuron	38.265508	167244	0.0002288	28. 7732483	125743
Methoprene	-	-	0.0006738	0. 2285781	339
Bti	21.7253912	3.3	6.6076021	66. 6887168	10
Azadirachtin	93.3783344	20	4.6867982	4.5035887	1
Ethofenprox	0.2348346	29	0.0082146	0.2604370	31
Deltamethrin	0.0481368	23	0.0020853	0.0038200	1.8

* Multiples of larval LC_{50}

Table 33. Comparison of LC_{90} values of various insecticides on the eggs, larvae and pupae of *Cx. tritaeniorhynchus*

Insecticide	**LC_{90} values in ppm**				
	Eggs	*X	4th stage Larvae	Pupae	*X
Diflubenzuron	197.9877	48290	0.0041	489.7338	119447
Methoprene	-	--	0.0067	6.2518	933
Bti	110.5554	5.3	20.8106	187.3660	9
Azadirachtin	163.5041	19.2	8.4951	11.3119	1.3
Ethofenprox	0.7611	19.3	0.0393	1.9121	49
Deltamethrin	1.5715	242	0.0065	0.0202	3.1

* Multiples of larval LC_{90}

Table 34. Order of relative toxicity between insecticides on eggs of *Cx. tritaeniorhynchus* based on LC_{50} values

Sl. No	Name of insecticide	LC_{50}	*Relative toxicity
1	Deltamethrin	0.0481368	Most effective
2	Ethofenprox	0.2348346	5 X
3	*Bti*	21.7253912	451 X
4	Diflubenzuron	38.2655082	795 X
5	Azadirachtin	93.3783344	1940 X
6	Methoprene	No effect	No effect

* In comparison to the most toxic insecticide

When the efficacy of the various insecticides were compared based on LC_{90} values it was seen that proportionately higher concentrations were needed to produce the same effect on eggs as well as on pupae.

The order of relative toxicity of insecticides when used against eggs, larvae and pupae are presented in Tables. 34, 35 and 36 respectively.

Table 35. Order of relative toxicity of insecticides on the 4th stage larvae of *Cx. tritaeniorhynchus* based on LC_{50} values

Sl. No	Name of insecticide	LC_{50}	*Relative toxicity
1	Diflubenzuron	0.0002288	Most effective
2	Methoprene	0.0006738	3 X
3	Deltamethrin	0.0020853	9 X
4	Ethofenprox	0.0082146	36 X
5	Azadirachtin	4.6867982	20,377 X
6	*Bti*	6.6076021	27,055 X

* In comparison to the most toxic insecticide

Table 36. Order of relative toxicity of insecticides on the pupae of *Cx. tritaeniorhynchus* based on LC_{50} values

Sl. No	Name of insecticide	LC_{50}	*Relative toxicity
1	Deltamethrin	0.0038200	Most effective
2	Methoprene	0. 2285781	60 X
3	Ethofenprox	0.2604370	68 X
4	Azadirachtin	4.5035887	1179 X
5	Diflubenzuron	28. 7732483	7532 X
6	*Bti*	66. 6887168	17,458 X

* In comparison to the most toxic insecticide

The insecticides used in this study are serially numbered according to the order of toxicity based on the LC_{90} values obtained when applied against eggs, 4th stage larvae and pupae. These are presented in tables 37, 38 and 39.

Table 37. Order of relative toxicity of insecticides on the eggs of *Cx. tritaeniorhynchus* based on LC_{90} values

Sl. No	Name of insecticide	LC_{90}	Relative toxicity
1	Ethofenprox	0.7611	Most effective
2	Deltamethrin	1.5715	2 X
3	*Bti*	110.5554	145 X
4	Azadirachtin	163.5041	215
5	Diflubenzuron	197.9877	260
6	Methoprene	No effect	No effect

* In comparison to the most toxic insecticide

Table 38. Order of relative toxicity of insecticides on the 4th stage larvae of *Cx. tritaeniorhynchus* based on LC_{90} values

Sl. No	Name of insecticide	LC_{90}	*Relative toxicity
1	Diflubenzuron	0.0041	Most effective
2	Deltamethrin	0.0065	1.6 X
3	Methoprene	0.0067	1.7 X
4	Ethofenprox	0.03931	9.7 X
5	Azadirachtin	8.49510	2087 X
6	*Bti*	20.8106	5113 X

* In comparison to the most toxic insecticide

Table 39. Order of relative toxicity of insecticides on the pupae of *Cx. tritaeniorhynchus* based on LC_{90} values

Sl. No	Name of insecticide	LC_{90}	Relative toxicity
1	Deltamethrin	0.0202	Most effective
2	Ethofenprox	1.9121	95 X
3	Methoprene	6.2518	310 X
4	Azadirachtin	11.3119	561 X
5	*Bti*	187.3660	9294 X
6	Diflubenzuron	489.7338	23499 X

* In comparison to the most toxic insecticide

4. 4. 5. Cost of Application in Unit Volume

Field dosages were calculated four times the LC_{50} values on larvae. The cost of application of the various insecticides in one lakh litres of water was calculated based on this and are shown in Table 40.

Table 40. Cost of application of different insecticides in a unit volume of water

Sl. No.	Insecticide	MRP (Rs.)	Field dosage (ppm)	Cost of application/ 1 lakh litres (Rs.)
1.	**Hilmilin** (Diflubenzuron 25%)	375/100 g	0.00092	1.38
2	**Altosid** (Methoprene 5%)	255/100 ml	0.00272	13.87
3	**HIL *Bti*** (*Bti* 1.2%)	105/100 ml	24.91	2179
4	**Neem Azal** (Azadirachtin 1%)	87/100 ml	18.747	1630
5	**Primo** (Ethofenprox 10%)	75/100ml	0.03276	0.03
6	**Butox** (Deltamethrin 1.25%)	77/100 ml	0.00836	0.51

When the MRP alone was considered, ethofenprox was found to be the cheapest insecticide, costing Rs.0.02/- per one litre of water followed by deltamethrin (Rs. 0.51/-). The most expensive one was *Bti* (Rs. 2179/-) followed by azadirachtin (Rs. 1630/-). Among the IGRs, diflubenzuron was much cheaper (Rs. 1.38/-) than methoprene (Rs. 13. 87/-).

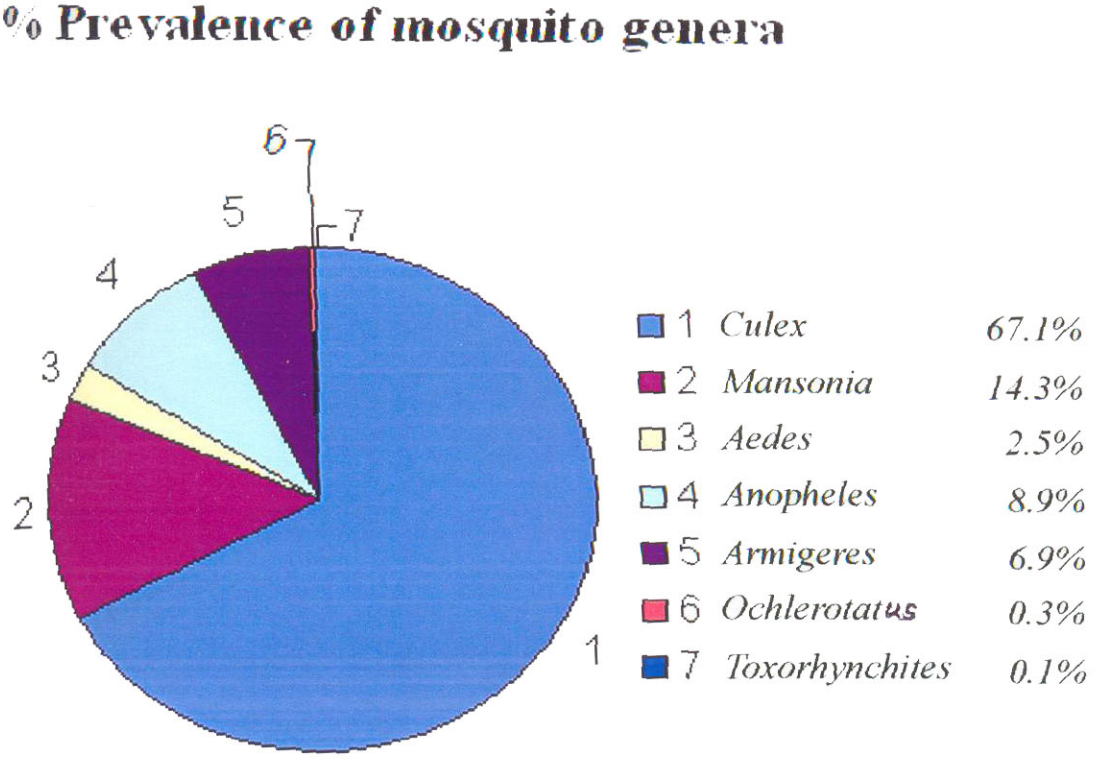


Fig. 5. Generic prevalence of mosquitoes (%)

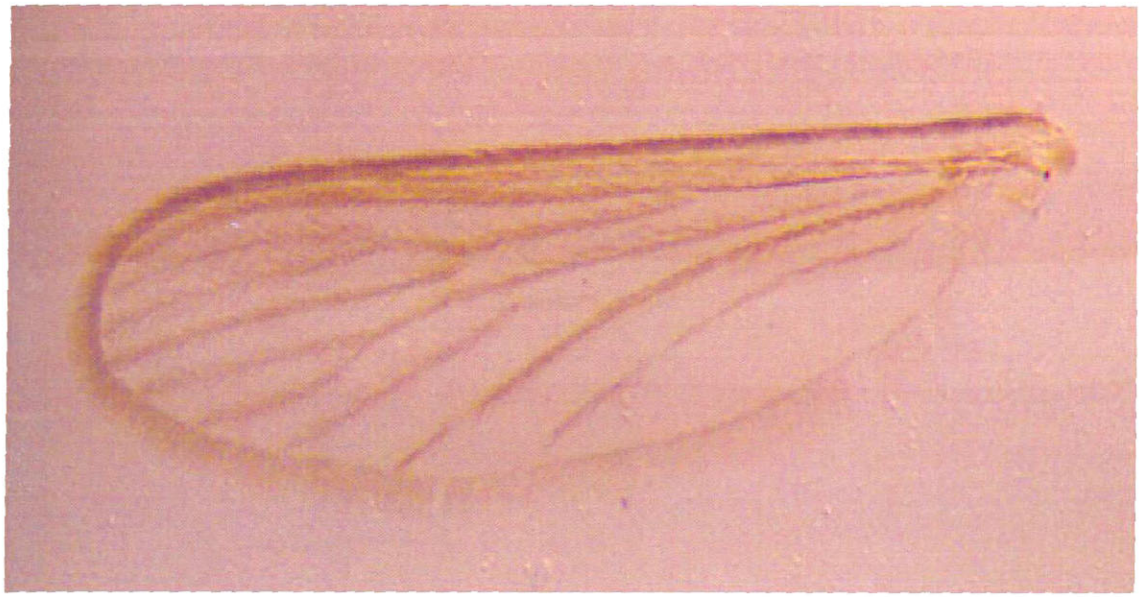


Fig. 6. Mosquito wing clothed with scales

Fig.7. *Toxorhynchities splendens* female

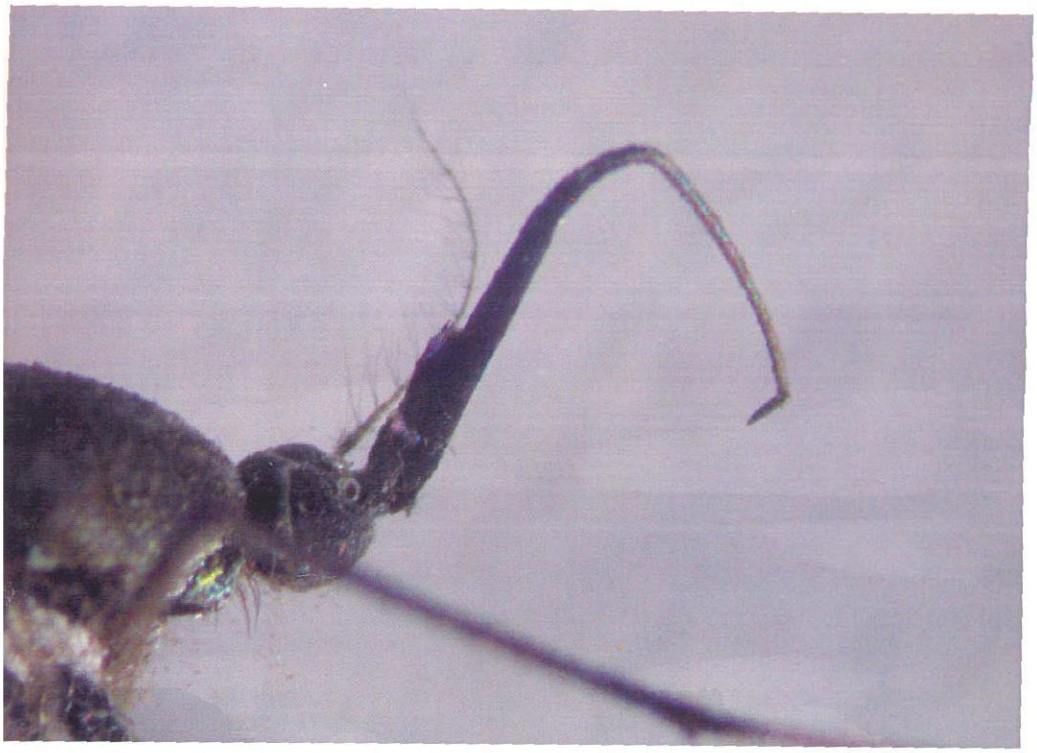


Fig. 8. *To. splendens* female mouthparts

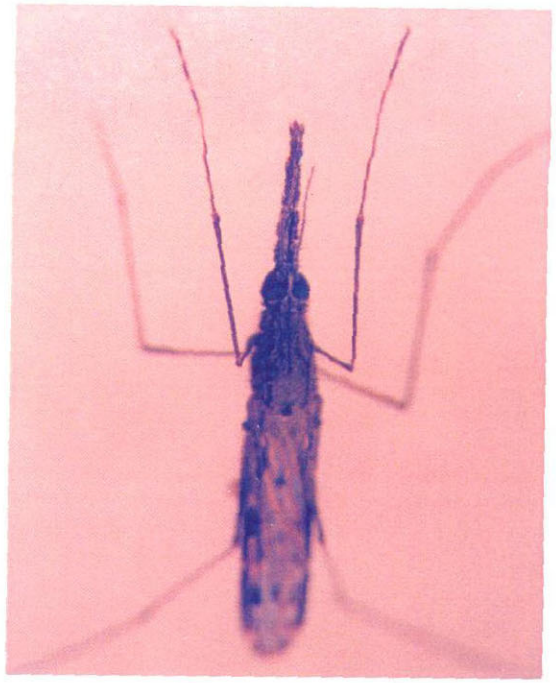
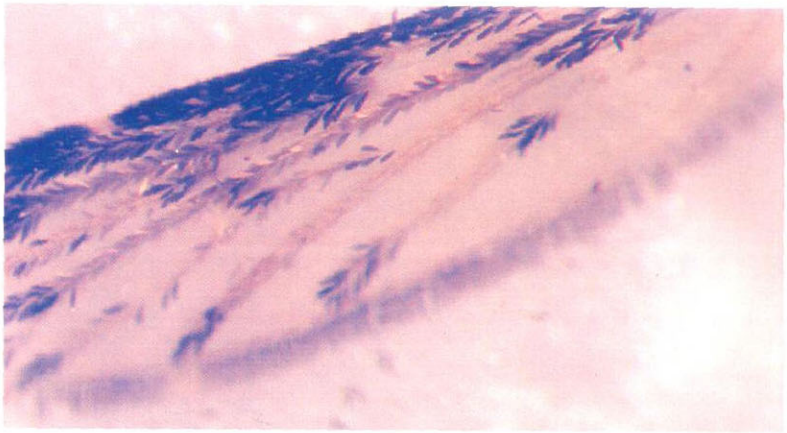
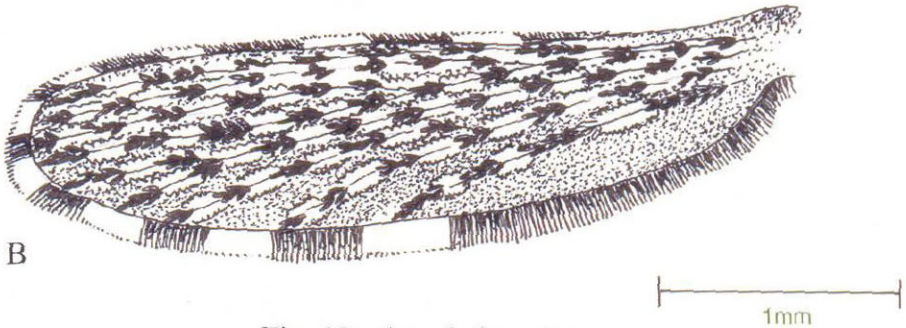


Fig. 9. *Anopheles* female



A



B

Fig. 10. *Anopheles* wing
(A. Photograph. B. *Camera Lucida* drawing)

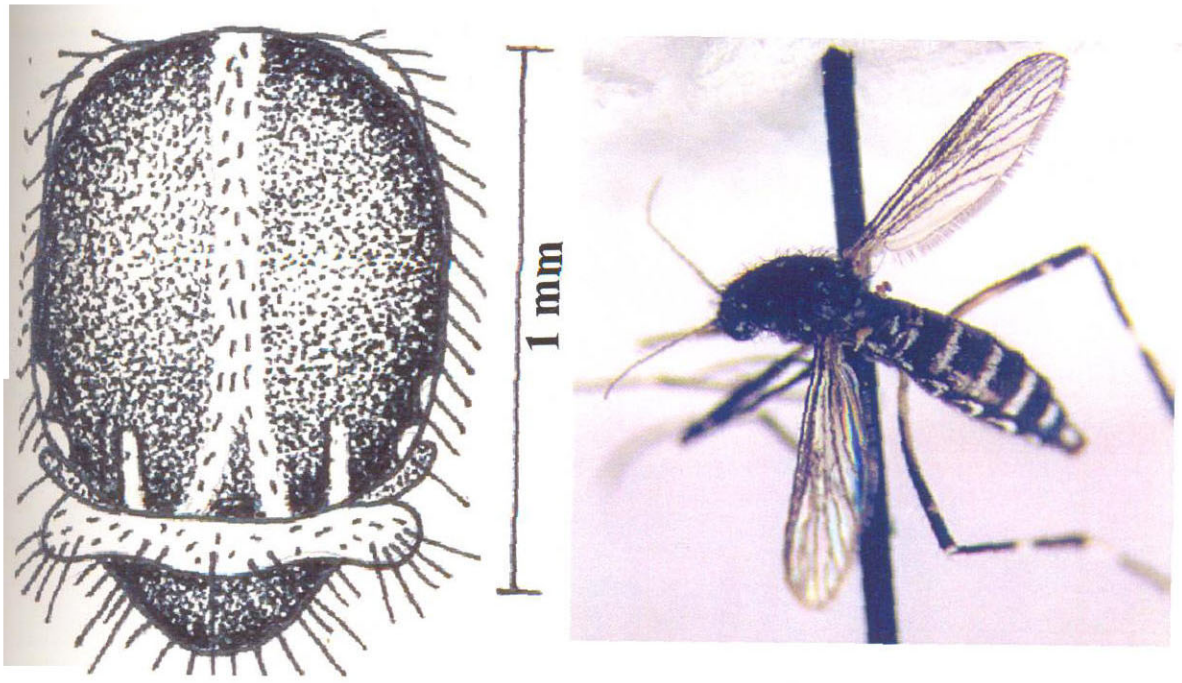


Fig. 11. *Aedes* (A. Thorax - *Camera Lucida* Drawing B. Photograph)

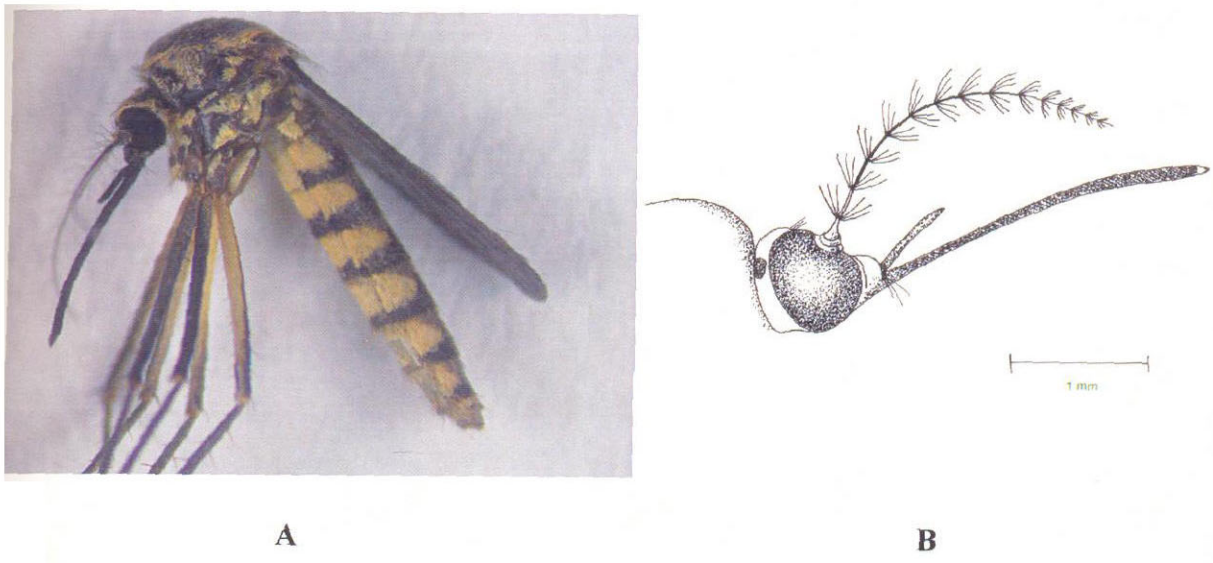
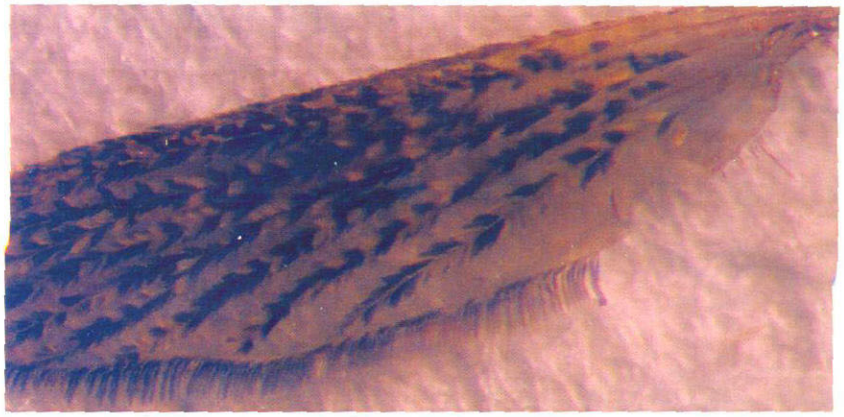


Fig. 12. *Armigeres* Female (A. Gross B. Mouth parts, *Camera Lucida* Drawing)

Fig. 13. *Mansonia* Female



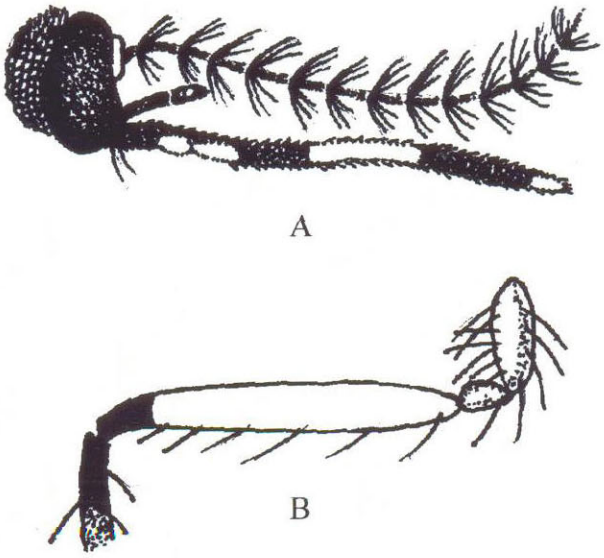
A

B

Fig. 14. *Mansonia* Wing. (A. Photograph B. *Camera Lucida* Drawing)

A B

Fig. 15. *Culex tritaeniorhynchus* (A. Female B. Male)



A

B

Fig. 16. *Culex tritaeniorhynchus* (A. Mouth parts B. Hind Femur)

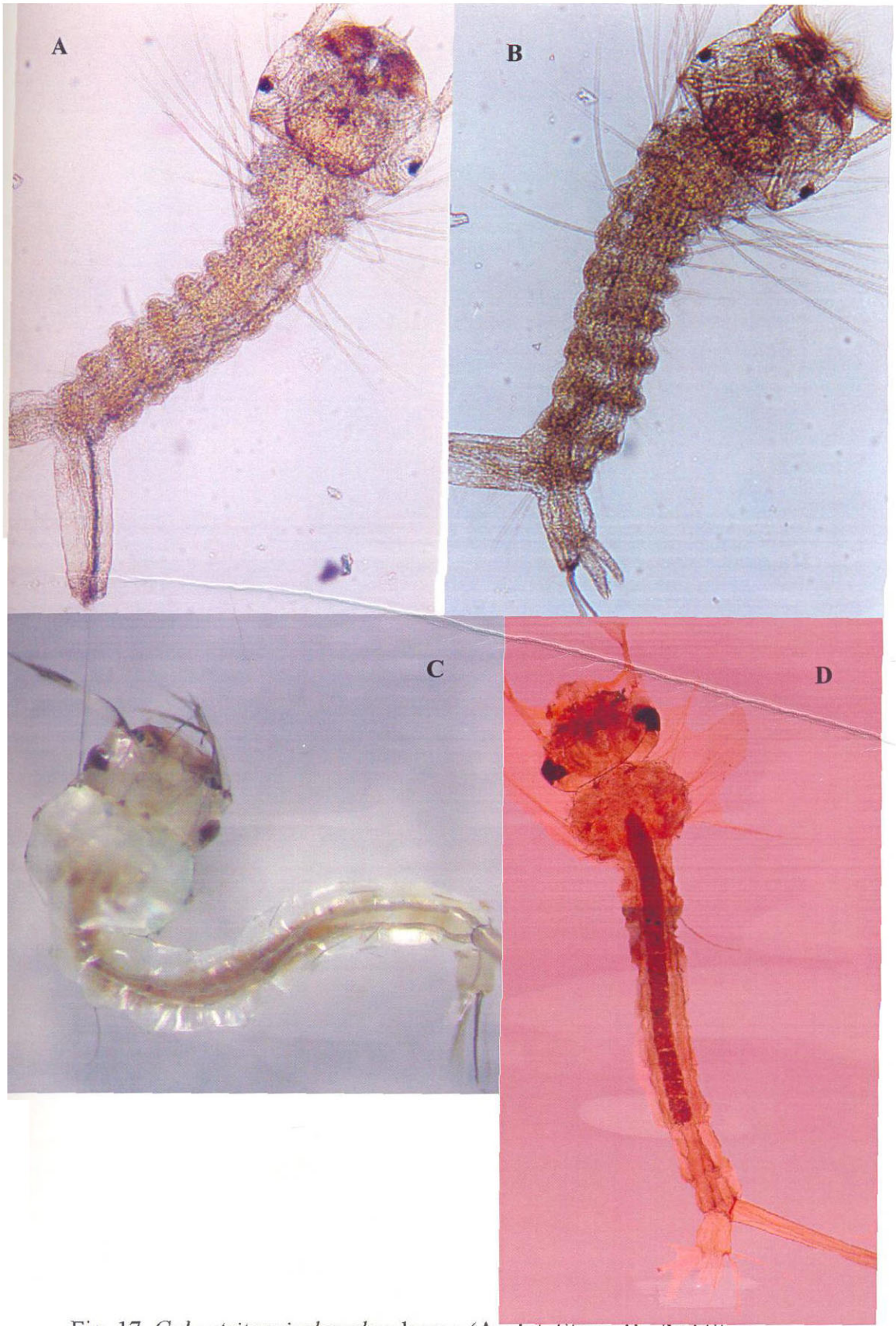


Fig. 17. *Culex tritaeniorhynchus* larvae (A - 1st Stage, B - 2nd Stage, C - 3rd Stage and D-4th Stage)

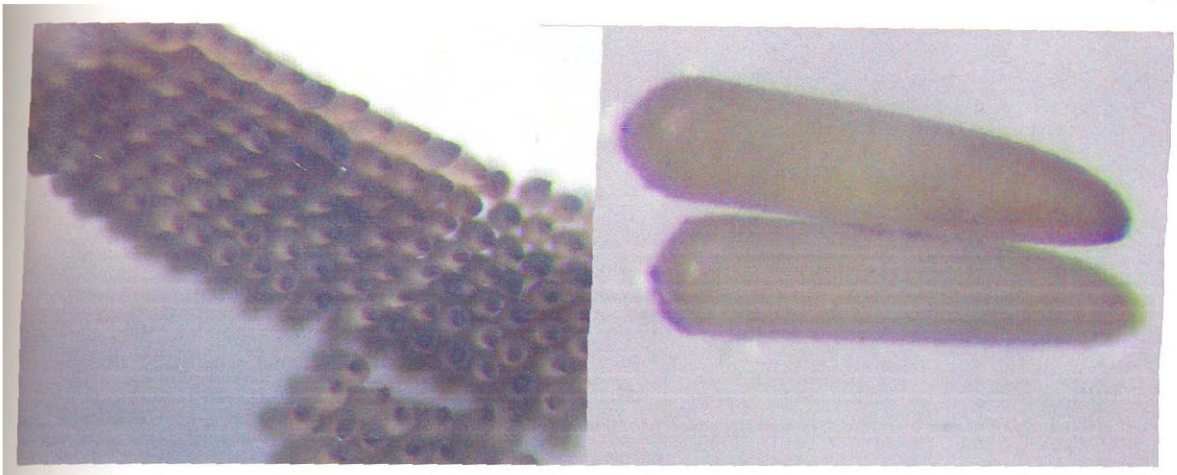
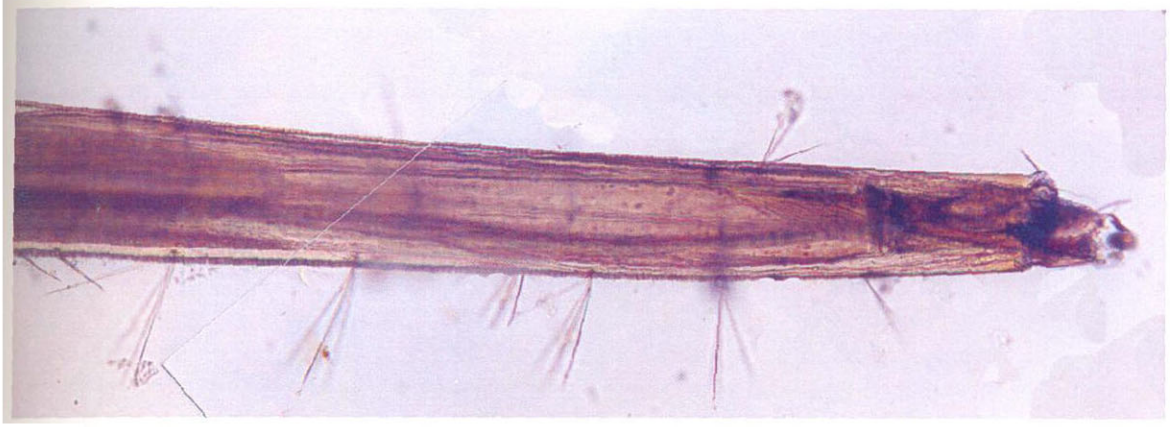


Fig. 18. *Culex* (A-Egg raft, B-individual eggs)



A

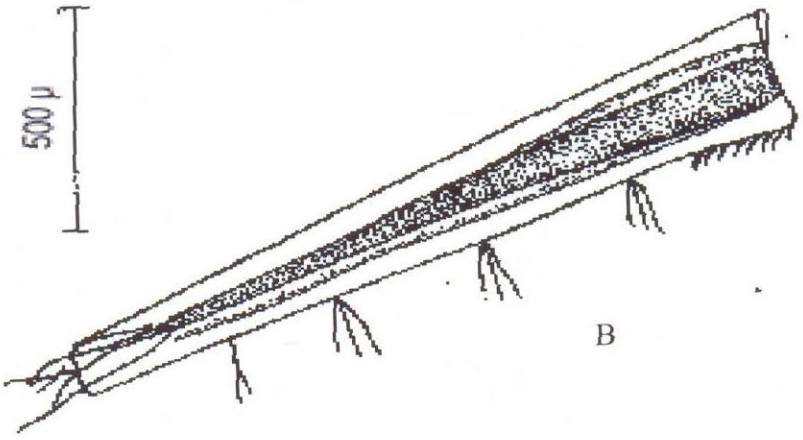
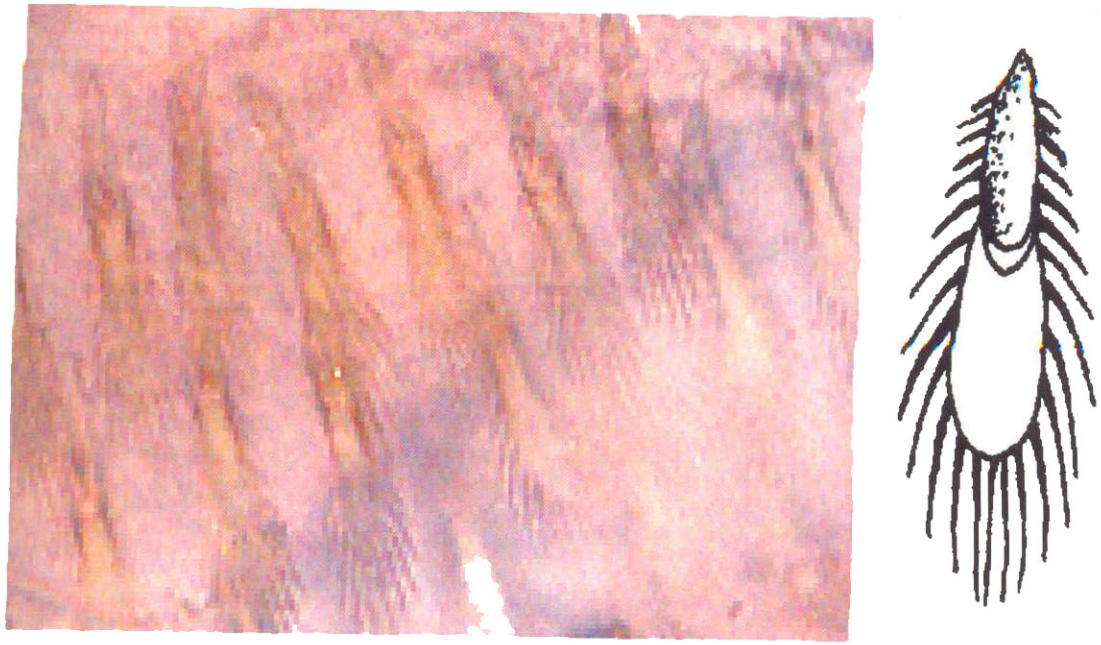


Fig. 19. *Cx. tritaeniorhynchus* larva - siphon tufts
(A-photograph, B- Camera lucida drawing)



A B

Fig. 20. *Cx. tritaeniorhynchus* larva- comb scales (A-photograph, B-camera lucida drawing)

Fig. 21. *Cx. tritaeniorhynchus* pupa

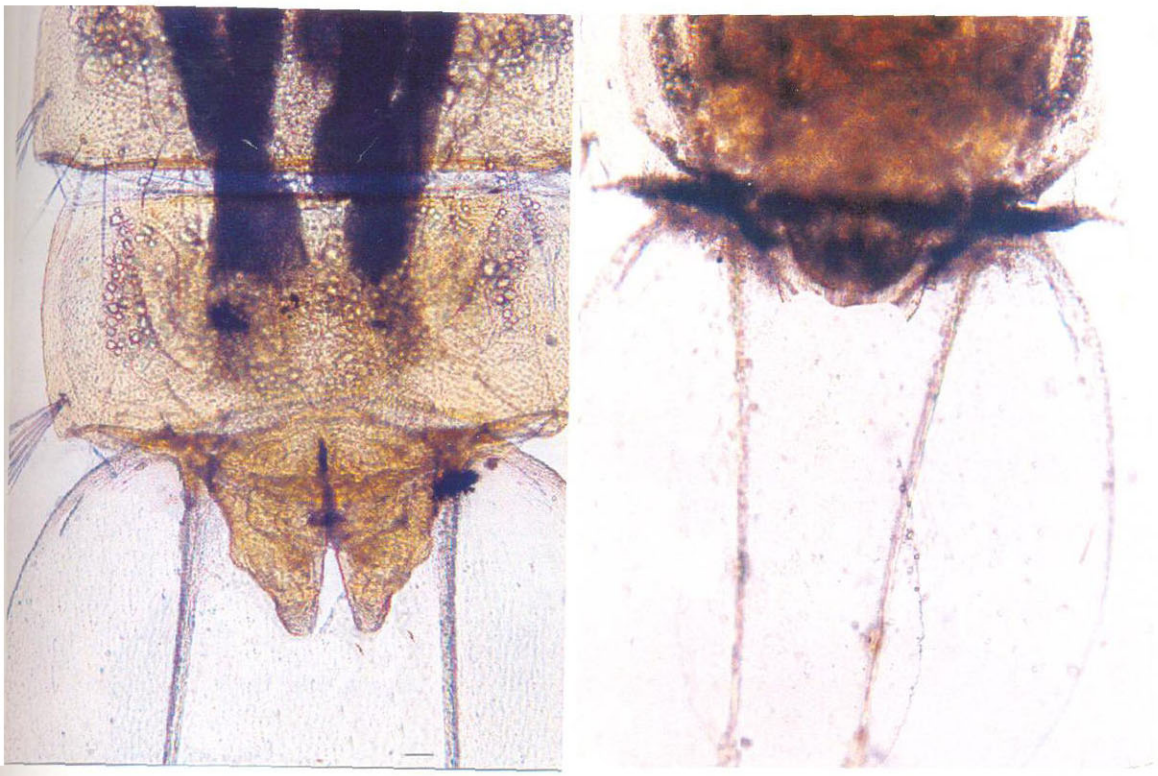


Fig. 22 *Cx. tritaeniorhynchus* pupa posterior pouch (A-Male, B- Female)

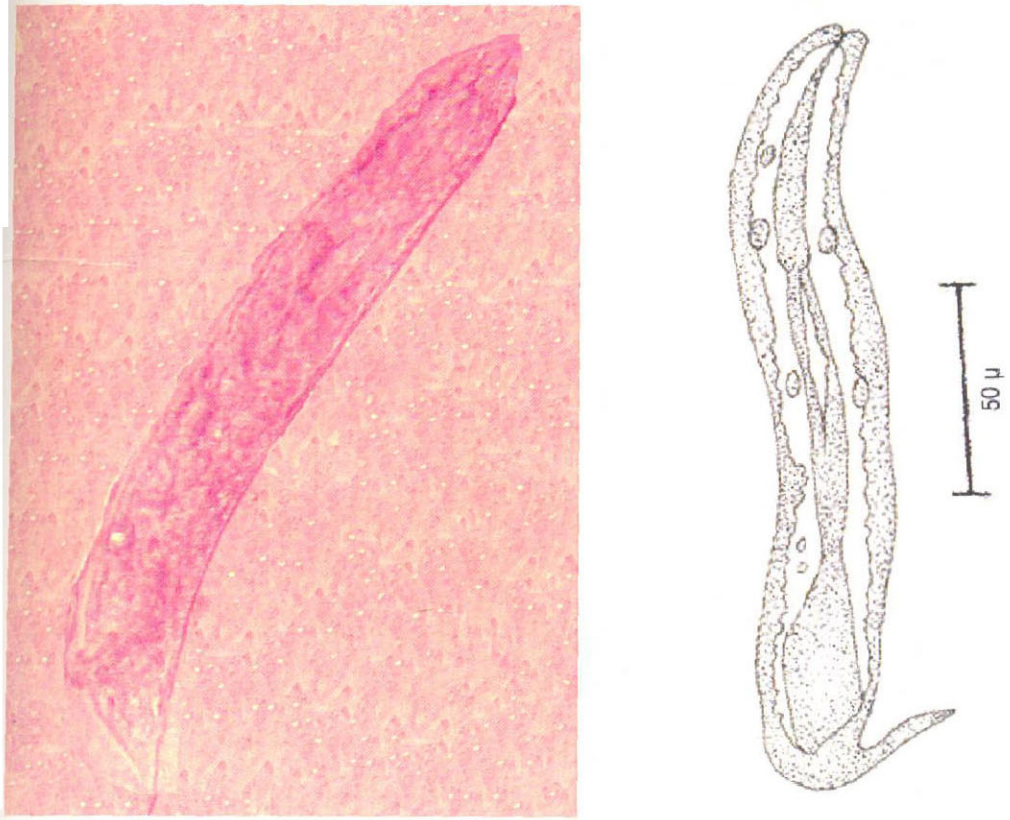


Fig 23. Second Stage Larva dissected from mosquitoes (A-photograph, B-camera lucida drawing)

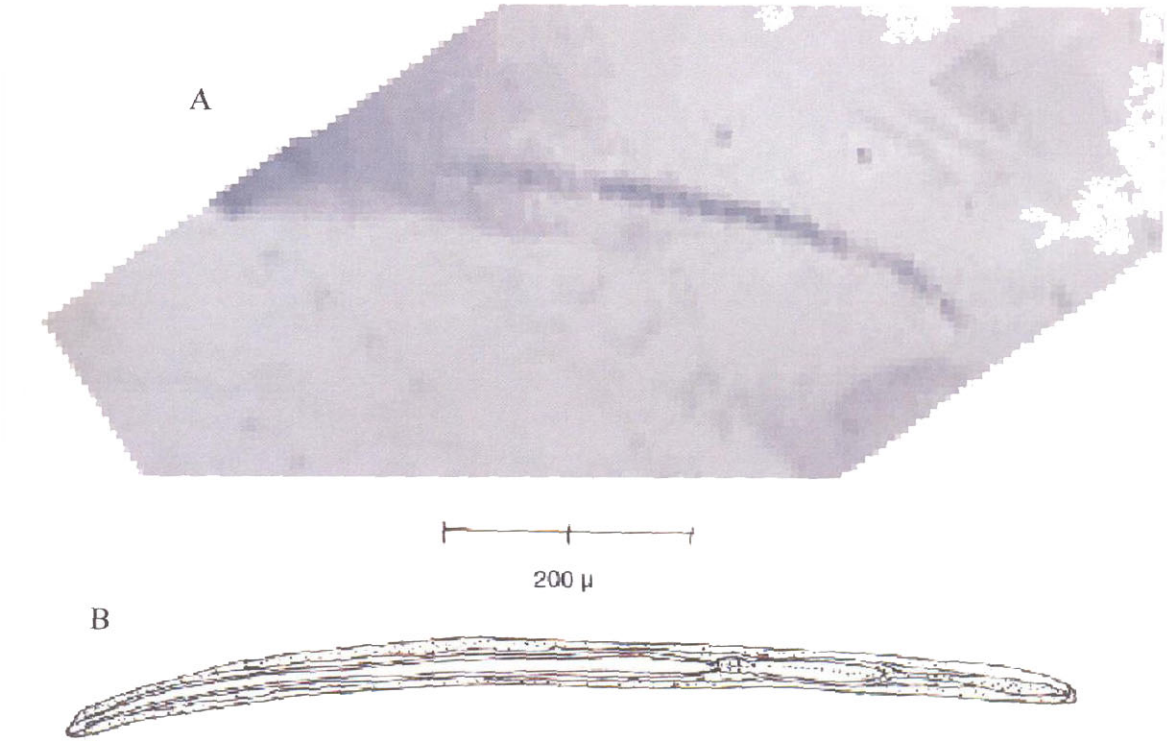


Fig. 24. Third stage larva dissected out from mosquito
(A- photograph, B-camera lucida drawing)

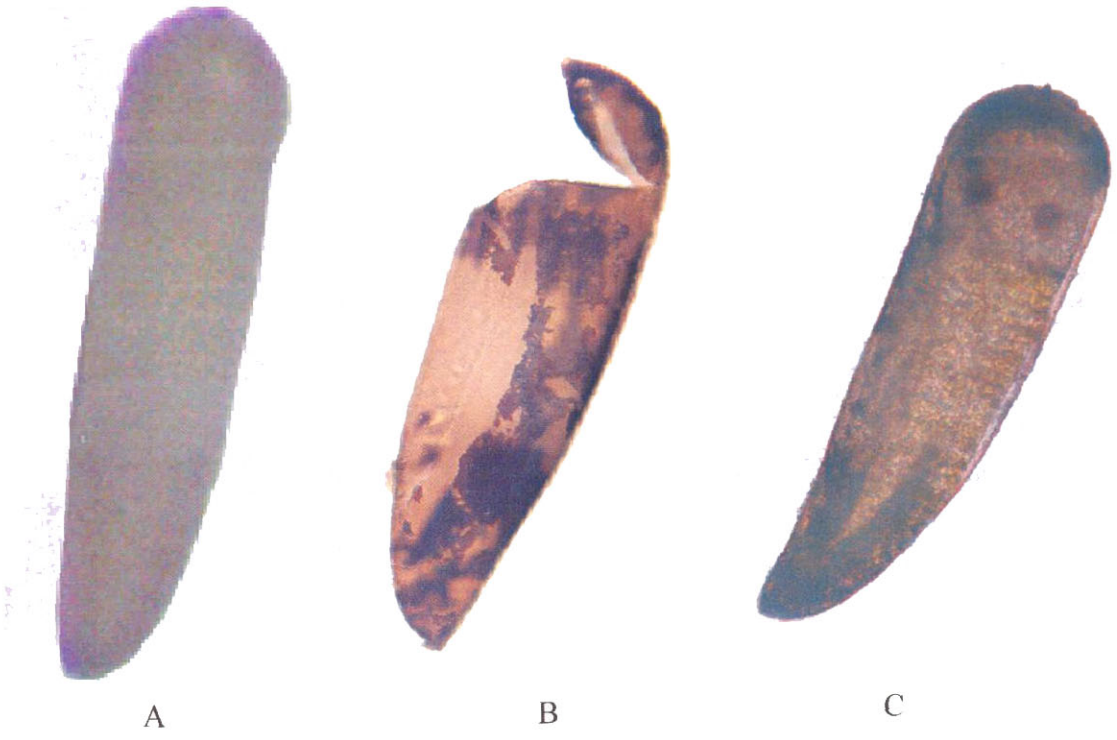


Fig. 25. A. Before hatch B. Normal hatch C. Unhatch

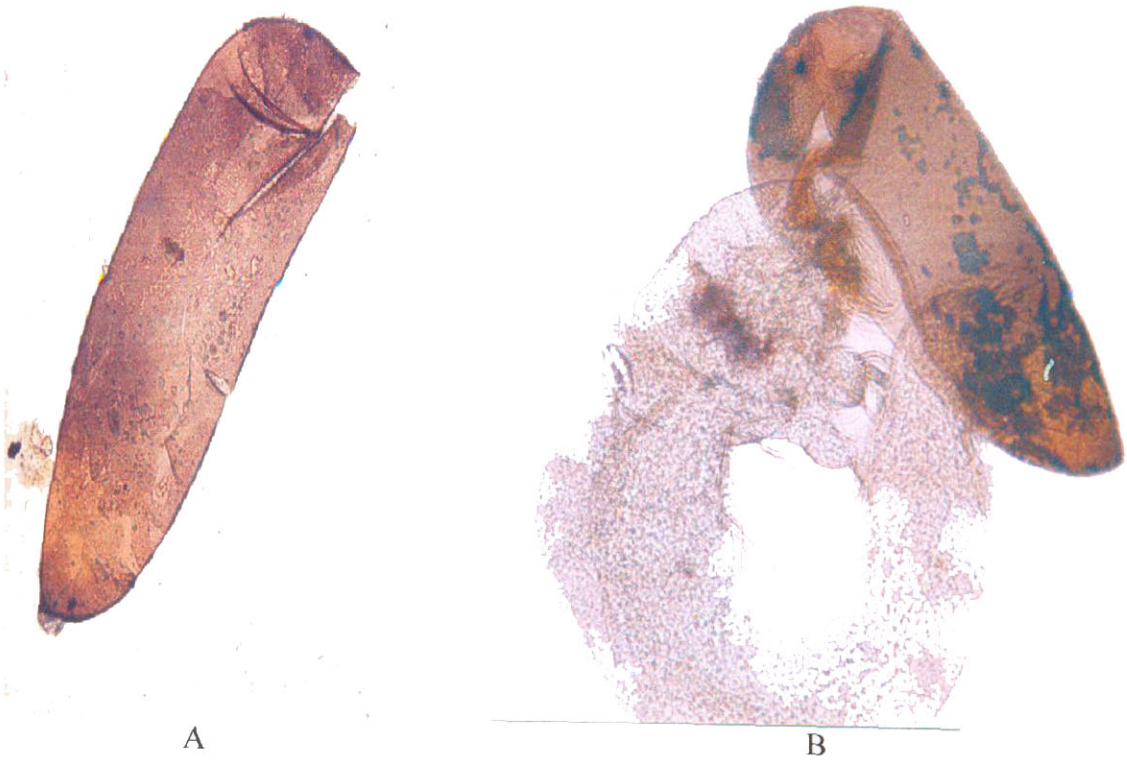


Fig. 26. A. Abnormal hatch; B. Partial side hatch

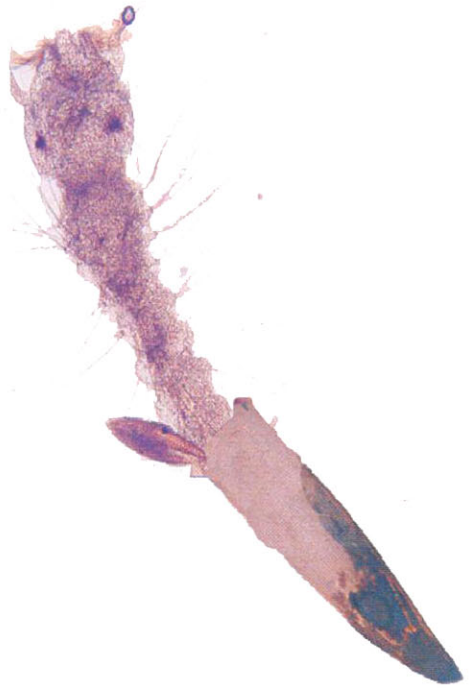
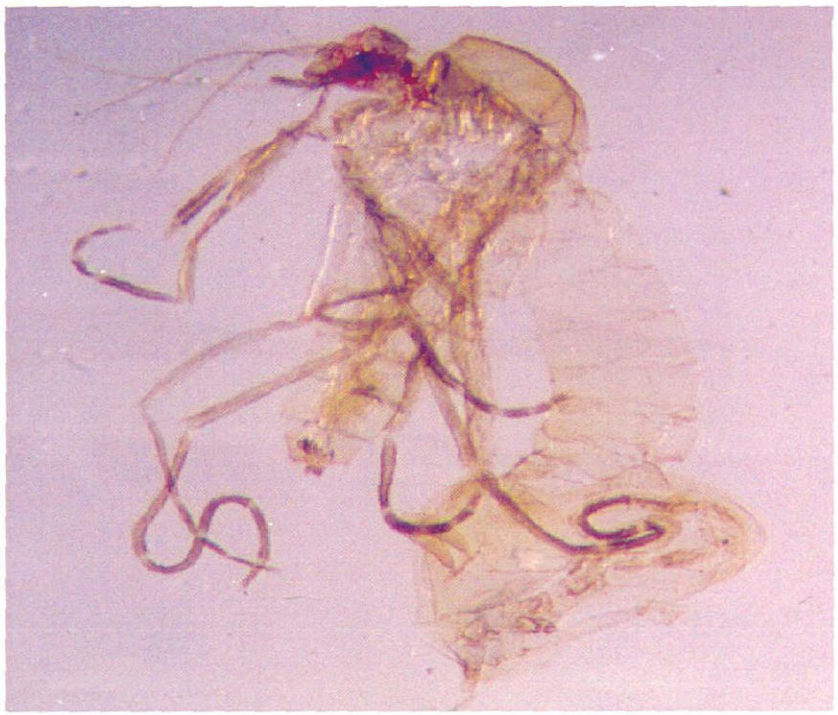


Fig. 27. Partial hatch

Fig. 28. Pupal exuvia

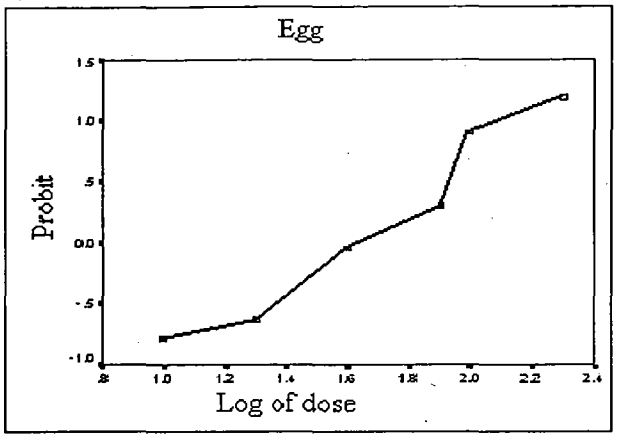
A



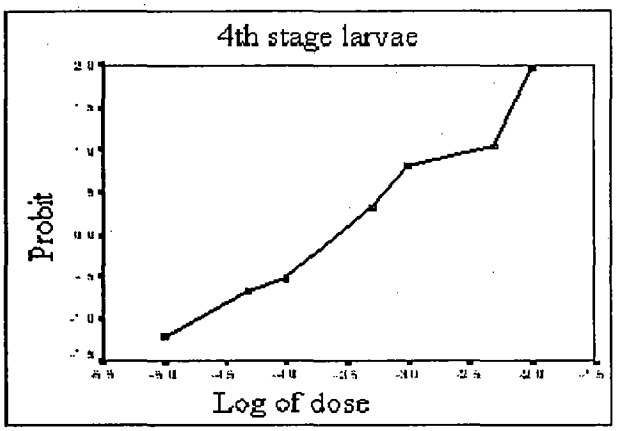
B

Fig. 29. A. & B. Partial eclosion

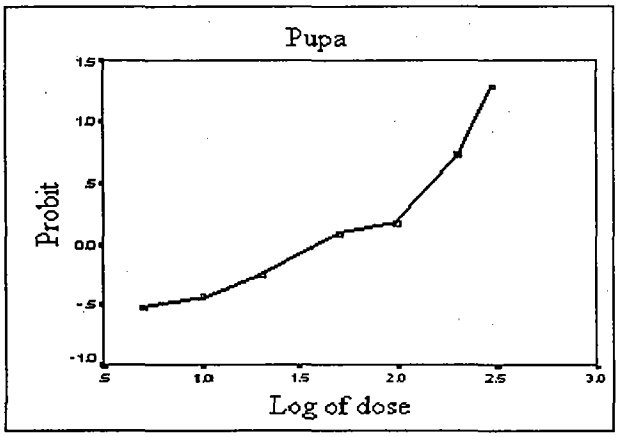
Fig. 30. Probit curves (ld-p lines) of diflubenzuron on *Cx. tritaeniorhynchus*



a= -0.5219
b = 324.289

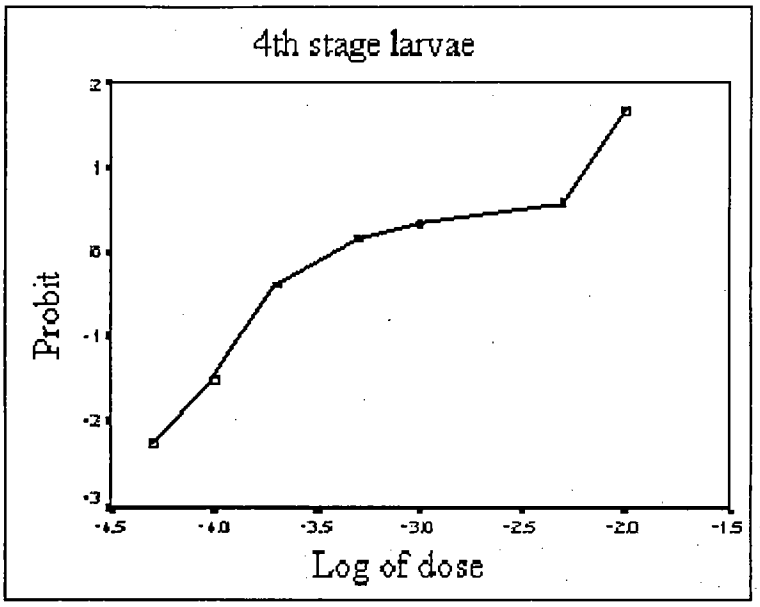


a = 3.7318
b = 1.0251

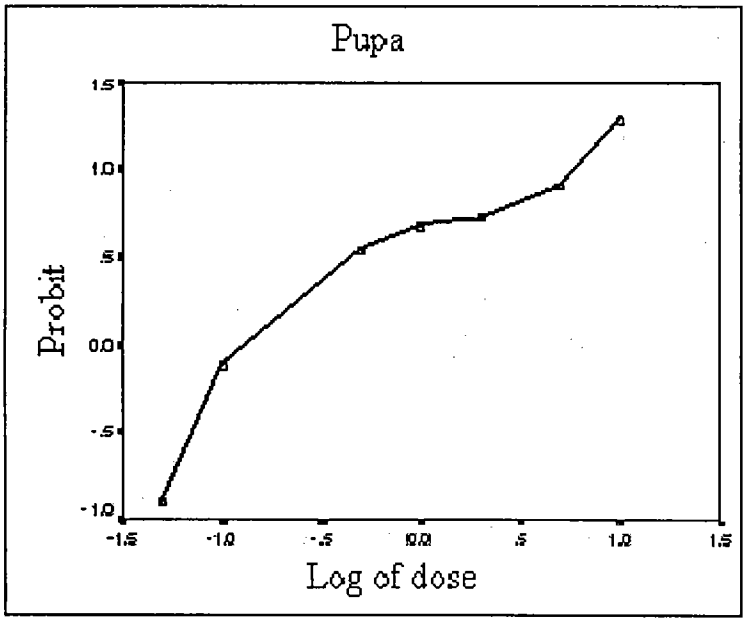


a = -1.5189
b = 1.0410

Fig. 31. Probit curves (ld-p lines) of methoprene on *Cx. tritaeniorhynchus*

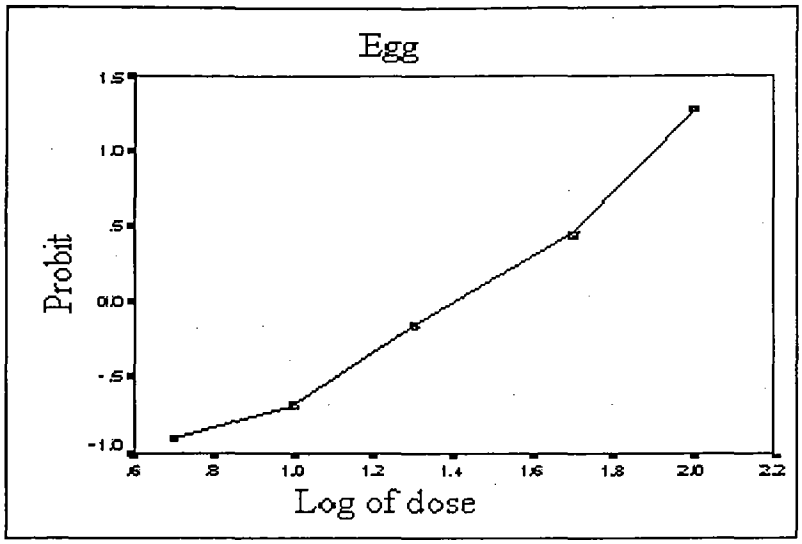


a = 4.0839
b = 1.2903

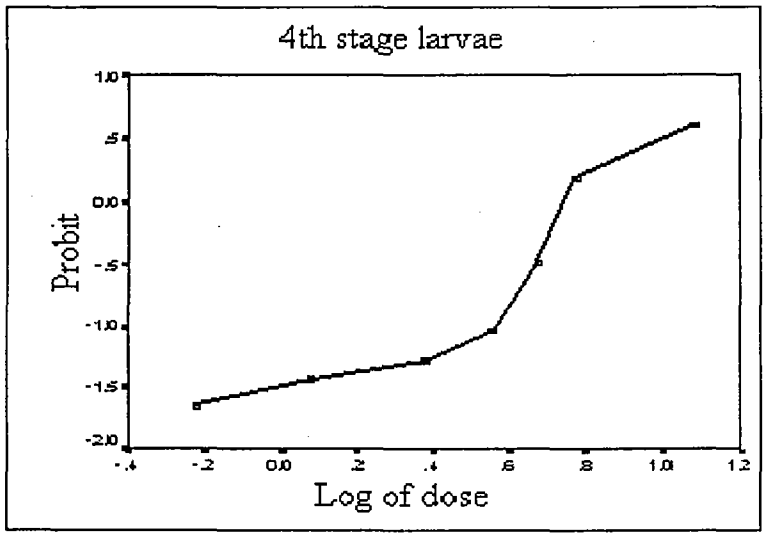


a = -0.5716
b = 0.8918

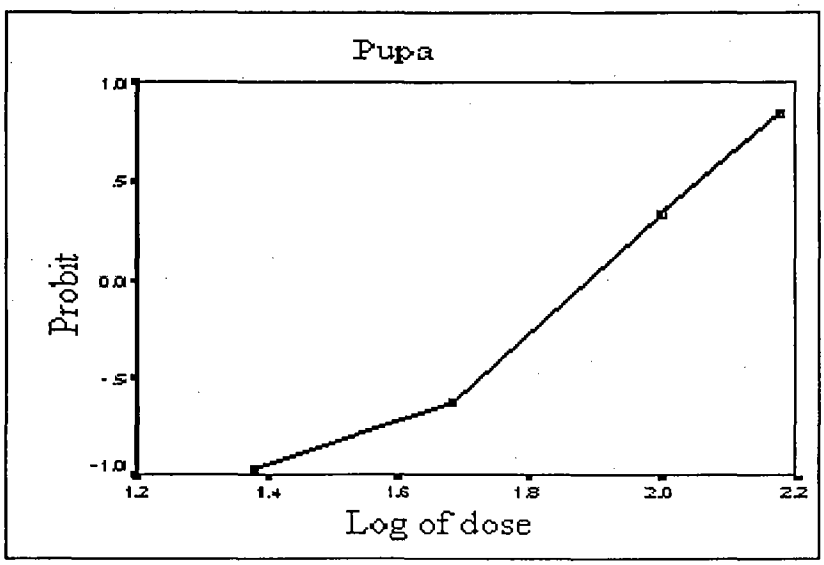
Fig. 32. Probit curves (ld-p lines) of *Bti* on *Cx. tritaeniorhynchus*



a = -1.6074
b = 0.0183

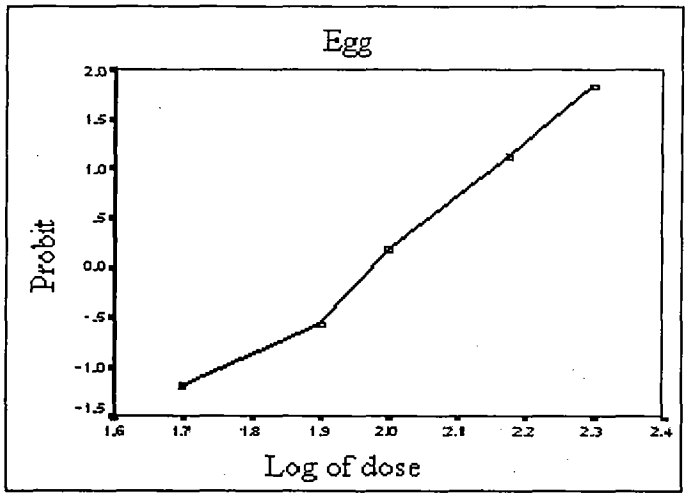


a = -1.9420
b = 2.4452

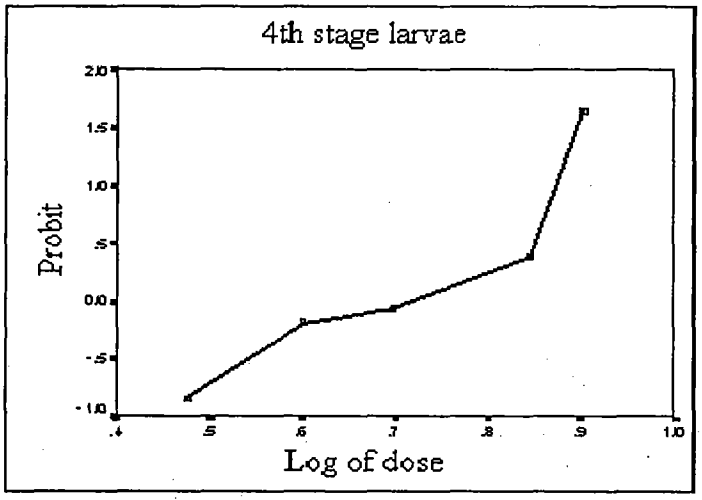


a = -5.2103
b = 2.8564

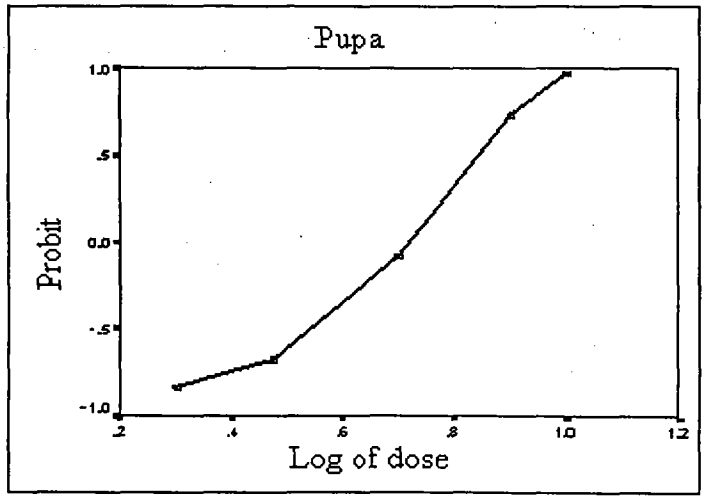
Fig. 33. Probit curves (ld-p lines) of azadirachtin on *Cx. tritaeniorhynchus*



a = -2.1644
b = 0.0323

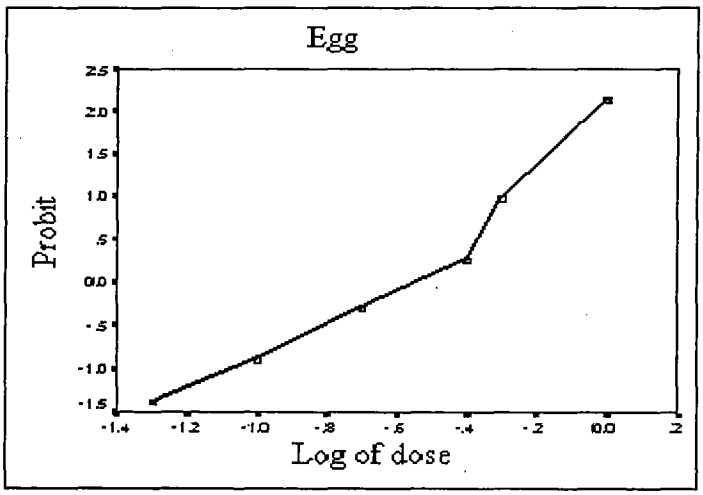


a = -3.3288
b = 4.9616

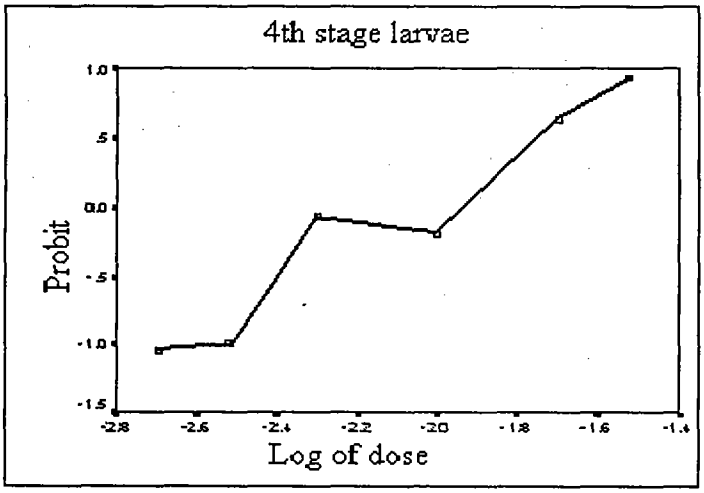


a = -2.0940
b = 3.2040

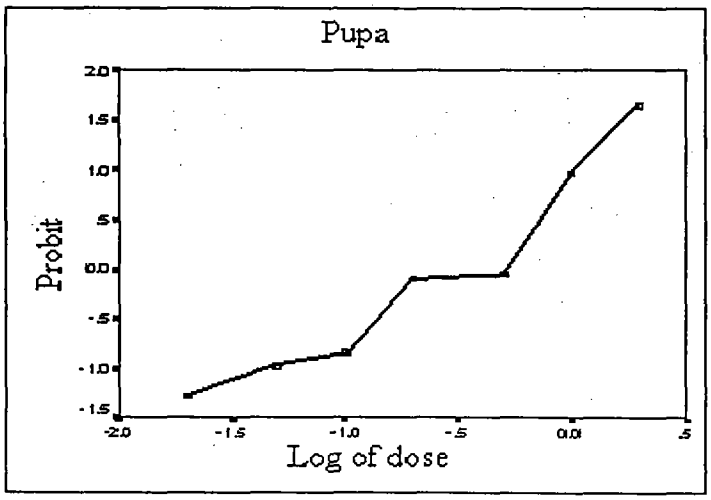
Fig. 34. Probit curves (ld-p lines) of ethofenprox on *Cx. tritaeniorhynchus*



a = -0.9987
b = 1.6984

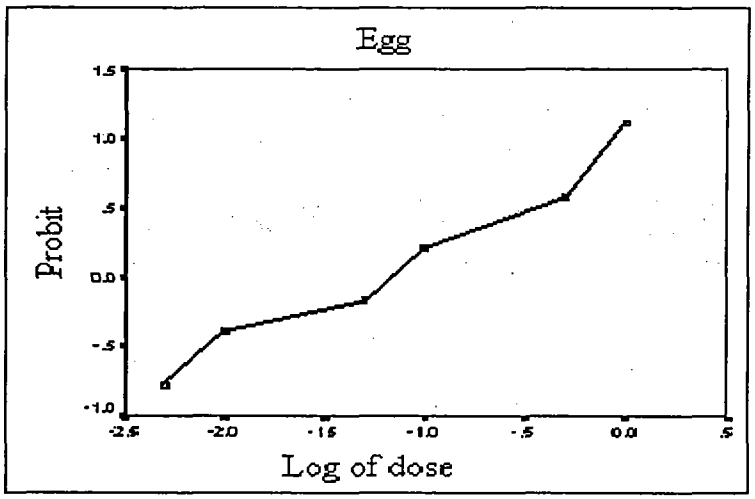


a = -1.0635
b = 77.6564

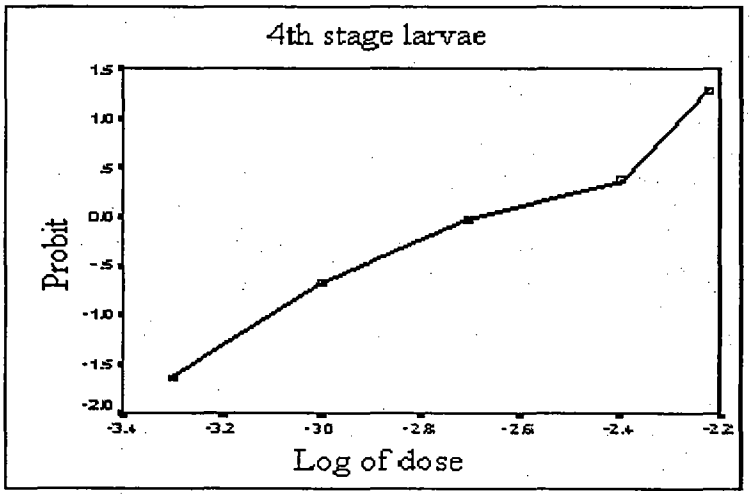


a = 0.8648
b = 1.4801

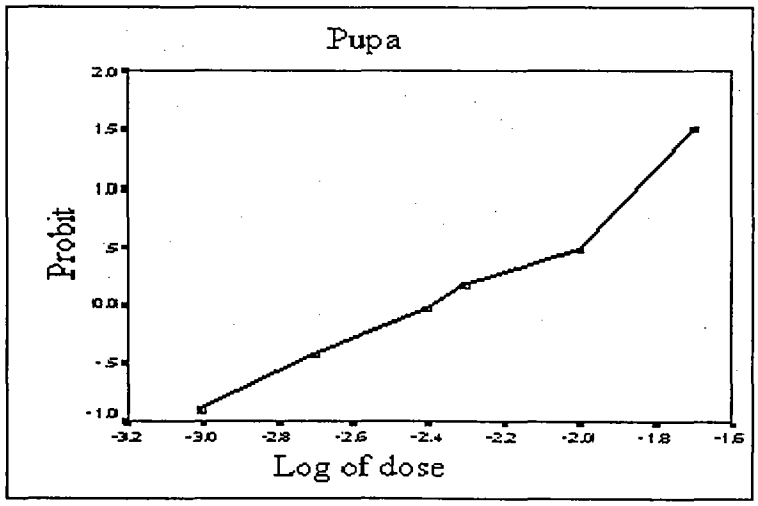
Fig. 35. Probit curves (ld-p lines) of deltamethrin on *Cx. tritaeniorhynchus*



a = -1.4279
b = 482.4126



a = 6.9222
b = 2.5821



a = 4.2891
b = 1.7739

Discussion

5. DISCUSSION

5. 1. PREVALENCE

Although around 3450 species of mosquitoes under 37 genera are known to exist globally (Alan Walker, 1994), only 369 species coming under 21 genera have been reported from India (Rajavel, 2004). The epidemiology of vector borne diseases is related to the vector distribution in each area and hence the species identification of this medically important vector deserves much attention. In the present study 27 species under seven genera namely, *Aedes, Anopheles, Armigeres, Culex, Mansonia, Ochlerotatus* and *Toxorhynchites,* belonging to the three subfamilies (*Toxorhynchitinae, Anophelinae* and *Culicinae*) of mosquitoes could be identified.

However, only a single female specimen was found in the subfamily *Toxorhynchitinae.* Since the collection in the present study was restricted to dusk, the non-biting species of mosquitoes such as the *Toxorhynchites* are unlikely to enter houses/animal houses especially during the evening hours. The species was identified as *To. splendens*. This in fact was the largest and most colourful of the entire collection. Barraud, 1934 also described it as the largest Indian species of mosquito known. The proboscis was recurved and it was incapable of sucking blood. It does not require further description as they have neither medical nor veterinary importance.

The genus *Anopheles* is the single blood-sucking genus known under the subfamily *Anophelinae.* The six species of *Anopheles* mosquitoes collected in the present study were *An. peditaeniatus, An. nigerrimus, An. jamesi, An. tesellatus, An. jeyporensis,* and

An. barbirostris. When mosquito populations were observed, *Anopheles* spp. could be spotted in relatively large numbers a few days after rains. The study carried out by Prakash (1998) in Assam also revealed that the density of *Anopheles* was significantly correlated with the monthly rainfall and high prevalence in monsoon months. This is because *Anopheles* breeds in fresh water unlike other mosquitoes, which breed in polluted/contaminated water.

As per the Malaria Research Centre Report, 2004, fifty eight species of *Anopheles* are known to occur in India. Of this, seven have been incriminated as vectors of malaria. An interesting observation was that none of the medically important vectors of malaria was there in the collections made during the current study. It is also worth mentioning that in the collections/observations made in human dwellings during the period of research, only a single specimen of *Anopheles* mosquito could be collected. This observation will justify the relative absence of malaria in the state though it is highly endemic in the neighboring states of Tamil Nadu, Karnataka and Andhra Pradesh.

The genus *Culex* accounted for 67.1 per cent of the entire collection followed by *Mansonia, Anopheles, Armigeres, Aedes, Ochlerotatus* and *Toxorhynchites*. Eleven species of *Culex* mosquitoes were identified in the present study. *Culex tritaeniorhynchus* was the most predominant species forming 43 per cent of the mosquitoes collected from cattle sheds. Kulkarni (1986) found this as the most abundant mosquito species forming 36 per cent of the total collection in the study carried out in Goa. Schreiber *et al.* (1988) also found *Cx. tritaeniorhynchus* as the most prevalent species

caught in surveillance traps when mosquito population trends associated with dairies were studied in Southern California.

Under the genus *Aedes,* 61 species have been reported from India so far (Rajavel, 2004). However, only four species were obtained in the present study. *Aedes* mosquitoes are more exophilic and diurnal in their habits and this could be the reason for the relative low prevalence of this genus in the present study.

The number of species under the genus *Armigeres* reported from India so far comes to sixteen. But the present study could identify only a single species, *Ar. subalbatus*.

5. 1. 1. Kerala

Compiling from the scanty studies carried out in Kerala on the species prevalence of mosquitoes (Daniel. *et al.,* 1986; Mariappan *et al.*, 1996; Mariappan *et al.,* 1997; Hiriyan, *et al.,* 2003; Sabesan *et al.*, 1991; Arunachalam *et al.*, 2004), 46 species under ten genera have been reported. This includes different species under various genera as shown in Table 41.

The present study revealed only seven genera such as *Aedes, Anopheles, Armigeres, Culex, Mansonia, Ochlerotatus* and *Toxorhynchites*. Though ten genera have been reported from Kerala, the occurrence of *Toxorhynchites* has not been reported in any of the previous studies. The reason for this might be that the previous workers concentrated on mosquitoes of medical importance and could have missed the non biting *Toxorhynchites*.

Table 41. Number of genera and species of mosquitoes recorded from Kerala (Compiled)

Sl. No.	Genera	Number of species recorded
1	*Anopheles*	11
2.	*Aedes*	5
3.	*Culex*	17
4.	*Armigeres*	1
5.	*Mimomyia*	2
6.	*Mansonia*	3
7.	*Uranotaenia*	4
8.	*Orthopodomyia*	1
9.	*Ficalbia*	1
10.	*Coquilettida*	1
Total		**46**

All the other species except *Ochlerotatus* have been reported from Kerala in the previous studies. It may be remembered that *Ochlerotatus* was elevated to the genus status only recently mainly based on the morphology of the genitalia (Reinert, 2000).

Culex species represented 77 per cent of the total collection made in Kuttanad, Kerala with *Cx. tritaeniorhynchus* as the most dominant among them representing 63 per cent (Hiriyan *et al.*, 2003). Mariappan *et al.* (1997) and Arunachalam *et al.* (2004) also found *Cx. tritaeniorhynchus* as the most abundant species as in the present study. *Culex quinquefasciatus*, the southern house mosquito, very abundantly present in human dwellings

was virtually absent in all cattle farms despite the very close location of some of the cattle sheds to human dwellings. Reuben *et al.* (1994) indicated the strong anthropophilic nature of this species.

Though 16 species of *Armigeres* were known to occur in India, only a single species, *Ar. subalbatus,* has been reported from Kerala. This species alone could be detected in the present study also. They were larger sized biting mosquitoes and were detected in all the localities including human dwellings. The prevalence in different locations varied from 1.5 to 8.5 per cent in cattle sheds and 34.5 per cent in human dwellings. Mariappan *et al.* (1997) recognized them as the predominant nuisance mosquito in Kochi. The blood fed females could be easily recognized on the walls of the cattle sheds owing to their large size. They were also present in large numbers around dusk in human dwellings and were a major biting nuisance.

Three species of *Mansonia* were known to occur in Kerala as early as 1938 which were involved in the transmission of brugian filariosis (Iyengar, 1938). The same three species alone could be identified in the present study also. *Mansonia uniformis* was present in all farms and human dwellings, while *Ma. indiana* could be collected from two farms only, and *Ma. annulifera* from Surya Farm alone. Only two specimens of *Mansonia* mosquito (*Ma. uniformis*) could be seen in human dwellings. Daniel *et al.* (1986) found that more than 60 per cent of the total collections in Trivandrm city were *Ma. uniformis.*. They collected mosquitoes from a cattle shed in the vicinity of a canal with much aquatic plants that were the breeding sites of *Mansonia* sp. *Mansonia uniformis, Ma. annulifera* and *Ma. indiana* - the three species of *Mansonia* described

by earlier workers (Iyengar, 1938; Sabesan *et al.*, 1991; Krishnaamoorthy *et al.*, 1993; Hiriyan *et al.*, 1996) from different parts of Kerala alone could be identified in the present survey. These three species are known vectors of human filariosis due to *Brugia malayi* widely prevalent in certain pockets of coastal Kerala.

5.2. BREEDING CULICINE MOSQUITOES IN THE LABORATORY

The highly prevalent species of mosquitoes found in cattle sheds identified as *Cx. tritaeniorhynchus* was bred in the laboratory. In many of the previous studies carried out, various species of *Aedes* mosquitoes or *Cx. quinquefasciatus* were maintained in the laboratory, as much of the work was in relation to human hosts. In tropical countries when temperature and humidity are high, breeding occurs at a faster rate (10 to 14 days). When temperature was low especially during June- July and November- December, the period for emergence of adults from eggs took up to 18 days. To regulate temperature and humidity, the rearing units were kept inside wooden cupboards and the life cycle could be completed in 10 to 18 days. The engorged adults were found to lay eggs by 48 to 72 hours. The eggs hatched by 24 to 36 hours. Each moulting took 48 to 72 hours. From a population of pupae, the first ones to emerge were always males. Horsfall (1972) and Service (1980) also reported the life span of mosquitoes in tropical countries to take a period of one to two weeks. The characters described for the eggs, larvae and pupae of mosquitoes by the above authors agreed with the observations made during the present work.

5.2.1 Culex tritaeniorhynchus

The accessory pale patch basal to the pale band on the ventral surface of proboscis and the narrow apical dark ring on the hind femur were the characters used to identify the most commonly prevalent species, *Cx. tritaeniorhynchus.* The fourth stage larvae were identified by the apically rounded (fan-shaped) comb scales fringed with sub equal spicules and weak siphonal tufts having two to five branches as described by Reuben *et al.* (1994).

5.3. SCREENING MOSQUITOES FOR LARVAL STAGES OF HELMINTHS

Though 1001 mosquitoes belonging to five genera namely, *Culex, Aedes*, *Armigeres, Anopheles* and *Mansonia* collected from cattle sheds were dissected to find developmental stages of helminth parasites, none was found infected. The helminth larvae expected to exist in them can only be those of *Setaria cervi*, a relatively nonpathogenic filarid worm affecting ruminants (Verma *et al.*1971). The prevalence of *Setaria* sp. infection in cattle is relatively low. Examination of 154 blood smears of cattle in the Department of Veterinary Parasitology during the years 2002 and 2003 had revealed the presence of microfilariae in only one sample. The number of mosquitoes dissected formed only a very small percentage of the entire population during a particular period in the cattle shed. The absence of helminth larvae in any of the mosquitoes examined during the present study can be attributed to the two factors mentioned above.

Out of the 1019 mosquitoes collected from human dwellings in the vicinity of dog houses, dissected and examined, two were found to harbour helminth larvae. In the *Armigeres* sp. sausage shaped second stage larvae and in the *Culex* sp. elongate 3rd stage larvae of *D. repens* were observed in the Malpighian tubules. Both *Culex* and *Armigeres* sp. have been reported as suitable vectors for the development *D.repens* (Wright *et al.* 1989; Aranda *et al.* 1998). Various studies conducted in and around Thrissur in dogs have shown the prevalence of *D. repens* infection to vary from 7 to 13 per cent (Saseendranath *et al.,* 1986; Radhika, 1996).

Tolbert and Johnson (1982) found two percent of the mosquitoes infected with helminth larvae in Alabama, while Parker (1986) observed it as 0.7 per cent. Aranda *et al.* (1998) found 20 per cent of the dogs as microfilariaemic in Barcelona, Spain, while none of the 2001 mosquitoes sampled were positive for infection. Singh *et al.* (2000) also could not find any larval helminth in the 339 *Cx. quinquefasciatus* dissected. Most of the mosquitoes caught for dissection from human dwellings in the vicinity of dog houses were either found non fed or fed for the first time and this could be the reason for the very low number of positive cases.

5. 4. EVALUATION OF NEWER GENERATION INSECTICIDES ON THE DEVELOPMENTAL STAGES OF MOSQUITOES

All the previous studies on the developmental stages of mosquitoes were conducted on species other than *Cx. tritaeniorhynchus*. However, in the present study, *Cx. tritaenirhynchus* was used, as it was the most abundant species found in cattle sheds.

5.4.1. Eggs

5. 4. 1. 1. Insect Growth Regulators

5. 4. 1. 1. 1. Diflubenzuron

The chitin inhibiting compounds are believed to disturb endocuticular deposition and thereby interfere with normal cuticle formation (Post and Vincent, 1973). Diflubenzuron has been reported to be effective against all stages of mosquitoes and is relatively safe to other organisms associated with mosquito breeding habitats. The effect of diflubenzuron toxicity was exhibited as unhatch, abnormal hatch (side hatch) and various types of partial hatch. In partial hatch, the larvae died during hatching and various parts of the larva were seen caught in the shell. Miura *et al.* (1976) also found such abnormalities when eggs of *Cx. quinquefasciatus* were exposed to various concentrations of diflubenzuron.

The concentration of diflubenzuron producing 50 per cent emergence inhibition was assessed as 38. 27 ppm based on probit analysis while Miura *et al.* (1976) did not use probit analysis but found that 42 per cent eggs were affected by a concentration of 0.01 ppm of diflubenzuron. Unexposed eggs hatched by 36 hr while, treated eggs were found to take longer time for hatching. This phenomenon was also reported by Miura *et al.* (1996) who attributed it to slower embryonic growth rate. Unhatched eggs contained fully developed embryos which failed to hatch-apparently died just before hatching. Segmentation, eyespots, setae and spine were visible through the eggshell. In normal eggs a portion of the anterior end of the eggshell is forced open transversely at a line of dehiscence forming an egg cap, which is not completely detached but

hinged to the remaining eggshell (Miura *et al.,* 1976; Vasuki, 1990). While LC_{50} on 4th stage larva was 0.00023 ppm, a concentration of 38.27 ppm cannot be recommended for use against eggs of mosquitoes.

5. 4. 1. 1. 2. Methoprene

Methoprene is a juvenile hormone analogue and the various preparations available in the market are recommended for control of mosquitoes due to their action on the larval stages. However in the present study conducted, a concentration of 500 ppm (10,000 times the LC_{100} on fourth stage larvae) was not found to induce any effect on the eggs of *Cx. tritaeniorhynchus.* There is also no report available on the effect of methoprene on the eggs of any species of mosquitoes and methoprene is recommended for use against larval stages of mosquitoes.

5. 4. 1. 2. *Bacillus thuringiensis israelensis* (HIL *Bti*)

Bti is recommended for mosquito control for its action on larval stages. However in the present study, LC_{50} on eggs was found to be 21.7252 ppm while the LC_{90} was 110.5554 ppm. These values in fact are around three and ten times respectively of the LC_{50} values of *Bti* on fourth stage larvae. It was found that higher concentrations had ovicidal action though such high concentrations are not employed in the field for mosquito control.

5. 4. 1. 3. Azadirachtin

Though azadirachtin, a triterpinoid derived from neem tree is a well known feeding and oviposition deterrent and repellent to many insects, Su and Mulla (1988) and Elhang *et al.* (2001) tested their efficacy on the hatchability of mosquito eggs. According to the former authors one ppm azadirachtin could induce cent percent mortality in eggs. Elhang *et al.* (2001) found that reduction in hatchability in concentrations as low as 0.02 per cent. However in the present study 93 ppm azadirachtin was required to produce fifty per cent non hatchability in the eggs of *Cx. tritaeniorhynchus.* The evident variations in the concentrations could be due to the difference in the contents of the preparations used.

5. 4. 1. 4. Ethofenprox

There are no reports on the effect of ethofenprox on the eggs of any species of mosquitoes. The LC_{50} arrived at in the present study was 0.23482 ppm and the LC_{90} was 0.76112 ppm. These values are about 29 and 19 times as that of the LC_{50} and LC_{90} concentrations on the fourth stage larvae respectively. The results suggest that ethofenprox has definite ovicidal action.

5. 4. 1. 5. Deltamethrin

The LC_{50} observed in the present study was 0.04814 ppm while the LC_{90} was 1.57149 ppm. However, Sahgal and Pillai (1993) could not find egg mortality above 50 per cent even at a concentration of one ppm which in fact was the highest concentration tried by them. They rated the ovicidal activity as moderate when compared to the activity on larval stages. In the

present study, a concentration of 0.005 ppm could produce 22 per cent non hatchability.

5. 4. 2. Fourth Stage Larvae

5. 4. 2.1. Insect Growth Regulators

5. 4. 2. 1. 1. Diflubenzuron

The sensitivity of larvae to a given compound varied with age of the fourth stage larvae (Schaefer and Wilder, 1972). In the present study, freshly emerged fourth stage larvae were employed. The LC_{50} was estimated as 0.00023 ppm on probit analysis. Such concentrations (LC_{50}) varied from 0.00016 to 0.01 ppm when diflubenzuron was studied against four species of mosquitoes on the 4^{th} stage larvae (Bakshi *et al.*, 1982). Similar values ranging from 0.00024 to 0.00145 ppm could inhibit 50 per cent adult emergence in *Ae. albopictus* (Kawada, 1993; Prakash, 1993; Ali *et al.,* 1995; Baruah and Das, 1996). The treated larvae died due to various types of deformities caused in the course of development or had delayed development often resulting in mortality in emerging adults as reported by Tyagi *et al.*, (1987). Bulk of the mortality occurred at the larval stage as seen by Mulla and Darwazeh (1985) and Baruah and Das (1996). But Bakshi *et al.* (1982) found that when 4^{th} stage larvae were exposed to diflubenzuron, the total mortality accounted was equally due to larval and adult mortality. Delayed larval development and discolouration of cuticle of immatures observed in the present study are in agreement with the observations made by Mulla *et al.* (1975), Miura *et al.* (1976), Bakshi *et al.* (1982) and Prakash (1992).

The LC_{90} of diflubenzuron on 4th stage larvae was 0.00409 ppm. This is 17.7 times the LC_{50} obtained in the present study. Relatively higher concentrations ranging from 1.22 to 34. 66 ppm have been reported by Prakash (1993) against four species of mosquitoes. Lower concentrations at par with the present study have been obtained by Ali *et al.* (1995), Ali *et al.* (1999) and Baruah and Das (1996).

5. 4. 2. 1. 2. Methoprene

Methoprene at a concentration of 0.00067 ppm was estimated as the LC_{50} on the 4^{th} stage larvae of *Cx. tritaeniorhynchus*. Baruah and Das (1996) found that the LC_{50} of methoprene on *Cx, quinquefasciatus* and *Ae. albopictus* as 0.001 and 0. 0018 ppm respectively. These concentrations are about two to three times the values obtained in the present study. When Hsich and Steelman (1974) conducted comparative susceptibility of five insect development inhibitors against 12 species of mosquitoes, reported that the susceptibility of each species within a genus relative to the type of compound varied to such a degree that no comparative generalizations of genus susceptibility could be made. The wide variation observed in the effective dosages by earlier investigators have been attributed to different strains of mosquito larval instars, rearing temperatures, type of water, food *etc.* as suggested by Baruah and Das (1996). Different types of deformities like decolorization, failure of eclosion or partial eclosion, prolonged larval period and pale larvae and pupae as observed by Hsich and Steelman (1974) and Das *et al.* (1981) were observed in the study.

The LC_{90} obtained in the present study was 0.00673 ppm, which is about 10 times the LC_{50} on the 4^{th} stage larvae. Slightly lower

concentrations (0. 0022 and 0.0027 ppm) of methoprene were found to produce 50 per cent mortality in *Ae. albopictus* and *Cx. quinquefasciatus* respectively (Baruah and Das,1996).

5. 4. 2. 2. *Bacillus thuringiensis israelensis*

The LC_{50} was estimated as 6.6076 ppm in the present study. Significant variation in the responses ranging from 4.5 to 100 ppm was reported by Farghal (1987) and Skovmand *et al.* (1998) on different species of mosquitoes. They attributed the variation to be associated with factors such as age, stage and strain of larvae as well as the amount and type of food provided to the larvae. Vectobac® containing *Bti* was found to give a LC_{50} of 0.181 and 0.0104 ppm respectively (Ali *et al.*, 1995; Ponce *et al.*, 2002). The same product when tried by another scientist on two different species of mosquitoes (*Ae. aegypti* and *Ae. albopictus)* needed 17 times the concentration to produce 50 per cent mortality. Skovmand *et al.* (1998) demonstrated variation in the LC_{50} between similar products from different companies.

Similarly, the LC_{90} obtained in the present study was 20. 8106 ppm. While Ali *et al.* (1995) found it as 0.380 ppm, Ponce *et al.* (2002) got the LC_{95} as 0.1843. These values are much less than those obtained in the present study. Such variations have been reported to occur when different products were tried on different species of mosquitoes.

5. 4. 2. 3. Azadirachtin

There are no reports on the action of azadirachtin on mosquito larvae though various studies have been carried out with different types of extracts from neem seed kernel. A concentration of 4.68681 ppm was found as LC_{50} while 8.49508 ppm was the LC_{90} obtained in the present study. Boschitz and Grunewald (1994) and Elhang *et al.* (2001) have also reported suppression of larval development, pupation and adult emergence when the 4^{th} stage larvae of *Cx. quinquefascitus* were exposed to various concentrations of different types of extracts from neem seed kernel. Delayed pupation was also noticed in the present study. Also the successfully emerged adults survived for lesser periods when compared to the control group. This emphasizes the delayed effect of azadirachtin on mosquito larvae. Since azadirachtin is a plant derived product, least environmental contamination is produced (Elhang *et al.,* 2001).

5. 4. 2. 4. Ethofenprox

Though ethofenprox has been shown to be an insecticide with comparable toxicity and similar mode of action to other pyrethroids, very few references are available on its effect on mosquito larvae. Vasuki *et al.* (1995) observed the LC_{50} as 0.579 ppm while in the present work it was 0.00819 ppm. The LC_{90} arrived at in the present study (0.03931 ppm) was much lower than that observed by Vasuki *et al.* (1995).

5. 4. 2. 5. Deltamethrin

Various pyrethroids have been recognized as potential alternatives to replace OC and OP compounds in mosquito abatement programmes due

to their excellent effect on various stages and remarkably low mammalian toxicity. The LC_{50} and LC_{90} values obtained in the present study were 0.00209 and 0.00654 ppm respectively. Comparable concentrations have been obtained by several earlier workers (Chakraboti *et al.,* 1993; Ali *et al.*, 1999; Ganesh *et al.,* 2003). Though the various pyrethroid compounds have been proven to have definite toxicity against all stages of mosquitoes, WHO (2002) strongly recommends that pyrethroids should never be used for larviciding as they are invaluable adulticides.

5. 4. 3. Pupae

5. 4. 3. 1. Insect Growth Regulators

The IGRs are more effective when applied during larval stages as they act by inhibiting larval development and therefore, very few reports are available on the effect of IGRs on the pupae of mosquitoes. When pupae are treated, the exposure time before eclosion is minimum and hence they have least action on pupae.

5. 4. 3. 1. 1. Diflubenzuron

A concentration of 28.774 ppm of diflubenzuron was found to produce 50 per cent emergence inhibition when pupae were treated. The fourth stage larvae have been reported to be more sensitive to IGRs (Schaefer and Wilder, 1973) and for this reason very few records are available on their effect on pupae of mosquitoes. Prakash (1993) found the LC_{50} value on pupae of four species of mosquitoes to range from 0.89 to 2.18 ppm. When several IGRs were tested against 1^{st} and 4^{th} instars and pupae of *Cx. quinquefasciatus*, Mulla *et al.* (1974) found that 10 to 150

times the LC_{50} on 4th stage larvae were needed to produce the same effect on pupae. The LC_{90} on pupae was much higher being 489.7338 ppm.

5. 4. 3. 1. 2. Methoprene

The material used in the present study (Altosid Liquid Larvicide®) is a product of Zoecon, Wellmark International, Illinois, USA. According to the product information provided by the manufacturer, methoprene has no effect when applied to pupae or adult mosquitoes. But Das *et al.* (1981) studied the effect of methoprene on the larvae and pupae of *Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti.* A concentration of two ppm of methoprene applied to larval stages produced total inhibition of adult emergence, while the same on application to pupal stages could inhibit only 21. 5 per cent adult emergence. However, in the present study the LC_{50} was 0.22858 ppm and LC_{90} was 6.25179 ppm. These values are 336 and 929 times respectively of the same for 4th stage larvae. Such high concentrations are never used in practical mosquito control and hence methoprene cannot be recommended against pupal stages of mosquitoes.

5. 4. 3. 2. *Bacillus thuringiensis israelensis*

Bti protoxins are insecticidal crystal proteins requiring solubilisation and activation in the insect midgut. Hence their effect on pupae will be least as the pupae are non feeding. Similarly, the LC_{50} (66. 68765) and LC_{90} (187.36604) against pupae obtained in the present study were about ten times the same on 4th stage larvae. Ponce *et al.* (2002) calculated the field dose of *Bti* as four times the LC_{50} on larvae. Higher concentrations effective against pupae are unlikely to be used in practical mosquito control

programs and for this reason *Bti* cannot be recommended against pupae of mosquitoes.

5. 4. 3. 3. Azadirachtin

Azadirachtin has been shown to have repellency and growth disruption in a number of species of insects. Mong Ting Tan and Sudderuddin (1978) noticed inhibition of pupal development and presence of deformed wings in surviving adults on exposure to azadirachtin. The LC_{50} and LC_{90} obtained in the present study were 4.50358 and 11.31192 ppm respectively. These values are quite comparable with those for 4^{th} stage larvae obtained during the present study. As azadirachtin is a plant derived product and again comparable concentrations are able to produce similar effects on pupae, azadiractin can be recommended as a pupicide on mosquitoes.

5. 4. 3. 4. Ethofenprox

There are no reports on the effect of ethofenprox on pupae. The LC_{50} and LC_{90} obtained in the present study were 0.26044 and 1.91214 ppm respectively. These values are very high when compared to those for 4^{th} stage larvae. And hence ethofenprox cannot be recommended as a pupicide of mosquitoes.

5. 4. 3. 5. Deltamethrin

The LC_{50} and LC_{90} on pupae were 0.00382 and 0.02016 ppm respectively. These concentrations are quite comparable with those for 4^{th} stage larvae. Though deltamethrin is as effective on pupae as larvae, the

recommendations of WHO (2002) excludes it to be used against mosquito immatures as all pyrethroids are invaluable adulticides.

5. 4. 4. Comparison of Effective Values of Insecticides on Different Stages of *Cx. tritaeniorhynchus*

The least concentrations were obtained as LC_{50} and LC_{90} when the various insecticides were used against 4th stage larvae. Schaefer and Wilder (1972) pointed out that the 4th stage larvae are more sensitive to insecticides than the other stages in the life cycle. When the LC_{50} on 4th stage larvae were minimum in case of all the insecticides tried in the present study, several multiples of the same was needed to produce the same effect on eggs and pupae. While methoprene had no effect on eggs, the other IGR (diflubenzuron) used in the study was observed to have least effect on the eggs and pupae of *Cx. tritaeniorhynchus*. At the same time the LC_{50} of these two IGRs on larvae were the least being only 0.00023 and 0.00067 ppm respectively (Table 28).

For the other insecticides (*Bti,* ethofenprox and deltamethrin) a 1.8 to 31 times the larval LC_{50} could cause the same rate of mortality in pupae. But in case of azadirachtin, the LC_{50} on larvae and pupae were comparable. Relative higher multiples of the larval LC_{50} were found to cause 50 per cent non hatchability in eggs in case of all the insecticides studied.

In the strict sense, IGRs cannot be compared with chemical insecticides, though they weigh over the latter in several concepts. Still it was found that the LC_{50} of the IGRs were the least in comparison to all other insecticides used in the study. Hence they need to be used in very less concentrations in practical mosquito control. IGRs being more compatible

with biologicals, do not interrupt the natural regulatory mechanisms and can be strongly recommended for mosquito control in IPM programs.

Based on the effective concentrations on 4th stage larvae, the chemical insecticides – ethofenprox and deltamethrin - were next in order. Deltamethrin was more potent than ethofenprox on eggs, larvae and pupae. Since they are protoplasmic poisons, relatively lesser concentrations when compared to IGRs could produce 50 per cent mortality/non hatchability in pupae/eggs. However, when the field dosages were calculated as four times the larval LC_{50}, deltamethrin will have effect on pupae as the pupal LC_{50} of deltamethrin was only a 1.8 multiple.

Though the other insecticides used in the study –*Bti* and azadirachtin- are equally environment friendly as the IGRs, the LC_{50} and LC_{90} values obtained were quiet high. However, the same concentration of azadirachtin was found to produce 50 per cent mortality in 4th stage larvae as well as pupae, though the LC_{50} on eggs was 20 times the former.

Bti was found to have better action on the eggs than on the pupae of *Cx. tritaeniorhynchus*.

5. 4. 5. Cost of Application in Unit Volume

When the cost of application of the various insecticides in a unit volume was calculated based on the MRP of the commercial products used in the study (Table 36), ethofenprox (Primo) was found to be most economical costing Rs. 0. 03/- only per one lakh litres of water, followed by deltamethrin and diflubenzuron. Both the IGRs were better than

azadirachtin and *Bti* in terms of cost. The most expensive insecticide was *Bti* costing Rs. 2179/- per one lakh litres of water.

Both the chemical insecticides used in the study have a mode of action similar to pyrethroids. The World Health Organization (2002) report strongly recommends that pyrethroids should never be used against larval stages of mosquitoes as they are invaluable adulticides. Hence both ethofenprox and deltamethrin cannot be recommended against larval mosquitoes though they have been found effective in terms of cost and concentration. At the same time they have also been shown to cause detrimental effects on beneficial insects and natural predators.

As there is a definite lack of commercial interest in the development of new pesticides on account of exorbitant financial commitment involved, the existing insecticides should be used judiciously to conserve them for the future. So in the IPM programs widely recommended for the control of mosquitoes, the various insecticides should be used to limit the population density to a level that is acceptable as determined by economic factors and environmental concern. As envisaged in the IPM strategy, the natural control measures must be maximized, the concentration of pests must be monitored and appropriate techniques must be adopted to suppress the pest only when necessary, to attain stable long term mosquito control.

Summary

6. SUMMARY

1. Mosquitoes were collected from five cattle sheds in and around Thrissur, at monthly intervals from January to December 2002. Specimens were also collected from human dwellings for identification. Mosquitoes coming under three subfamilies were identified *viz.*, Anophelinae (*Anopheles*), Culicinae (*Aedes, Armigeres, Culex, Mansonia, Ochlerotatus*) and Toxorhynchitinae (*Toxorhynchites*). The collection comprised of 27 species coming under seven genera. The genus *Culex* constituted 67.1 per cent of the total collection.

2. *Culex tritaeniorhynchus* was identified as the most prevalent species in the collection from all five cattle sheds with an overall prevalence of 43.1 per cent. The most prevalent species in human dwellings was *Cx. quinquefasciatus* (36.4 %), closely followed by *Ar. subalbatus* (34.5%). *Culex quinquefasciatus,* the southern house mosquito could not be found in the collection from any cattle shed while, *Armigeres subalbatus* was equally distributed in both cattle sheds and human dwellings.

3. *Culex tritaeniorhynchus,* the most commonly prevalent species in cattle sheds was successfully colonized in the laboratory. The eggs were laid as egg raft containing 77 to 166 with an average of 117 eggs per raft. The development of the adult mosquitoes from the eggs took 7-18 days under laboratory conditions. The average number of eggs laid by a female was 42. The average survival time was 45 days. The male mosquitoes always emerged first from the pupae.

4. Dissection of 1001 mosquitoes collected from cattle sheds did not reveal any helminth larvae. Meanwhile, 1019 mosquitoes collected from human dwellings in the vicinity of dog houses when dissected revealed helminth larvae in two mosquitoes. One *Armigeres* sp. had 56 sausage shaped second stage larvae and a *Culex* sp. had five elongate infective larvae. Based on morphology the larvae were identified as those of *Dirofilaria repens*.

5. Six different insecticides *viz.*, diflubenzuron, methoprene, *Bti*, azadirachtin, ethofenprox and deltamethrin were evaluated for their effect on the eggs, fourth stage larvae and pupae of *Cx. tritaeniorhynchus*. At least five concentrations producing mortality ranging from 5 to 100 per cent were worked out. The mortality data were subjected to computerized probit analysis of log dosages using SPSS to arrive at the LC_{50} and LC_{90} values. The 4^{th} stage larvae were more susceptible to all the insecticides with the least concentration of LC_{50} and LC_{90}. The IGRs used in the study were most efficient. In terms of LC_{50} concentration, the various insecticides in the order of efficacy were diflubenzuron (0.0002288 ppm), methoprene (0.0006738), deltamethrin (0.0020853) ethofenprox (0.0082146), azadirachtin (4.6867982) and *Bti* (6.6076021).

6. On treating the pupae with the various insecticides, the most effective one was found to be deltamethrin (0.00382) followed by methoprene (0.228578), ethofenprox (0.260437), azadirachtin (4.503588), diflubenzuron (28.77353) and *Bti* (66.68765). However, several multiples of the larval LC_{50} was required to produce the same effect on pupae except azadirachtin.

7. When eggs were exposed to the insecticides, deltamethrin was the most toxic followed by ethofenprox, *Bti,* diflubenzuron and azadirachtin. Methoprene had no effect on the egg hatchability. Several thousand multiples of the larval LC_{50} of diflubenzuron were needed to produce the same effect on eggs. But in the case of *Bti,* 3.3 times of the larval LC_{50} could produce 50 per cent non hatchability in eggs.

8. The LC_{50} values of azadirachtin on the fourth stage larvae and pupae were almost same. However a 20 times concentration was needed to effect 50 per cent non hatchability in eggs. The IGRs -diflubenzuron and methoprene- were the most effective insecticides when used against 4^{th} stage larvae and are recommended for use in mosquito larval control as only minimum concentration need to be used. These compounds do not interrupt natural regulatory mechanisms as they are least toxic to beneficial insects and natural predators.

9. When MRP alone was considered, ethofenprox was found to be the cheapest insecticide, costing Rs.0.03/- per litre of water followed by deltamethrin (Rs.0.51/-). The most expensive one was *Bti* (Rs.2179/-) followed by azadirachtin (Rs.1630/-). Among the IGRs diflubenzuron was much cheaper (Rs.1.38/-) than methoprene (Rs.13.87/-).

10. To avoid selection pressure on immature stages of mosquitoes, deltamethrin and ethofenprox should not be used for larval mosquito control as they are invaluable adulticides.

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EFFECT OF CERTAIN NEWER GENERATION INSECTICIDES ON THE DEVELOPMENTAL STAGES OF MOSQUITOES

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Mosquitoes collected from five cattle sheds and human dwellings in and around Thrissur were identified into 27 species coming under seven genera in three subfamilies. Only one genus each could be found in the subfamilies of Toxorhynchitinae and Anophelinae, while there were five genera in the subfamily Culicinae. The genus *Culex* constituted 67.1 per cent of the total collection. *Culex tritaeniorhynchus* was identified as the most prevalent species in cattle sheds while in human dwellings it was *Cx. quinquefasciatus*.

Culex tritaeniorhynchus was successfully colonized in the laboratory. The development of adults from eggs took 7-18 days under laboratory conditions. The average number of eggs laid by a female was 42. The average survival time was 45 days. The male mosquitoes always emerged first from the pupae.

Dissection of 1001 mosquitoes collected from cattle sheds did not reveal any helminth larvae. Meanwhile, 1019 mosquitoes collected from human dwellings in the vicinity of dog houses when dissected revealed helminth larvae in two mosquitoes. The larvae were identified as those of *Dirofilaria repens* based on morphology.

Six different insecticides namely, diflubenzuron, methoprene, *Bti*, azadirachtin, ethofenprox and deltamethrin were evaluated for their effect on the eggs, 4th stage larvae and pupae of *Cx. tritaeniorhynchus*. The 4th stage larvae were more susceptible to all the insecticides with the least concentration of LC_{50} and LC_{90}.

The IGRs used in the study were most efficient. In terms of LC_{50} concentration on larvae, the various insecticides in the order of efficacy were diflubenzuron (0.0002288 ppm), methoprene (0.0006738), deltamethrin (0.0020853) ethofenprox (0.0082146), azadirachtin (4.6867982) and *Bti* (6.6076021). The IGRs -diflubenzuron and methoprene- are recommended for use in mosquito larval control as only minimum concentration need to be used. Methoprene had no effect on the eggs whereas several multiples of the other insecticides were needed to produce the same effect on eggs or pupae. In case of azadirachtin, the LC_{50} on larvae and pupae were almost same.

When MRP alone was considered, ethofenprox was found to be the cheapest insecticide, costing Rs.0.03/- per one lakh litres of water followed by deltamethrin (Rs. 0.51/-). The most expensive one was *Bti* (Rs. 2179/-) followed by azadirachtin (Rs. 1630/-). Among the IGRs diflubenzuron was much cheaper (Rs. 1.38/-) than methoprene (Rs. 13.87). To avoid selection pressure on immature stages of mosquitoes, deltamethrin and ethofenprox should not be used for larval mosquito control as they are invaluable adulticides.